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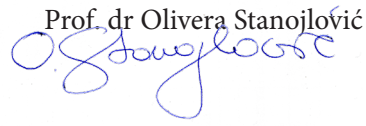

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ORIGINAL ARTICLE

The role of 18F-FDG PET/CT in the follow-up of laryngeal cancer after treatment

Jelena Petrović[1,3], Dragana Šobić Šaranović[1,3], Jovica Milovanović[2,3], Ana Jotić[2,3], Strahinja Odalović[1,3], Isidora Grozdić Milojević[1,3], Milica Stojiljković[1,3], Vera Artiko[1,3]

[1] Center for Nuclear Medicine with PET, University Clinical Center of Serbia

[2] Clinic for Otorhinolaryngology, University Clinical Center of Serbia

[3] University in Belgrade, Faculty of Medicine

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Correspondence to:
dr Jelena Petrović
Center for Nuclear Medicine with PET, University Clinical Center of Serbia
26 Visegradska Street
11 000 Belgrade, Serbia
Tel: +381 64 147 4901
E-mail: jelena_petrovic18@yahoo.com

Summary

Introduction: The aim of this investigation was to estimate the diagnostic performance of positron emission tomography/computed tomography using fluorine-18 fluoro-deoxyglucose (18F-FDG PET/CT) in the follow-up of post-treatment laryngeal squamous cell carcinoma (SCC) and the survival rate.

Material and methods: Fifty seven patients (50 males, 7 females), mean age (68.3±6.7), with post-treatment laryngeal SCC were investigated. Indications for 18F-FDG PET/CT were: post-surgery staging, post-therapy restaging with positive/uncertain CT, follow-up, suspected recurrence on CT. 18F-FDG PET/CT findings were compared to the clinical follow-up of up to 10 years after imaging. The degree of metabolic activity was analyzed visually and semi-quantitatively using the maximum standardized uptake value (SUVmax).

Results: A high accumulation of radiopharmaceutical was found in 41 (71.9%) patients who were considered true positive, physiological in 14 patients (24.5%) and only two males (3.5%) were false positive. Overall sensitivity of 18F-FDG PET/CT was 95.3%, specificity 100.0%, positive predictive value 100.0%, negative predictive value 87.5% and accuracy 96.5%. In 19 cases (33.3%) PET/CT findings significantly influenced further management of the patients. Progression-free survival (PFS) in the 18F-FDG positive group was 41.1±12.7 months. Statistically significant correlation between SUVmax and PFS was not observed (p>0.05).

Conclusion: 18F-FDG PET/CT is a valuable tool for the follow-up of laryngeal SCC due to its high sensitivity, specificity, PPV, NPV and accuracy. It can influence the patients' management in a significant number of cases. Patients with negative 18F-FDG PET/CT findings had longer PFS than those with positive ones, but without statistical significance. SUVmax was not proven to be a strong predictor of patients' disease-free survival.

Keywords: PET/CT, laryngeal carcinoma, 18F-FDG, SUVmax

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INTRODUCTION

The majority of laryngeal cancers are squamous cell carcinoma (SCC) that can develop in any part of the larynx. Most laryngeal cancers originate from the glottis, while supraglottic, or subglottic tumors are less frequent (1). The cure rate depends on the location of the tumor (2). Laryngeal cancer may spread by direct extension to adjacent structures, by metastases to regional cervical lymph nodes, or more distantly, through the blood stream. Distant metastases to the lungs are most common (3). A five-year survival rate is around 60.3% and it depends on the stage (4). The diagnosis is made based on medical history, physical examination and tissue biopsy. In addition, various imaging methods are performed, such as chest x ray, contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI) and ^{18}F-2-fluoro-2-deoxy-D-glucose positron-emission tomography/computed tomography (18F-FDG PET/CT), especially in detection of the presence and the stage of the tumor and in the follow-up of the treatment (5-7). Current indications for the application of 18F-FDG PET/CT, being even superior to CT and MRI, are: the detection of carcinoma of unknown primary localization, cervical lymph node metastases, distant metastases, residual/recurrent cancer and second primary tumors. In a certain number of cases, this investigation leads to alterations in patient management, provides an overall assessment of tumor aggressiveness with prognostic implications based on the spread of the disease and a calculation of maximal standardized uptake value (SUVmax) (8, 9). The aim of this investigation is to estimate the diagnostic performance of 18F-FDG PET/CT in the follow-up of the post-treatment laryngeal SCC, as well as the survival rate.

MATERIAL AND METHODS

Patients

In the period from November 2012 to October 2018, all patients with the diagnosis of laryngeal carcinoma who were referred to 18F-FDG PET/CT, at the National PET Center with PET of the University Clinical Center of Serbia, were included in this cohort retrospective study. Indications for 18F-FDG PET/CT scan were: staging after surgery, restaging after chemo- or radiotherapy with positive or uncertain CT results, the follow-up, and suspected recurrence based on CT. The criteria for inclusion were histological verification of laryngeal carcinoma during surgical resection, and CT scans done prior to 18F-FDG PET/CT. Exclusion criteria were patients with another neoplasm, and glycaemia over 11mmol/l. Fifty-seven patients (50 males and 7 females), mean age (68.3 ± 6.7, median 69, range 55-85), satisfied the criteria for entering this study.

All the patients included underwent surgical resection of the tumor with some form of the neck dissection in case of cervical lymphadenopathy. The choice of adjuvant treatment was decided upon based on the guidelines recommended and used at the Clinic for Otorhinolaryngology and Maxillofacial Surgery and the Institute for Oncology and Radiology of Serbia in Belgrade (10, 11). Radiotherapy consisted of external radiotherapy with the total dose of 60 to 70 Gy in 30–35 fractions for 6–7 weeks. Patients received concomitant chemotherapy consisted of at least three courses of cisplatin (CDDP) with 5-fluorouracil (5-FU) intravenously.

The reference standards were the clinical follow-up, and a surgery with histological finding of laryngeal carcinoma. The results of 18F-FDG PET/CT were compared to the results of the clinical follow-up of up to 10 years, mean (42.7 ± 14.9 months, median 40, range 17-97 months) after imaging. All the patients signed written consent for the investigation according to the Decision of the Ethical Committee of the University Clinical Center of Serbia (No. 668/6 since April 19th, 2018.).

Acquisition and interpretation of 18F-FDG PET/CT findings

All patients underwent 18F-FDG PET/CT examination on a 64-slice hybrid PET/CT scanner (Biograph, TruePoint64, Siemens Medical Solutions, Inc. USA). Having fasted for at least 6h, the patients received an intravenous injection of 5.5MBq/kg of 18F-FDG. Following the injection, the patients had rest in a quiet and darkened room for 60min, after which the images of PET/CT were obtained. Low-dose non-enhanced CT scans (120kV with automatic, real-time dose modulation amperage, slice thickness of 5mm, pitch of 1,5 and a rotation time of 0.5s) and 3-dimensional PET scans (6-7 fields of view, 3min/field) were acquired from the base of the skull to the mid-thigh. Non-corrected and attenuation-corrected CT, PET and fused PET/CT images were displayed for analysis on a Syngo Multimodality workplace (Siemens AG). The level of ^{18}F-FDG uptake was analyzed visually and semi-quantitatively using maximal standardized uptake value (SUVmax). 18F-FDG PET/CT findings were considered positive in case of a higher accumulation of 18F-FDG in comparison to surrounding parenchyma, mediastinal blood vessels and the liver. For assessment of glucose metabolism level in the areas of active disease, SUVmax was used, calculated as: the activity in tissue (count/pixel/s) multiplied by the calibration factor and divided by the dose applied (MBq/kg of body weight). Tumor lesions were defined by the volume of interest (VOI) placed around every suspected focus of an increased 18F-FDG uptake, with 50% threshold. The measurements of SUVmax, were done on reconstructed images, after using ordered subsets expectation maximization (OSEM) as statistical reconstruction method, but no absolute cut-off value of SUVmax was used for the diagnosis. Images were interpreted

Table 1. Patients' characteristics

Characteristics	Value
Total number of patients, n (%)	57 (100%)
Male	50 (87.7%)
Female	7 (12.3%)
Age (years)	
Mean ± SD	68.3 ± 6.7
Median	69
Range	55 - 85
Treatment, n (%)	
Surgery	57 (100%)
Chemotherapy	4 (7.0%)
Radiotherapy	21 (36.8%)
Chemoradiotherapy	11 (19.3%)
PET/CT indication, n (%)	
Staging after surgery	21 (36.8%)
Restaging after therapy with positive/uncertain CT	24 (42.1%)
Follow-up	5 (8.8%)
Suspected recurrence based on CT	7 (12.3%)

separately by two nuclear medicine physicians, unaware of the results of other imaging modalities. In cases of discrepancy, images were presented to multidisciplinary team and experts' opinion was adopted.

Statistical analyses

The results were shown as mean ± standard deviation (SD) and percentages. The 18F-FDG PET/CT diagnostic output was evaluated by calculating specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy. Chi-square test was used for testing statistical differences between positive and negative 18F-FDG PET/CT findings between male and female patients. Kaplan Mayer survival analyses was used to assess progression free survival (PFS), as well as Log-rank test to compare the survival times between positive and negative 18F-FDG PET/CT groups. Cox proportional regression analyses was used to identify predictive value of relevant prognostic factors on progression of the disease. Statistical significance was considered at $p < 0.05$.

RESULTS

Characteristics of the patients

The study included 57 patients (50 males and 7 females) with laryngeal SCC of mean age (68.3 ± 6.7 years, median 69, range 55-85). All patients underwent surgical resection of the tumor with some form of the neck dissection in case of cervical lymphadenopathy, followed by chemotherapy (4 patients), radiotherapy (21 patients) or chemoradiotherapy (11 patients). Restaging after therapy was in most cases an indication for 18F-FDG PET/CT **(Table 1)**.

18F- FDG PET/CT results

Regions of high accumulation of radiopharmaceutical were found in 41 (71.9%) patients (37 males and 4 females) and they were considered true positive (TP). There were 9 patients with local recurrences (SUVmax 10.0 ± 3.7) **(Figure 1)**, 18 with neck and/or mediastinal

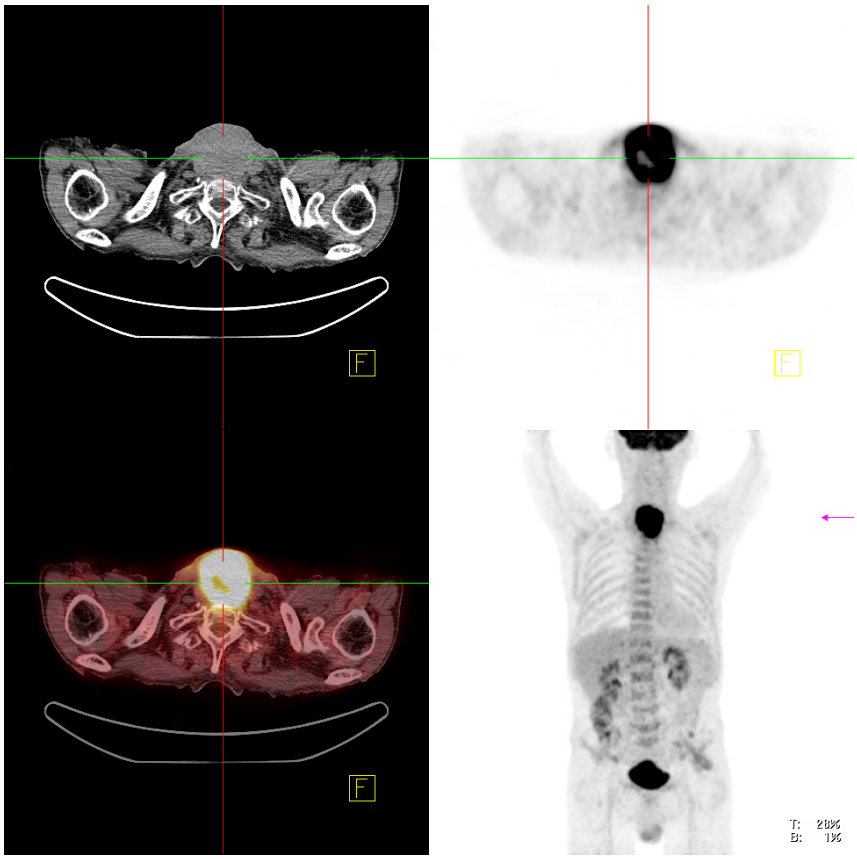

Figure 1. Transversal plane of CT, PET and fused PET/CT, as well as whole body MIP (maximal intensity projection) using 18F-FDG: Large local recurrence of laryngeal carcinoma, after total laryngectomy and bilateral neck dissection, with an increased uptake of radiopharmaceutical.

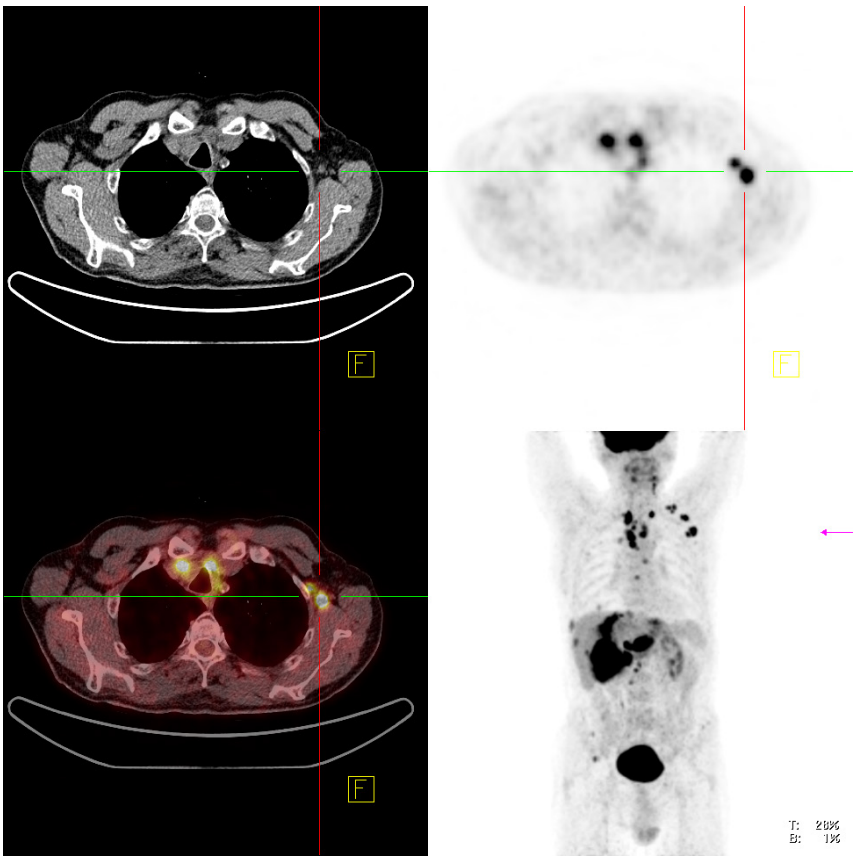

Figure 2. Transversal plane of CT, PET and fused PET/CT, as well as whole body MIP (maximal intensity projection) using 18F-FDG: Disseminated metastases of laryngeal carcinoma in neck, mediastinal, axillar and abdominal lymph nodes, as well as the liver and bones.

lymph node metastases (SUVmax 8.2 ± 2.9), 13 with lung metastases (SUVmax 11.3 ± 4.4), one with disseminated disease in lymph nodes, bones and the liver (SUVmax up to 25.0) **(Figure 2)**.

Physiological accumulation was found in 14 (24.5%) patients (11 males and 3 females) and those were considered true negative (TN). Only two male patients (3.5%) were found false positive (FP) due to the presence of discrete uptake of 18F-FDG in neck lymph nodes after radiation therapy in which biopsy showed no signs of an active disease. Significant statistical difference was not found in positive and negative findings between male and female patients (Chi-square test 0.322, p=0.570, p >0 .05).

Overall sensitivity of 18F-FDG PET/CT in this study was 95.3%, specificity 100.0%, positive predictive value (PPV) 100.0%, negative predictive value (NPV) 87.5% and accuracy 96.5% **(Table 2)**. In 19 cases (33.3%) PET/CT findings significantly influenced further management of the patients, who were mostly up-staged (14 cases) and the rest were down-staged.

Kaplan Meier survival analyses showed that survival time to progression in the 18F-FDG PET/CT positive group was 41.1 ± 12.7 months **(Figure 3)**, while in negative group PFS was 46.6 ± 19.3 months, with the 95% confidence interval. Statistical significance was not found between PFS of 18F-FDG PET/CT positive and negative patients' groups (p= 0.30, p>0.05). Cox proportional regression analyses showed a weak positive correlation between PFS in the 18F-FDG PET/CT positive group and SUVmax (R=0.1072, P=0.5048, P>0.05) **(Figure 4)**.

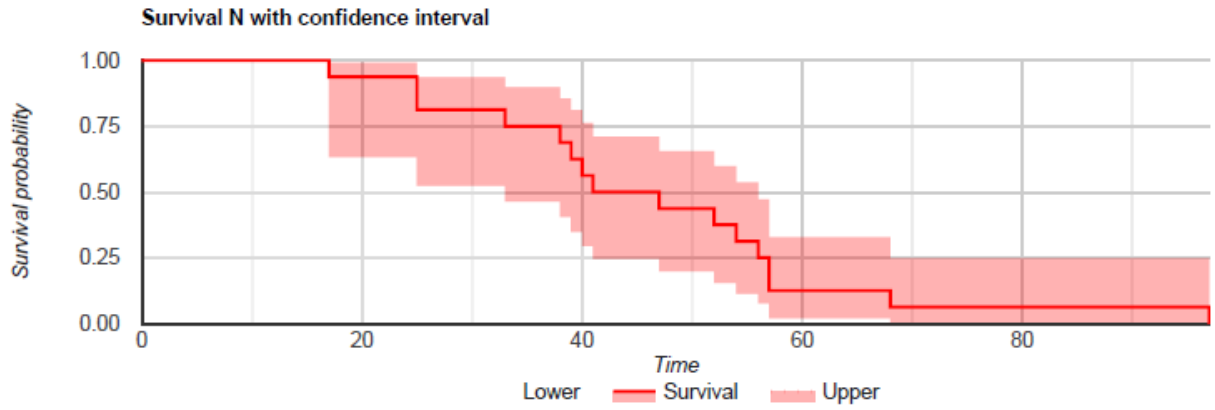

Figure 3. Progression free survival in 18F-FDG PET/CT positive group

Table 2. Diagnostic performance of 18F-FDG PET/CT findings in patients with laryngeal SCC

Parameter	Value (%)	95% CI
Sensitivity	95.3	84.2- 99.4
Specificity	100.0	76.8- 100.0
Positive predictive value	100.0	100.0
Negative predictive value	87.5	64.4 - 96.4
Accuracy	96.5	87.9 - 99.6

DISCUSSION

The results of other authors, regarding sensitivity, specificity, PPV, NPV and accuracy, are similar to ours. Thus, Tatar et al. (7) concluded that for primary tumor detection, the sensitivity of PET/CT was higher (100%) than magnetic resonance images (MRI) or CT (93.3%). Also, Suenaga et al. (12) established that sensitivity, specificity, and accuracy of 18F-FDG PET/CT was higher comparing to CT (72.9%, 96.8% and 92.1 % respectively vs. 52.9%, 98.6%, and 89.6%, $p < 0.01$) using discriminative SUVmax cutoff of 3.65.

For response assessment, Slevin et al (13) reported ^{18}F-FDG PET/CT sensitivity, specificity, PPV and NPV of primary and nodal sites were respectively 100%, 73%, 46%, 100% and 83%, 95%, 83%, 95%. He concluded that response assessment on ^{18}F-FDG PET/CT of laryngeal and hypopharyngeal carcinomas after (chemo) radiotherapy had a high NPV for both primary site and lymph nodes and could be used to guide treatment decisions. Sagardov et al. (14) conducted a retrospective analysis of 46 patients with larynx, hypopharynx, or cervical lymph node location of SCC treated with chemoradiotherapy, with sensitivity, specificity, PPV and NPV at the primary site of 86.7%, 90%, 76.5%, 93.1% and 100%, 97.2%, 87.5%, 100%, in the neck. They demonstrated that 18F-FDG PET/CT seemed effective in detecting residual disease and in predicting recurrent disease within the first 2 years of follow-up after a nonsurgical treatment. During this investigation we observed only two cases of false positive findings due to reactive lymph nodes after radiation. Cengiz et al (15) also noted a possibility of false positive findings in case of hypermetabolic focus in the larynx and lungs consistent of tuberculosis.-

In our study, in 19 cases (33.3%) PET/CT findings significantly influenced further management of the patients. Similarly, Lv et al. (16), proved that 18F-FDG PET/CT findings resulted in a change of staging in 34.9% of the patients. According to Khodary et al. (17), ^{18}F-FDG PET/CT altered further clinical management in 18.4% patients already treated for laryngeal tumor and induced a change in already planned therapeutic approach in 23.6%. Rohde et al. (18) recommended palliative treatment instead of curative treatment in 32% of patients after 18F-FDG PET/CT.

Even tough survival rate with disease progression was worse in patients with positive 18F-FDG PET/CT finding, statistical significance was not found between PFS of 18F-FDG PET/CT positive and negative patients groups in our study. However, some authors (19) claimed that complete response on 18F-FDG PET/CT was associated with an overall survival benefit (50.7 versus 10.3 months; $p<0.001$). Contrary to our results, Taghipour et al. (20) showed a significant difference and association in overall survival between patients with positive versus negative post-therapy 18F-FDG PET/CT scan (hazard ratio, 5.65; 95% CI, 2.48–12.83; log rank Mantel-Cox $p< 0.001$). Similarly, Wichman et al. (21) concluded that 18F-FDG PET/CT significantly contributed to post-therapy assessment of patients analyzing long-term laryngectomy-free, tumor-specific and overall survival in patients with locally advanced laryngeal or hypopharyngeal cancer.

Statistically significant correlation between PFS and SUVmax value was not found in our study, which is in accordance with the literature data where it was emphasized that the SUVmax of the primary tumor could be used for prediction of the course of the disease, and not SUVmax of recurrences or metastases like in our investigation. When SUVmax of recurrences and metastases were compared, Schwartz et al. (22) concluded that a primary tumor's 18F-FDG SUV of greater than 9.0 predicted inferior local recurrence-free survival ($p = 0.02$) and

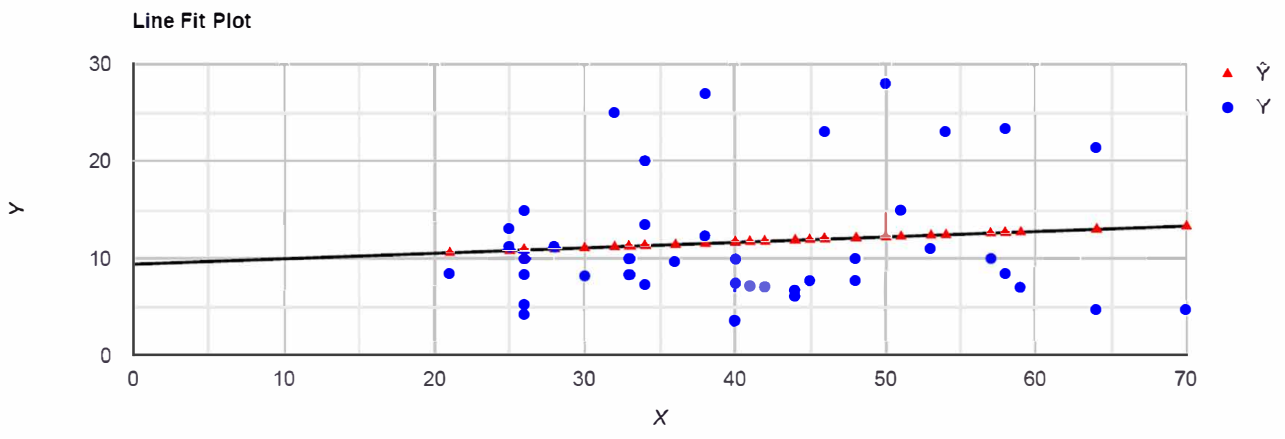

Figure 4. Linear regression line between progression free survival and SUVmax in 18F-FDG positive patients (R=0.1072, P=0.5048, P>0.05)

disease-free survival (p =0.03), while nodal SUV was not predictive. Castelli et al (23) and Paone et al (24) concluded that among many quantitative PET parameters, SUVmax did not show promising results as a good outcome predictor. However, Chun et al. (25) claimed that there was a significant difference in SUVmax between cervical lymph nodes with and without extracapsular spread (6.39 ± 4.53 vs 1.21 ± 1.70; p < .001) in patients with laryngeal cancer who had undergone 18F-FDG PET/CT before the surgery. The cutoff value for differentiating nodes with extracapsular spread from those without it was 2.8, with a sensitivity of 85.7% and specificity of 85.6%.

Considering very complex pathology, authors in general recommend combined imaging with different modalities for obtaining the highest accuracy. Nikagawa et al. (26) concluded that the combination of a very high-signal mass on T2-weighted MRI and lower 18F-FDG uptake might be diagnostic image characteristics for distinguishing laryngeal cartilaginous tumor from laryngeal cancer, emphasizing the 18F-FDG PET/CT's high negative predictive value. Bozzato et al. (27) emphasized the synthesis of the findings from endoscopy, biopsy and imaging as prerequisite for initiation of stage-appropriate treatment. Chu et al. (28) summarized that the role of 18F-FDG PET, in the evaluation of laryngeal tumors clinically classified as N0, did not show consistent usefulness due to resolution limitations of the camera. In the post-therapy setting, however, 18F-FDG PET has consistently demonstrated a high NPV in the identification of recurrent disease, both during the course of therapy and follow-up. In addition, contrast material-enhanced CT in conjunction with 18F-FDG PET has demonstrated a complementary role by allowing superior anatomic co-registration and therefore more definitive diagnosis.

CONCLUSIONS

18F-FDG PET/CT is a valuable tool for the follow-up of laryngeal carcinomas due to its high sensitivity, specificity, PPV, NPV and accuracy. Statistically significant difference in positive and negative PET/CT findings was not found between male and female patients. The results of 18F-FDG PET/CT can influence patient management in a significant number of cases. Patients with negative ^{18}F-FDG PET/CT findings had longer PFS than those with positive, although not statistically significant, it could be used with caution for prediction. Even though SUVmax values in recurrences and metastases were very high, it is not proven to be a strong predictor for patients' disease-free survival.

LIMITATIONS OF THE STUDY

The study was retrospective, so the choice of the patients and the ways of acquisition and interpretation have not been completely controlled. The indications for the investigation were retrospectively analyzed from previously obtained medical records. The exact perspective of the clinician requesting the study was not known. We used low dose CT and not contrast enhanced CT, which may have led to an increased number of indeterminate results. In addition, the biopsies that were performed to confirm residual/recurrent disease were done by surgical excision or under image guidance, and those performed with image guidance had limitations such as sampling error. The overall survival was estimated according to the last date of the record during follow up in our institution and with direct contact with the patient, and this can affect the exact date of PFS results. 18F-FDG PET/CT was performed only once after the therapy, even though multiple PET/CT examinations would provide us with more exact data and allow us more precise analysis.

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AUTHOR CONTRIBUTIONS

Petrovic Jelena, as a corresponding author, have made substantial contribution to the concept and design, Milica Stojiljković contributed to data acquisition, Isidora Grozdić Milojević and Strahinja Odalović dealt with analysis and interpretation of the data, Ana Jotić and Vera Artiko were involved in drafting the manuscript and revising it critically for important intellectual content, Dragana Šobić Šaranović and Jovica Milovanović gave the final approval of the revised version of the manuscript to be published

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ULOGA 18F-FDG PET/CT U PRAĆENJU PACIJENATA SA LARINGEALNIM SKVAMOCELULARNIM KARCINOMOM NAKON TERAPIJE

Jelena Petrović[1,3], Dragana Šobić Šaranović[1,3], Jovica Milovanović[2,3], Ana Jotić[2,3], Strahinja Odalović[1,3], Isidora Grozdić Milojević[1,3], Milica Stojiljković[1,3], Vera Artiko[1,3]

Sažetak

Uvod: Cilj ovog istraživanja je procena dijagnostičke vrednosti pozitronske emisione tomografije/kompjuterizovane tomografije koristeći fluor-18 fluorodeoksiglukozu (18F-FDG PET/CT) u post-terapijskom praćenju laringealnog skvamocelularnog karcinoma (SCC) i preživljavanju.

Metode: Pedeset sedam pacijenata (50 muškaraca, 7 žena), proseka godina (68.3±6.7), sa laringealnim SCC nakon terapije je ispitivano u ovoj studiji. Indikacije za 18F-FDG PET/CT su bile: stejdžing nakon operacije, post-terapijski restejdžing sa pozitivnim/ekvivokalnim CT, praćenje, sumnja na recidiv na osnovu CT. 18F-FDG PET/CT nalazi su poređeni sa kliničkim praćenjem do 10 godina nakon snimanja. Stepen metaboličke aktivnosti je analiziran vizuelno i semikvantitativno koristeći maksimalnu standardizovanu vrednost preuzimanja radiofarmaka (SUVmax).

Rezultati: Pojačana akumulacija radiofarmaka je viđena kod 41 (71.9%) pacijenta koji su smatrani stvarno pozitivnim, fiziološka kod 14 stvarno negativnih pacijenata (24.5%), dok je dvoje (3.5%) smatrano lažno pozitivnim. Sveukupna senzitivnost 18F-FDG PET/CT je 95.3%, specifičnost 100.0%, pozitivna prediktivna vrednost 100.0%, negativna prediktivna vrednost 87.5% i tačnost 96.5%. U 19 slučajeva (33.3%) PET/CT nalazi su značajno uticali na dalji tok lečenja pacijenta. Preživljavanje bez progresije bolesti (PFS) u 18F-FDG pozitivnoj grupi je 41.1±12.7 meseci. Statistički značajna korelacija između SUVmax i PFS nije uočena (p>0.05).

Zaključak: 18F-FDG PET/CT je značajna metoda u praćenju laringealnih SCC sa visokom senzitivnošću, specifičnošću, PPV, NPV i tačnošću. Može uticati na tok lečenja pacijenta u značajnom broju. Pacijenti sa negativnim 18F-FDG PET/CT su imali duži PFS od onih sa pozitivnim nalazom, bez statističke značajnosti. SUVmax se nije pokazao kao bitan prediktor PFS kod ovih pacijenata.

Ključne reči: PET/CT, laringealni karcinom, 18F-FDG, SUVmax

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ORIGINAL ARTICLE

Assessment of timely detection of abdominal aortic aneurysm in serbian population based on the data from serbvasc registry of operated patients

Slobodan Pešić[1], Slobodan Tanasković[2,3], Nemanja Stepanović[4,5], Vuk Joković[6], Aleksandar Tomić[7,8], Srđan Babić[2,3], Andrija Roganović[9], Miroslava Popović[10], Igor Končar[9,3]

[1] General Hospital of Subotica, Department of Surgery/Vascular Surgery Division, Subotica, Serbia
[2] Vascular Surgery Clinic, Dedinje Cardiovascular Institute, Belgrade, Serbia
[3] University of Belgrade, Faculty of Medicine, Belgrade, Serbia
[4] Vacular Surgery Clinic, University Clinical Center of Niš, Niš, Serbia
[5] University of Niš, Faculty of Medicine, Niš, Serbia
[6] Center for Vascular Surgery, University Clinical Center Kragujevac, Kragujevac, Serbia
[7] Clinic for Vascular and Endovascular Surgery, Military Medical Academy, Belgrade, Serbia
[8] University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia
[9] Clinic for Vascular and Endovascular Surgery, University Clinical Centre of Serbia, Belgrade, Serbia
[10] Clinic for Surgery, Department for Nephrovascular Surgery, Zvezdara University Medical Center, Belgrade, Serbia

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Correspondence to:
Slobodan Pešić, General Hospital of Subotica, 3, Izvorska Street, 24 000 Subotica, Serbia, Phone: +381 24 555 222, mob +381 61 6303360. E-mail: spesic90@gmail.com

Summary

Introduction Screening of males over 65 years of age for abdominal aortic aneurysm (AAA) has been proved effective in some countries. The incidence of AAA in Serbian population has not been evaluated yet.

The aim of the study was to assess the ratio between elective and urgently operated patients with AAA and to estimate hospital incidence of treated AAA based on the population of 100 000 inhabitants.

Methods Data were obtained from Vascular surgery registry - Serbvasc which has been created by 17 institutions. Data containing demographic characteristics were obtained from the publication of the Statistical office of the Republic of Serbia. For statistical analysis, the methods of descriptive statistics were used and the values were expressed per 100 000 inhabitants.

Results During the year 2021, 422 operations were performed in 7 hospitals due to asymptomatic, symptomatic and ruptured AAA in 323 (76,54%), 37(8,76%) and 62 (14,69%) patients, respectively. Hospital incidence of electively operated AAA is 8,06 cases per 100 000 inhabitants, whereas the incidence of operated ruptured AAA is 1,55 cases per 100 000 inhabitants. Overall hospital incidence of all operatively treated AAA is 10,53 cases per 100 000 inhabitants. Out of the total number of operated patients with AAA, 134 (31,75%) were younger than 65 years, while 22 (35%) out of 62 patients treated for rAAA, where younger than 65 years.

Conclusion The incidence of operatively treated elective AAA is lower than the values reported in literature, while the rate of ruptured AAA is more than twice as high as the rate reported by recent papers. One third of treated patients were younger than 65 years. The most efficient strategy for reducing the number of urgently treated AAA is the implementation of screening for AAA in groups with higher risk.

Key words: Abdominal aortic aneurysm, registry, hospital incidence

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INTRODUCTION

Abdominal aortic aneurysm (AAA) is defined as abnormal dilatation of the abdominal aorta ≥ 3.0 cm, or dilatation of more than 50% of its diameter.[1] According to literature it is more common in men, in cigarette smokers and in persons with hypertension or obesity. During the past two decades a drop in newly discovered cases of AAA per year has been noticed, from the previous incidence of 30,1 - 40 per 100.000 inhabitants to the currently estimated incidence of 13,2 - 16 per 100.000 inhabitants.[2,3,4,5,6] From the moment the diagnosis of AAA is made, life expectancy is 11 years.[7] Aneurysm diameter above 5,5 cm is a threshold above which the operative treatment is recomended.[8]

The most serious risk of living with AAA is the risk of aneurysm rupture which is the most common and potentially fatal complication. The only chance for surviving AAA rupture is emergency operative treatment. Cumulative mortality in AAA rupture is above 80% because most patients cannot make to the healthcare institutions which are involved in definitive treatment. Hospital mortality is up to 50%.[1] AAA rupture is the 13th cause of death in men over 55 years of age in western world. On the other hand, mortality as a result of elective surgery of AAA is below 5%,[5] and in high volume centers it is below 2%.[9]

This high mortality rate can be prevented by early detection and elective treatment of aneurysm. Internationally, there have been four large randomized studies in general population which estimated the effects of AAA screening by one time ultrasound (US) examination in men over 65 years of age(*Chichester*[7] - Great Britan, MASS[10] - multicentric screening study, Great Britain, *Viborg*[11] - Denmark, *WA*[12] - Western Australia). The studies showed that screening program had resulted in reducing AAA related mortality by 40% and reducing ruptured AAA incidence by 38%[13], after 3-5 years of follow up, but that it had not contributed to lowering the overall mortality. A long term follow-up showed an increase in the number of elective AAA procedures, a decrease in the number of emergency AAA procedures as well as a significant reduction in 30-day postoperative mortality due to all AAA procedures.[14] Literature data about frequency and the total number of ruptured AAA in our country are scarce and so far a study on justification of AAA screening has not been conducted. Certainly, data on the number of electively treated patients with AAA and the number of urgently treated patients due to ruptured AAA (rAAA) would be one of the first indicators for the necessity of AAA screening in our country.

The aim of this study was to assess the ratio between electively and urgently operated patients with AAA and to estimate hospital incidence of treated AAA based on the population of 100 000 inhabitants.

MATERIAL AND METHODS

Data on primary procedures for treating AAA in the year 2021 were prospectively collected in vascular surgery registry - *Serbvasc*. Primary procedures included surgically treated (open and endovascular) asymptomatic, symptomatic and ruptured AAAs. Overall, 17 healthcare institutions took part in the creation of *Serbvasc* registry (Clinic for Vascular and Endovascular Surgery of University Clinical Center of Serbia, Institute for Cardiovascular Diseases Dedinje, Clinic for Vascular Surgery of Military Medical Academy, Clinical Centre (CC) of Nis, Clinical Centre of Kragujevac, Zvezdara University Medical Centre, Zemun University Medical Centre, General Hospital Subotica, General Hospital Užice, General Hospital Kruševac, General Hospital Valjevo, General Hospital Zrenjanin, General Hospital Kikinda, General Hospital Sremska Mitrovica, General Hospital Požarevac, General Hospital Gornji Milanovac), however AAA are treated only in 7. Data containing demographic characteristics of the population that gravitates towards the institutions that create Serbvasc register are gathered from (currently latest) publication of the Statistical Office of the Republic of Serbia.[15]

Serbian vascular registry (SERBVASC) was founded at the beginning of the year 2020, although full data collection began in 2021, delay being caused by COVID-19 pandemic. The registry was developed in cooperation with the international network of vascular registries, VASCUNET, which collects data from most European Union countries, New Zeland, Australia and Serbia and collaborates with similar organizations in North America. Most Serbian hospitals agreed to contribute to the collection of data in SERBVASC registry and gained Ethical approval in their institutions.

Descriptive statistics methods were used for statistical analysis and values are showed per 100.000 population. Correlation and regression analysis were used for the analysis of variables. Statistical hypothesis testing was made at the level of significance of $p < 0,05$.

RESULTS

According to the data from the Statistical Office of the Republic of Serbia, the total number of inhabitants in administrative areas which gravitate towards healthcare institutions that create SERBVASC registry is 4.006.463. A total of 679.165 are older than 65 years. **(Table 1.)**

In the period January - December 2021, 422 operations of AAA were performed. Out of the total number of treated, 323 (76,54%) operations of AAA were performed in elective settings, 37 (8,76%) to treat symptomatic AAA and 62 (14,69%) to treat ruptured AAA. The total number of urgently treated patients for symptomatic and ruptured AAA is 99 (23,46%).

Table 1. The number of inhabitants according to administrative areas that gravitate towards healthcare institutions which create Vascular surgery registry - *Serbvasc*

Administrative area (Healthcare institution towards which the population gravitates)	The total number of inhabitants	The number of inhabitants older than 65 years
Belgrade district (CVEH UCCS*, ICVDD#, MMA°, UMC¥ Zvezdara, UMC Zemun)	1 659 440	266 762
Nišava district (CC¢ Niš)	376 319	71 563
Šumadija **district (CC Kragujevac)**	293 308	49 925
North Bačka district (GH§ Subotica)	186 906	31 973
Zlatibor district (GH Uzice)	286 549	50 345
Rasina district (GH Krusevac)	241 999	34 837
Kolubara district (GH Valjevo)	174 513	33 123
Srem district (GH Sremska Mitrovica)	312 278	51 168
Kikinda (GH Kikinda)	59 453	9 786
Central Banat district (GH Zrenjanin)	187 667	32 119
Braničevo district (OB Požarevac)	183 625	39 065
Gornji Milanovac (GH Gornji Milanovac)	44 406	8 499
Total	4 006 463	679 165
	*CVEH UCCS – Clinic for vascular and endovascular surgery of University Clinical Center of Serbia # ICVDD – Institute for cardiovascular diseases Dedinje ° MMA – Military Medical Academy ¥ UMC – University Medical Center ¢ CC – Clinical Center § GH – General Hospital	

Graphic 1. The number of treated patients with asymptomatic, symptomatic and ruptured abdominal aortic aneurysms according to months in year 2021.

The incidence of operatively treated rAAA in the examined population in the year 2021 is 1,55 cases per 100.000 inhabitants. The total incidence of all urgently treated AAA (symptomatic and ruptured) in the examined time frame is 2,47 cases per 100.000 inhabitants. The incidence of electively treated patients with AAA is 8,06 cases per 100.000 inhabitants. The overall incidence of all operatively treated patients for all AAA in the examined time frame is 10,53 cases per 100.000 inhabitants.

Out of the total number of operated patients with AAA (422), 288 (68,25%) were older than 65 years. The incidence of patients older than 65 years treated for all AAA is 42,40 cases per 100.000 inhabitants older than 65 years. Out all 62 patients treated for rAAA, only 40 (65%) of were older than 65 years. The incidence of treated rAAA in patients older than 65 years is 5,89 cases per 100.000 inhabitants older than 65 years.

DISCUSSION

This prospective study examined hospital incidence of operated AAA on the population of 100 000 inhabitants and the ratio of elective and urgent procedures (due to symptomatic and ruptured AAA) in the examined population without exclusion of subjects concerning sex and age. This enabled the evaluation of influence and share of emergent AAA operations with regard to overall hospital incidence of AAA operations. The estimated values differ from those in international publications.

In the examined population, the incidence of all treated AAA (elective and urgent) is 10,53 cases per 100.000 inhabitants per year. This incidence is lower than previously published hospital incidence on European population in other countries, which is within the range of 13,2 to 15,7 per 100.000 inhabitants.[5,6] The incidence of treated rAAA in this study (1,55 cases per 100.000 inhab-

itants) is also lower than the incidence of treated AAA ruptures in literature, which is 1,6 cases per 100.000 inhabitants.[6] According to literature data, it has also been shown that out of the total number of ruptured AAA, 41% to 52% of those admitted to hospital are not operatively treated.[6,16] Consequently, the incidence of rAAA is significantly higher than the number of treated rAAA. On the other hand, the rate of untreated patients admitted due to rAAA in Serbian hospitals is somehow lower due to local practice.[17,18]

This lower incidence rate of treated AAA cases can be explained by a lower rate of detection and treatment of existing AAA. Since there is no evidence of waiting lists in Serbian hospitals when it comes to the treatment of AAA, one can assume that the lower rate of detection is the cause of this unfavorable ratio between elective and urgent procedures for treating AAA. There is a significant difference between the incidence of elective treated AAA and the estimated values in literature, 8,06 cases per 100.000 inhabitants in the examined population versus 13,1 cases per 100.000 inhabitants in literature.[6]

Burden and share of emergently operated AAA, which have a higher mortality rate, is more than twice as high in our examined population in comparison with literature. From the total number of operated AAA, 23,46% is treated urgently which is more than twice as high as the share of 10,99% of urgently treated in literature[5].

When the distribution of AAA operations is assessed by age groups, it becomes evident that more than two thirds of operations are performed at patients older than 65 years. Also, an increase in the incidence of AAA operations is significant in subjects older than 65 years, with the value of 42,40 cases per 100.000 inhabitants other than 65 years. More than two thirds of treated ruptured AAA is in patients older than 65 years, while in reported European population this share of treated rAAA is in population older than 75 years.[19,20] This share of rAAA in the population which is a decade younger than the one reported in literature requires a deeper analysis. The presence of risk factors in Serbian population is higher than in Western Europe and the quality of life due to economic situation is lower whereas stress exposure is higher. Higher rates of rAAA means higher costs for healthcare system and a greater burden for vascular departments with urgent patients.

A possible strategy for reducing the rate of rAAA is the introduction of screening program for AAA. Internationally, screening for AAA is applied by ultrasonography of the abdomen in men older than 65 years. Ultrasonography of abdomen is a very sensitive and specific noninvasive test for detection of all AAA regardless of diameter. Ultrasound based screening for AAA fulfills all criteria for a screening program recommended by the World Health Organization (WHO).[21] Meta-analyses of randomized controlled performed on large population showed that screening for AAA reduces AAA related mortality by 40% after follow-up period of 3-5 years.[7,10,11,12] According to the data from large randomized studies, after the period of follow up of 13-15 years, screening reduces the prevalence of rAAA by 55% and increases the number of electively treated AAA by 1,35 times.[14] Estimated screening induced absolute risk reduction (ARR) in AAA mortality is the same or higher than ARR in death from breast cancer with mammography screening, ARR in death from colorectal cancer by fecal occult blood screening or ARR in death from prostate cancer screening.[13] Cost-benefit analyses showed that screening for AAA was cost effective for the incidence of AAA as low as 0,5%.[22,23]

Better understanding of epidemiology, risks and outcome of urgently operated AAA events in the examined population can shape a future screening strategy. Data from vascular surgery registry - *Serbvasc* can be of paramount importance for understanding the effectiveness and justification of screening for AAA in general population and therefore, it is important to continue to follow the number of operated patients and the inclusion of other healthcare institutions in the development of this registry.

The study has certain limitations. Not all hospitals performing aortic procedures were included in this analysis due to different reasons specific for this kind of data collection (noncompliance of medical stuff or administration of hospitals to enter data in the registry or participate in such a voluntary project). We estimate that the number of treated patients in these hospitals is not higher than 20% of all reported in this paper. The report from the National Statistical office are based on the data from 2011 and the number of inhabitants might have decreased due to constant migration in the Balkan countries. Still, younger population is more prone to migrations. Finally, the year 2021 was a pandemic year and potentially the number of patients treated by elective repair is lower than usual. However, we speculate that also the number of urgently treated patients could be lower due to the hesitation of patients to come to hospital regardless the symptoms or incapability of health care system to provide high quality care to all patients, which was the case in all countries during the pandemic.

CONCLUSION

Hospital incidence of electively treated AAA in the examined population is lower than the values reported in literature, which is probably due to lower detection rate. For the same reason, the rate of rAAA in the total number of treated AAA is more than twice as high as the one reported in literature. Still one third of patients in Serbian population is younger than 65 years which should be considered in the screening program. *Serbvasc* registry can be an effective tool for monitoring not only the number and effectiveness of surgical procedures but also for the

assessment of epidemiological data, justification and effectiveness of a screening program implementation and the effects of its conduction later as well.

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PROCENA PRAVOVREMENOG OTKRIVANJA ANEURIZME ABDOMINALNE AORTE U SRPSKOJ POPULACIJI NA OSNOVU PODATAKA IZ SERBVASC REGISTRA OPERISANIH PACIJENATA

Slobodan Pešić[1], Slobodan Tanasković[2,3], Nemanja Stepanović[4,5], Vuk Joković[6], Aleksandar[7,8], Tomić, Srđan Babić[2,3], Andrija Roganović[9], Miroslava Popović[10], Igor Končar[9,3]

Sažetak

Uvod Skrining muškaraca starijih od 65 godina na aneurizmu abdominalne aorte (AAA) se pokazao efikasnim u zemljama koje ga primenjuju. Do sada nije procenjivana incidenca AAA u srpskoj populaciji.

Cilj studije je bio da se proceni odnos elektivno i hitno operisanih bolesnika sa AAA i incidenca tretiranih AAA prema broju stanovnika tokom 2021. godine shodno populaciji u ispitivanim regionima Republike Srbije.

Materijal i metode Podaci su dobijeni iz registra vaskularnih operacija - Srbvasc u čijem kreiranju učestvuje 17 ustanova. Podaci o demografskim karakteristikama stanovništva za izračunavanje učestalosti dobijeni su iz publikacije Republičkog zavoda za statistiku Republike Srbije. Za statističku analizu podataka korišćene su metode deskriptivne statistike, a vrednosti su iskazivane na 100 000 stanovnika.

Rezultati Tokom 2021. godine izvršene su 422 operacije AAA u 7 ustanova, 323 (76,54%) zbog asimptomatske, 37 (8,76%) zbog simptomatske AAA i 62 (14,69%) zbog rupturirane AAA. Incidenca elektivno operisanih AAA je 8,06 slučajeva na 100 000 stanovnika, incidenca operativno tretiranih rupturiranih AAA je 1,55 slučajeva na 100 000 stanovnika. Ukupna incidenca svih operativno tretiranih AAA je 10,53 slučaja na 100 000 stanovnika. Od ukupnog broja tretiranih AAA njih 134 (31,75%) su bili mlađi od 65 godina a 22 (35%) od 62 tretirana pacijenata sa rAAA je bilo mlađe od 65 godina.

Zaključak Incidenca operativno tretiranih elektivnih AAA u ispitivanoj populaciji na 100 000 stanovnika je manja u odnosu na prijavljene vrednosti u literaturi ali je udeo rupturiranih AAA više od dva puta veći od publikovanih vrednosti u literaturi. Značajan broj elektivno i hitno tretiranih bolesnika je mlađi od 65 godina. Najefikasniji način za smanjenje broja hitno operisanih AAA je uvođenje skrininga AAA u grupama populacije sa većim rizikom.

Ključne reči: aneurizma abdominalne aorte, registar, incidencija

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ORIGINAL ARTICLE

Evaluating the clinical application of PAMD score in the assessment of TRUS-biopsy positive outcomes in patients with PSA 4-10 ng/ml treated in Serbia

Milan Radovanović[1,2], Aleksa Zubelić[2], Veljko Šantrić[1,2], Bogomir Milojević[1,2], Uroš Bumbaširević[1,2], Branko Stanković[1,2]

[1]Clinic of Urology, University Clinical Centre of Serbia, Belgrade, Serbia
[2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

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Correspondence to:
Milan Radovanović
Clinic of Urology, University Clinical Centre of Serbia, Resavska 51, 11000 Belgrade, Serbia
Phone number: +381637795055
E-mail: milan_950@hotmail.com

Summary

Introduction: Transrectal ultrasound-guided prostate biopsy (TRUS-biopsy) is the "gold standard" in the diagnosis of prostate cancer (PC). There is much divided opinion on the need for biopsy in patients with prostate-specific antigen (PSA) between 4 and 10 ng/ml. The positive biopsy outcome (PC) in these patients ranges from 20 to 39%. Low sensitivity and specificity of PSA in predicting positive biopsy outcome results in a large number of unnecessary biopsies and treatments. In order to better select candidates for biopsy, several risk stratification models for PC have been proposed in recent years, among them the PAMD score.

Aim: The aim of this study was to examine the value of the PAMD score in the assessment of positive biopsy outcomes in our population of patients, as well as to examine individual risk factors for PC in patients with PSA values between 4 and 10 ng/ml treated in Serbia.

Material and methods: The study involved 50 patients at the Clinic of Urology, University Clinical Centre of Serbia, whose PSA value were measured in the range from 4 to 10 ng/ml. In all the patients we measured PSA and %fPSA, and performed DRE, as well as magnetic resonance imaging (MRI) to evaluate prostate volume (PV) and PI-RADS score. All patients underwent TRUS-guided systemic prostate biopsy. In accordance with the data from literature, PAMD score was determined for all the patients.

Results: A PAMD score > 3 showed a high specificity in the prediction of PC, as well as an association with a higher frequency of high-grade PC. A positive finding on DRE, %fPSA< 16, age above 69 years and PI-RADS > 3 showed a statistically significant association with the existence of PC. A high individual predictive value in assessing the presence of PC was confirmed for DRE, %fPSA, PV, and PI-RADS score.

Conclusion: The PAMD scoring system may be of importance for better selection of candidates for TRUS-biopsy, in the population of patients with PSA values 4-10 ng/ml.

Keywords: prostate cancer, PSA, PAMD, risk factors.

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INTRODUCTION

Prostate cancer (PC) accounts for about 29% of all malignant tumors in men (1). Today PC is the 5th most common cause of cancer death after lung and colon cancer. 1.6 million of new cases of PC are diagnosed annually in worldwide (2). The frequency of new cases of PC is significantly higher in medium and highly developed countries compared to developing countries. The most important risk factors for PC are older age, obesity, smoking, lack of physical activity, sexually transmitted diseases and genetic predisposition (3). A significantly higher incidence of PC is recorded in African-American population (4).

Digital rectal examination (DRE), prostate specific antigen (PSA), ratio of free/total PSA (%fPSA), transrectal ultrasound-guided prostate biopsy (TRUS-biopsy) and pathophysiological (PH) verification are commonly used in diagnosing PC (5). Magnetic resonance imaging (MRI) has been increasingly important in diagnostics in recent years (6).

According to the recommendations of the European Association of Urology (EAU), the decision to perform a TRUS-biopsy is based on PSA values and DRE findings (7). A PSA value of 4 ng/ml is traditionally taken as the cut-off value where biopsy is indicated. However, there are divided opinions about the need for a biopsy in patients with PSA between 4 and 10 ng/ml, the so-called "gray zone" (8). The positive outcome of biopsy (PC) in these patients ranges from 20 to 39% (9). Low sensitivity and specificity of PSA in predicting a positive biopsy outcome in this population of patients results in a large number of unnecessary biopsies and treatments. Furthermore, lower predictive value in the PSA "gray zone" was also observed with other PSA-based indices, such as %fPSA and PSA density (9). %fPSA may be adversely affected by several pre-analytical and clinical factors (e.g., instability of fPSA, and variable assay characteristics). The biopsy procedure is not completely „benign" either, with an increasing incidence of infections (3–5%) and the potential for serious complications requiring hospitalization (10).

In order to provide an optimal and personalized treatment for patients, in recent years research proposed several blood- and urine-based assays for detecting PC, most notably Prostate Health Index, 4Kscore, PCA3, and Select Dx (11). For patients with PSA between 4 and 10 ng/ml, it has been suggested to implement risk stratification scoring systems predicting positive biopsy outcomes. Risk stratification model proposed by Fang et al., named PAMD implements ultrasound-determined prostate volume (PV), DRE findings, age and MRI results in assessing the positive outcome of TRUS-biopsy in patients with PSA in the "gray zone" (12).

The aim of this study was to assess individual risk factors for PC implemented in PAMD scoring system, and frequency of PC in our population of patients with PSA between 4 and 10 ng/ml, as well as to examine the value of PAMD score in predicting positive biopsy outcomes in our patients.

MATERIALS AND METHODS

In our study there were 50 patients, treated at the Clinic of Urology, University Clinical Centre of Serbia, from January 2020 to March 2021, whose initial PSA values were in the range from 4 to 10 ng/ml. The patients were admitted for further diagnosis and treatment due to elevated PSA values and/or suspicious findings on DRE. Data were collected only from the patients in whom a TRUS-biopsy and PH verification of results were ultimately performed.

Age information was recorded for all the patients. PSA values and %fPSA were determined before performing DRE. DRE was performed in each patient, and the findings were marked as positive (palpatorily present area of hardness, nodule, or consistency differences between the lobes of the prostate) or negative. Prostate dimensions were determined using MRI (1.5T), and prostate volume (PV) was calculated using the following formula (height x width x length x 0.52) and expressed in grams.

MRI scans were used to determine a Prostate Imaging–Reporting and Data System (PI-RADS) score ranging from 1-5. All results, in accordance with the data from literature (13), were divided into two groups, positive results (PI-RADS = 4-5) and negative results(PI-RADS < 3). All images were evaluated by an experienced radiology specialist.

In all patients, TRUS-guided biopsy of the prostate was performed, at least 12 samples were taken per patient, according to the zonal distribution of the prostate tissue. All PH samples were evaluated by a pathology specialist. According to the data from literature, PCs with a Gleason score> 7 were designated as high-grade PCs (14).

STATISTICAL ANALYSIS

The normality of distribution of continuous numerical data was tested with the Kolmogorov-Smirnov test and their values were expressed as the arithmetic mean ± standard deviation. The significance of the difference between two independent groups of continuous numerical variables was analyzed by Student's t-test. Categorical variables were analyzed using Pearson's chi-square test. A p-value below 0.05 was considered a statistically significant difference. Receiver operating characteristic (ROC) curves were generated to illustrate the predictive value of various parameters and to calculate the area under the curve (AUC).

Statistical analysis of the data was performed using the SPSS 17.0 program (Statistical Package for Social Sciences, SPSS incorporation Chicago, USA).

Table 1. Clinical characteristics of the examined patients.

	Prostate cancer		
Parameter	Yes	No	*p*
PSA (ng/ml)	7.54± 1.47	6.05± 1.46	0.221
%fPSA	0.11 ± 0.03	0.156 ± 0.02	**0.001***
Age	69.31± 3.85	68.23 ± 3.10	0.262
PV (g)	39.96± 8.34	54.57 ± 9.74	**0.001***
MRI	Positive (30) Negative (5)	Positive (2) Negative (13)	
DRE	Positive (28) Negative (7)	Positive (1) Negative (14)	

PSA - Prostate specific antigen; PV - Prostate volume;
MRI - Nuclear magnetic resonance imaging (positive = PI-RADS (4,5), negativ = PI-RADS (0-3)); DRE - Digital rectal exam;
The significance of the difference in numerical variables was analyzed by Student's t-test.

RESULTS

50 patients participated in the study, the average age of the examined patients was 68.8 ± 3.66 years, the youngest patient was 62 and the oldest was 76 years old. The average PSA values were 6.79 ± 1.54 ng/ml. PC was found in 35 out of 50 patients after TRUS-biopsy and PH verification. High-grade PC was HP verified in 26 patients. A statistically significant difference ($p < 0.05$) was found in %fPSA and PV between patients with PC and patients with a negative biopsy.There was no statistically significant difference ($p > 0.05$) in PSA values in these two groups **(Table 1)**.

Age above 69, positive DRE, %fPSA under 16 and positive MRI findings showed a statistically significant association with a positive biopsy outcome in our population ($p < 0.05$) **(Table 2)**.

ROC curves and AUC value showed that positive DRE (AUC = 0.937), %fPSA (AUC = 0.937), positive MRI finding (PI-RADS = 4-5) (AUC = 0.93) and PV (AUC = 0,87) have a high individual predictive value in assessing a positive biopsy outcome in patients with PSA 4-10 ng/ml. Lower predictive value of the PSA (AUC = 0.75) and the patient's age in the evaluation of the risk for PC (AUC = 0.57) was found.

PAMD - model for risk stratification

In accordance with the data from literature (12), we applied the risk stratification model proposed by Fang et al. to our population of patients with PSA values from 4-10 ng/ml. Each risk factor was scored as follows: PV > 50 mL = 0 points, PV ≤ 50 mL = 2 points; age ≤ 69 = 0 points, age > 69 = 2 points; negative MRI finding = 0 points, positive MRI finding = 2 points; negative DRE = 0 points, positive

Table 2. Analyzed variables in patients with and without prostate cancer.

		Prostate cancer			
	Total	Yes	No		
Patients	50	35	15	*p*	χ^2
PSA (7.21 ± 1.54 ng/ml)					
> 7 ng/ml	26	23	3	3.51	3.52
≤ 7 ng/ml	24	11	13		
%fPSA(0.123 ± 0.03)					
< 0.16	41	33	8	**0.005***	-7.87
≥ 0.16	9	4	5		
Age (69.8 ± 3.66)					
> 69	22	19	3	**0.001***	10.81
≤ 69	28	6	22		
MRI					
Positive (PI-RADS = 4-5)	30	30	0	**0.001***	17.23
Negative (PI-RADS ≤ 3)	20	5	15		
PV (46± 10.21 g)					
≤ 50 g	35	29	6	0.13	-2.33
> 50 g	15	9	6		
DRE					
Positive	28	28	0	**0.001***	10.80
Negative	22	7	15		

PSA - Prostate specific antigen; PV - Prostate volume;
MRI - Nuclear magnetic resonance imaging (positive = PI-RADS (4,5), negative = PI-RADS (0-3)); DRE - Digital rectal exam;
Statistical significance was determined using the Pearson chi-square test.

Table 3. PAMD model for risk stratification proposed by Fang et al.

		Prostate cancer					High-grade prostate cancer			
		Total	Yes	No	*P*	χ^2	Yes	No	*p*	χ^2
PAMD	Patients	50	35	15	**0.0007***	14.43	26	24	**0.0001***	18.22
	Low (0-1)	6	2	4			0	6		
	Medium (2-3)	14	3	11			0	14		
	High (4-7)	30	30	0			26	4		

DRE = 1 point. The PAMD score is defined as the sum of the individual scores. In relation to the PAMD score,all patients are divided into three risk groups: low (0-1), medium (2-3) and high (4-7). There was statistically significant difference in biopsy outcomes between these three groups ($p < 0.05$). PAMD score values >3, were associated with statistically significant higher number of positive biopsy outcomes, as well as with high-grade PC **(Table 3)**.

Statistical significance was determined using the Pearson chi-square test.

High specificity (AUC = 0.85) of the PAMD score (cut off value = 3) in assessing a positive biopsy outcome was observed.

DISCUSSION

TRUS-biopsies of the prostate in all patients with PSA values above 4 ng/ml are accompanied by a high rate of negative findings, while at the same time they represent a significant economic burden for a healthcare system. (15) The rate of negative biopsy findings is particularly high in the population of patients with PSA values between 4-10 ng/ml, various studies report rates between 30 and 70% (12). In our examined population, a negative biopsy result was found in 30% of patients, and a smaller number of patients compared to similar studies is a possible cause.

Age is one of the first well-studied risk factors for the development of PC. A large number of epidemiological studies have shown that the incidence of PC and mortality from PC increases with age (16). Recent studies have shown that age is an independent risk factor for the development of high-grade PC. This is explained by a lower screening rate, especially in the population of patients older than 75 years, which leads to late diagnosis, but changes in tumor biology in older people have also been demonstrated (17). The average age of the analyzed patients in our study was 68.8 ± 3.66 years,statistically significant association between the patients's age and the positive biopsy outcome was observed, which is in accordance with the results of similar studies (17).

PSA exists in several forms in the serum, and is predominantly bound to plasma proteins, however one form of PSA, free PSA, is not bound to proteins. Free PSA is produced as a product of proteolysis of the PSA molecule in normal prostate tissue (18). Increased PSA release and decreased proteolytic activity result in a lower percentage of free PSA in patients with PC compared to patients with a normal prostate or benign changes (19). A large number of studies have shown a good predictive value of the %fPSA in assessing the outcome of biopsy (19). However, the differences of %fPSA ratio were not significant between PCa and non-PCa group in some studies. The inconsistent results of %fPSA among studies may be caused by the unstable fPSA in serum (20). Bachour et al. proposed using ratio of serum human kallikrein-2 with fPSA, which gave significantly larger area under the curve (0.96 vs 0.41) in comparison with %fPSA, suggesting higher specificity (21). In its recommendations, the EAU still advises a routine determination of the %fPSAas a part of screening for PC (7). The results of our study showed a statistically significant difference between %fPSA values between patients with positive and negative biopsy results. Catalona et al. suggested using %fPSA ratio ≤ 15, which would detect all advanced, non-organ confined, and large volume tumors, while avoiding 80% of biopsies in men with insignificant disease particularly in the intermediate range of total PSA (4.1–10 ng/mL) (22). Other investigators have recommended cutoffs of 18–27% (23). In our study we tested cutoff value of %fPSA< 16, which is proposed by Fang et al. as a part of PAMD scoring system (12). High predictive value of %fPSA< 16 in detecting PC was observed.

The role of MRI in the early detection of PC has been in the research focus in recent years (24). Various studies have shown a high predictive value of MRI imaging and PI-RADS score in the selection of candidates for prostate biopsy (25). Perdona et al. showed that MRI results have the highest single predictive value for positive biopsy outcome in the population of patients with PSA 4-10 ng/ml (26). This is consistent with the results of our study, and indicates the importance of MRI imaging and PI-RADS score in screening for PC. The PI-RADS v2.1 scoring criteria differ according to the location of the lesion. In a recent study, PI-RADS v2.1 score had the best performance among the probable single predictive factors for PC in the population with PSA 4-10 ng/ml (27).

In order to better select candidates for biopsy, a large number of scoring systems have been developed. Two of the more popular scoring systems that have been validated are the Prostate Cancer Prevention Trial Risk Calculator 2.0 (PCPT RC) and the newer Prostate Biopsy Collaborative Group (PBCG) Risk Calculator (28). Jethwani et al. proposed implementing neutrophil-to-lymphocyte ratio in scoring systems in order to improve specificity in predicting PC (29).

Fang et al. developed a PAMD scoring system for patients with PSA 4-10 ng/ml implementing parameters usually taken while doing standard workup of patients with possible PC, including age, PV, findings on MRI and DRE (12), making it well-suited for implementation in our health-care system due to low cost and accessibility. With a cut-off value of 3, the results of our study showed a high specificity of the PAMD score in evaluating a positive outcome of TRUS-biopsy in our population of patients. Studies on a larger number of subjects are necessary to determine the optimal cut-off value. Higher values of the PAMD score showed a statistically significant association with high-grade PC, these data are in accordance with the data of Fang et al. (12).

Conclusion

The prevalence of PC in the examined population of patients with PSA 4-10 ng/ml was 70%. PAMD score showed high specificity in assessing positive biopsy outcomes in our population of patients with PSA 4-10 ng/ml. The PAMD scoring system requires further testing on a larger sample of patients, and in future it could be used to better select candidates for prostate biopsy.

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EVALUACIJA KLINIČKE PRIMENE PAMD SKORA U PROCENI POZITIVNOG ISHODATRUS-BIOPSIJE PROSTATE KOD PACIJENATA SA PSA 4-10 NG/ML LEČENIH U SRBIJI

Milan Radovanović[1,2], Aleksa Zubelić[2], Veljko Šantrić[1,2], Bogomir Milojević, Uroš Bumbaširević[1,2], Branko Stanković[1,2]

Sažetak

Uvod: Transrektalna ultrazvučno vođena biopsija prostate (TRUS-biopsija) predstavlja „zlatni standard" u dijagnostici karcinoma prostate (KP). Postoje podeljena mišljenja o potrebi za biopsijom kod pacijenata sa vrednostima prostata specifičnog antigena (PSA) između 4-10 ng/ml. Pozitivan ishod biopsije (KP) kod ovih pacijenata kreće se u rasponu između 20 I 39%. Niska senzitivnost i specifičnost PSA u predikciji pozitivnog ishoda biopsije rezultuje velikim brojem nepotrebnih biopsija i tretmana. U cilju što bolje selekcije kandidata za biopsiju, poslednjih godina predloženo je nekoliko modela stratifikacije rizika za KP, među njima je i PAMD skor.

Cilj rada: Cilj ovog rada je bio da se ispita vrednost PAMD skora u proceni pozitivnog ishoda biopsije u našoj populaciji pacijenata, kao i da se ispitaju pojedinačni faktori rizika za pozitivan ishod biopsije kod pacijenata sa vrednostima PSA između 4 i 10 ng/ml.

Materijal i metode: U studiji je učestvovalo 50 pacijenata, lečenih na Klinici za urologiju, Univerzitetskog kliničkog centra Srbije kod kojih je izmerena vrednost PSA u opsegu od 4 do 10 ng/ml. Svim pacijentima određene su vrednosti PSA, indeksa PSA (%fPSA), urađen je DRE, kao i snimanje nuklearnom magnetnom rezonancom (MRI) u cilju evaluacije volumena prostate (PV) i *PI-RADS* skora. Kod svih pacijenata urađena je TRUS-vođena sistemska biopsija prostate. U skladu sa podacima iz literature svim pacijentima određen je PAMD skor.

Rezultati: PAMD skor> 3 pokazao je visoku specifičnost u predikciji KP, kao i povezanost sa višom učestalošću KP visokog gradusa. Pozitivan nalaz na DRE, %fPSA< 16, starost veća od 69 godina i *PI-RADS*> 3 pokazali su statistički značajnu povezanost sa postojanjem KP. Visoka individalna prediktivna vrednost u proceni postojanja KP potvrđena je za DRE, %fPSA, PV i *PI-RAD S*skor.

Zaključak:. PAMD skoring sistem može biti od značaja u boljoj selekciji kandidata za TRUS-biopsiju, u populaciji pacijenta sa vrednostima PSA 4-10 ng/ml.

Ključne reči: karcinom prostate, PSA, PAMD, faktori rizika.

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ORIGINAL ARTICLE

Doppler assessment of splanchnic arterial flow in patients with liver cirrhosis: correlation with nitric oxide

Tijana Glišić[1, 2], Dušan Đ. Popović[1, 2], Milica Stojković-Lalošević[1, 2], Jelena Martinov[1, 2], Marija Stojanović[3], Vladimir Jurišić[4]

[1]Clinic for Gastroenterohepatology, University Clinical Center of Serbia, Belgrade, Serbia
[2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[3]Institute of Medical Physiology „Richard Burian", Faculty of Medicine, University of Belgrade, Belgrade, Serbia
[4]Faculty of Medical Science, University of Kragujevac, Kragujevac, Serbia

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Correspondence to:
Tijana Glišić, MD, PhD
Clinic for Gastroenterohepatology, University Clinical Center of Serbia, Belgrade, Serbia
Pasterova 2, 11000 Belgrade, Serbia
phone: +38164139 36 45; e-mail: tijana.glisic78@gmail.com

Summary

Introduction/aim: Nitric oxide (NO) is a key mediator which, paradoxically, regulates sinusoidal (intrahepatic) and systemic/splanchnic circulation. The main goal of this study was to measure NO and compare serum values of NO with flow data in visceral blood vessels of the liver, spleen, kidney and intestine in patients with cirrhosis.

Material and methods: This prospective study included 80 patients with cirrhosis of the liver. Doppler ultrasonography was used to assess flow velocity and resistive index (RI) in the hepatic (HA), right (RRA), and left renal (LRA), splenic (SA) and superior mesenteric artery (SMA). NO concentration was determined using the DetectX® Nitric Oxide colorimetric detection kit.

Results: We found a statistically significant difference in the mean NO value in the group of patients without ascites compared to the ascites group, as well as in the group of patients with A stage in relation to C stage of cirrhosis ($p < 0.05$). There is statistically significant negative correlation between NO and diameter, and maximal and minimal velocity in LRA. There is significant positive correlation between NO and minimal velocity in SMA.

Conclusions: In this study, we found that patients with cirrhosis of the liver were exposed to significantly higher RI LRA, RRA, SA and HA. In patients with cirrhosis complicated by ascites and in those with end stage liver disease, the NO level was significantly higher. The concentration of NO had an effect on the diameter and flow rate in the LRA and flow rate in SMA.

Keywords: liver cirrhosis, nitric oxide, Doppler ultrasonography

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INTRODUCTION

Hyperdynamic splanchnic and systemic blood flow is typical for patients with cirrhosis of the liver. The combination of arterial vasodilation and increased intravascular volume is necessary for full expression of hyperdynamic circulatory condition. Cirrhosis is associated with peripheral vasodilatation resulting from the effects of systemic vasodilator substances. Many vasoactive substances are involved in the development of portal hypertension. Among them, nitric oxide (NO) is a key mediator which, paradoxically, regulates sinusoidal (intrahepatic) and systemic / splanchnic circulation.

Doppler ultrasound has been in use for the assessment of arterial blood flow in patients with cirrhosis of the liver. Intrarenal vasoconstriction, caused by complex interactions between portal and systemic hemodynamics, occurs early in the non-ascitic phase of cirrhosis and before the occurrence of hepatorenal syndrome [1-3]. Arterial resistance index (RI) is the most widely used Doppler variable for the estimation of intrarenal vascular resistance in clinical studies. Available data suggest 0.70 as the upper limit for normal intrarenal RI [4].

NO has significant effects on renal blood vessels. Nitric oxide synthase (NOS) inhibition leads to an increased renal vascular resistance, during the stimulation of endothelial nitric oxide synthase (eNOS), and after the application of L-arginine, and it causes a reduction of the same resistance. Endothelial nitric oxide synthase and inducible nitro-oxide synthase (eNOS and iNOS) may be closely related to the pathophysiology of cirrhosis of the liver and kidney damage due to the fact that iNOS is expressed as an important part of the kidney tissue.

Nitric oxide is likely the most potent vasodilator molecule known today. In cirrhotic livers, NO production/bioavailability is significantly diminished, which contributes to increased intrahepatic vascular resistance [5,6-9]. Decreased NO production is explained by at least two mechanisms. Firstly, the NO synthesizing enzyme eNOS is inhibited by negative regulators (such as caveolin-1), which are up-regulated during cirrhosis; as a result, NO production is decreased [8]. Secondly, oxidative stress is increased in cirrhosis.

The role of NO in the modulation of intrahepatic vascular resistance (IHVR) has been well documented [10-12]. eNOS dysfunction in sinusoidal endothelial cells and consequent reduction in NO production (or bioavailability) plays an essential role [13]. This results in reduced vasodilation and a decreased capacity for antagonizing contractile factors such as endothelin-1, angiotensin II, norepinephrine, prostaglandin F2 and thromboxane A2 [14,15].

A hyperdynamic splanchnic circulatory state is a major accompaniment of portal hypertension (PHT). An increase in splanchnic blood flow and the subsequent increase in portal venous inflow aggravates and perpetuates PHT. The mechanisms underlying this phenomenon are not fully understood, but overproduction of endogenous vasodilators and decreased vascular reactivity to vasoconstrictors have been suggested [16].

NO plays a pivotal role in the pathogenesis of PHT. NO levels are differentially altered in cirrhosis, with a reduced production in the intrahepatic circulation and an increased NO production in the splanchnic bed. Ideally, a NO donor or drug delivery system that selectively targets liver cells without actions on systemic circulation is required to reduce PHT without adverse systemic effects.

Only few studies have examined renal blood flow in cirrhosis of the liver [17-20]. The clinical significance of these tests lies in the therapeutic approach to the phenomenon of arterial vasodilation, because of the changing views on the application of beta-blockers or beta-agonists in the treatment of portal hypertension. Once developed, portal hypertension affects extrahepatic vascular bed in splanchnic and systemic circulation, leading to arterial vasodilation and formation of collateral, which increases the flow of blood in the portal vein, and this is exacerbated by portal hypertension [5, 21].

We found no report of correlations between serum nitric-oxide (NO) level and visceral arteries resistive index in the available literature.

Therefore, the aim of the present study was to assess the relationship between NO levels, hepatic (HA), splenic (SA), right renal artery (RRA) and left renal artery (LRA) blood flow in patients with cirrhosis of the liver.

MATERIAL AND METHODS

Subjects

This prospective study included patients with cirrhosis of the liver in different clinical stages, diagnosed at the Clinic for Gastroenterohepatology, University Clinical center of Serbia, from June 2010 to September 2012. The criteria for exclusion from the study were hepatocellular carcinoma, cardiorespiratory diseases, hypertension, renal artery stenosis, acute and chronic kidney lesions, diabetes mellitus, recent alcohol abuse, vasoactive medication and diuretics use during the study, portal vein thrombosis, and patients under the age of 18. The diagnosis of cirrhosis of the liver was based on clinical and histologic findings. The etiology was alcoholic, viral, autoimmune, or metabolic. The liver function was assessed by Child-Pugh score. The control group included healthy subjects.

Methods

Nitric oxide concentration (reference values 11–76 μmol/L) was determined using the DetectX® Nitric Oxide colorimetrical detection kit which was designed to measure nitrate and nitrite that are present in different samples. Basically, the NO measured after the serum sample was incubated with a nitrate reductase and

Table 1. Descriptive characteristics of patients with cirrhosis of the liver

Variable	n (%)
Gender	
Male	58 (72.5%)
Female	22 (27.5%)
Etiology of cirrhosis	
Hepatitis B	7 (8.9%)
Hepatitis C	8 (10.13%)
Autoimmune	5 (6.33%)
Alcohol	52 (65.9%)
Other	8 (8.74%)
Clinical characteristics	
Esophageal varices	41 (51.25%)
Ascites	50 (62.5%)
Abdominal collateral pathways	9 (11.25%)
Hepatic encephalopathy	24 (30.0%)
Hypertension	26 (32.5%)

NADH. Reductase in combination with NADH reduces nitrate into nitrite. After 20 minutes of incubation at room temperature non-ferrous reagents A and B were added and incubated at room temperature for 5 minutes. The colored product was read and accounts subtracted the measured concentrations of nitrite from the total concentration of nitric oxide in the sample.

On the same day, together with biochemical analyses, the patients and controls underwent color-coded and pulsed wave Doppler measurements of blood flow velocity and RI in the right and left interlobar renal arteries, hepatic artery, splenic artery and superior mesenteric artery, using a Toshiba Xario SSA-660A or Toshiba Aplio SSA-790 ultrasonographic system (Toshiba, Tokyo, Japan), with a 2- to 6-MHz multifrequency convex probe. The RI was automatically calculated from the Doppler spectrum as (Vmax-Vmin)/Vmax, where Vmax was the maximum systolic blood flow velocity and Vmin was the maximum end-diastolic velocity.

Statistical analysis

Statistical analysis was performed by the SPSS 13.0 statistical package (IBM-SPSS, Armonk, NY). All results are expressed as mean±SD. Comparisons between subgroups were made using the Mann-Whitney U test and Kruskal Wallis test as appropriate. Correlations were evaluated using the appropriate Spearman's p coefficients. Values of $p<0.05$ were considered significant.

Ethical approval

This Manuscript is a part of the doctoral thesis''Noninvasive research of arterial splanchnic circulation in liver cirrhosis: correlation with serum nitric oxide (NO) and ammonia" that has been completed and mentored by deceased Professor Mirjana Perisic, MD, PhD and co-mentored by Professor Vladimir Jurišić, MD, PhD.

The preparation of the doctoral thesis was approved by the University of Belgrade, Decision No. 020-1883/33, 27.05.2010.

RESULTS

The study included 80 cirrhotic patients. The average age was 56.26±10.5 years in men and 48.86±14.5 years in women. The control group consisted of 11 men, 46.76±14.6 years old, and 9 women, 40.66±14.6 years old. Patient characteristics are presented in **Table 1** and Doppler variables are presented in **Table 2**.

RRA Vmax is similar in patients with cirrhosis of the liver and in controls, whereas LRA Vmax is higher in patients with cirrhosis then in healthy controls **(Figure 1)**. HA and SA Vmax are higher in patients with liver cirrhosis than in controls **(Figure 1)**.

RRA Vmin is lower in patients with cirrhosis of the liver than in controls, as Vmin in LRA. SA Vmin is similar in patients and in controls **(Figure 2)**. RRA, LRA, SA, and HA RI are higher in patients with cirrhosis than in controls **(Table 1)**.

Table 2. Doppler variables in patients with cirrhosis of the liver and in healthy controls

Blood vessel	Variable	Patients with liver cirrhosis (n = 80)	Healthy controls (n = 20)	p
Right renal artery	Vmax (cm/s)	88.9 ± 31.6	83.3 ± 16.7	0.286
	Vmin (cm/s)	24.3 ± 8.9	29.3 ± 70	0.022
	RI	0.72 ± 0.08	0.65 ± 0.04	<0.001
Left renal artery	Vmax (cm/s)	99.4 ± 30.5	80.0 ± 17.1	<0.001
	Vmin (cm/s)	27.4 ± 9.2	31.2 ± 7.8	0.1
	RI	0.71 ± 0.09	0.61 ± 0.05	<0.001
Hepatic artery	Vmax (cm/s)	125.7 ± 67.7	79.3 ± 20.3	0.001
	Vmin (cm/s)	35.5 ± 25.6	28.9 ± 10.4	0.766
	RI	0.72 ± 0.09	0.64 ± 0.07	<0.001
Splenic artery	Vmax (cm/s)	144.5 ± 54.9	94.5 ± 20.6	0.001
	Vmin (cm/s)	44.8 ± 19.0	38.1 ± 9.5	0.278
	RI	0.68 ± 0.09	0.59 ± 0.06	<0.001

RI - resistance index; Vmax - maximum systolic blood flow velocity; Vmin - maximum tele diastolic blood flow velocity

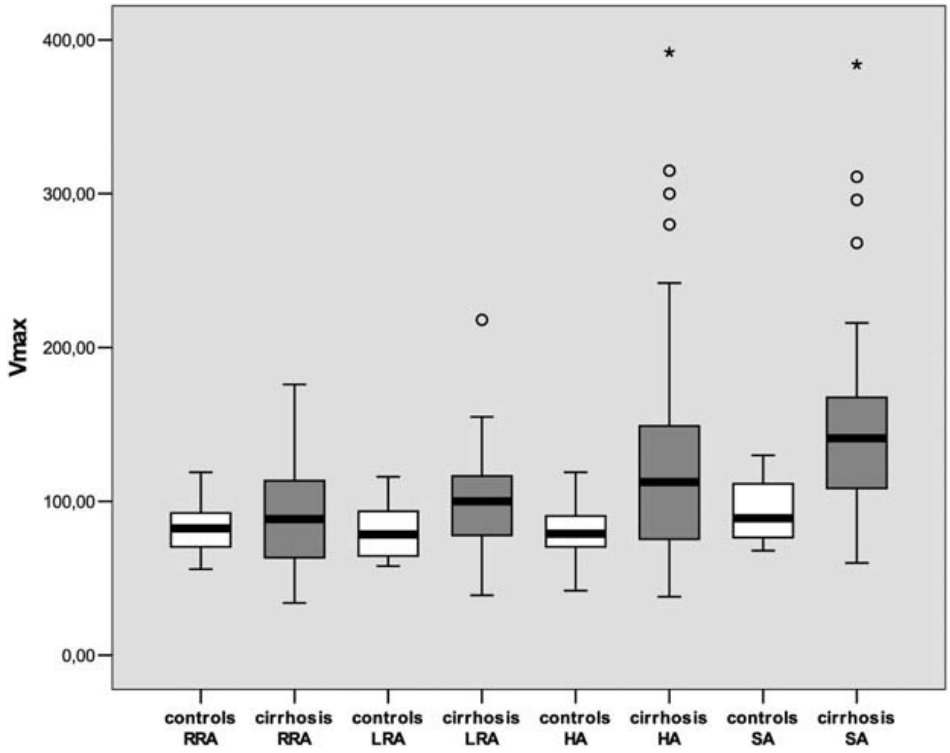

Figure 1. Box-and-whiskers plot showing maximum systolic blood flow velocity (Vmax) in patients with liver cirrhosis and in healthy controls
Vmax-maximum systolic blood flow velocity (cm/s), RRA- Right renal artery, LRA-Left renal artery, HA- Hepatic artery, SA- Splenic artery
The bottom and the top of the box are the first and third quartiles, respectively, while the horizontal band dividing the box is the median. The whiskers represent the lowest and highest data within 1.5 interquartile range, while outlying data are shown as small circles.
* Extreme values, which in the non-parametric analyzes have not changed the significance to a great extent

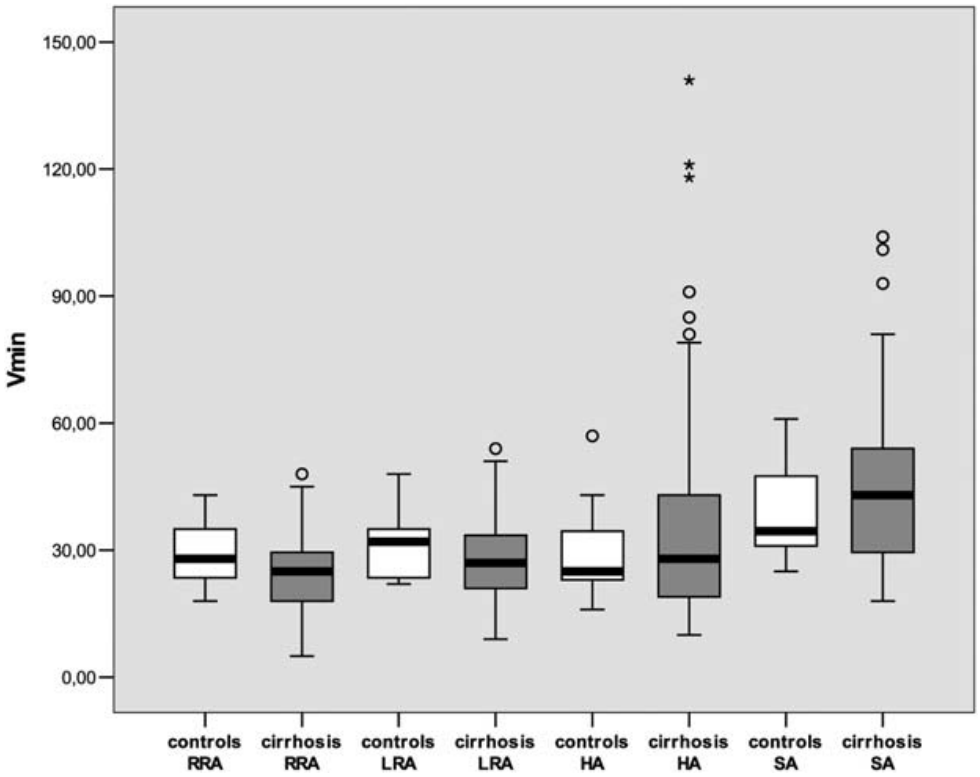

Figure 2. Box-and-whiskers plot showing maximum end-diastolic blood flow velocity (Vmin) in patients with cirrhosis and in healthy controls
Vmin-maximum end-diastolic blood flow velocity (cm/s), RRA- Right renal artery, LRA-Left renal artery, HA- Hepatic artery, SA- Splenic artery
The bottom and the top of the box are the first and third quartiles, respectively, while the horizontal band dividing the box is the median. The whiskers represent the lowest and highest datum within 1.5 interquartile range, while outlying data are shown as small circles

* Extreme values, which in the non-parametric analyzes have not changed the significance to a great extent

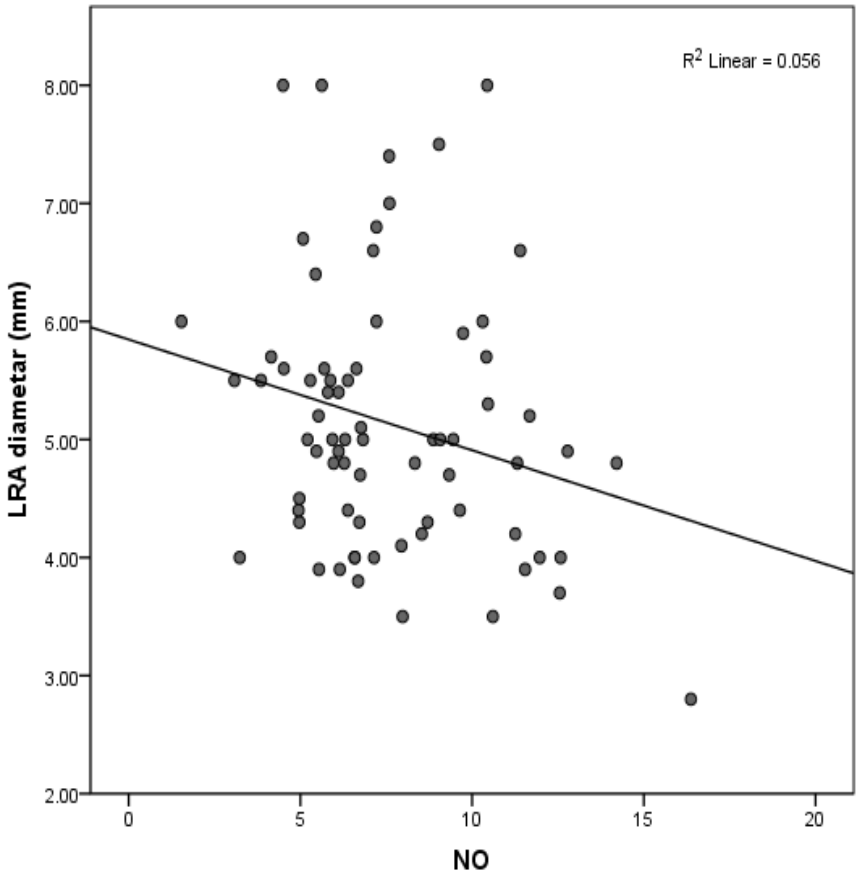

Figure 3. Correlation between nitric-oxide serum level and left renal artery diameter

LRA – left renal artery, NO – nitric-oxide

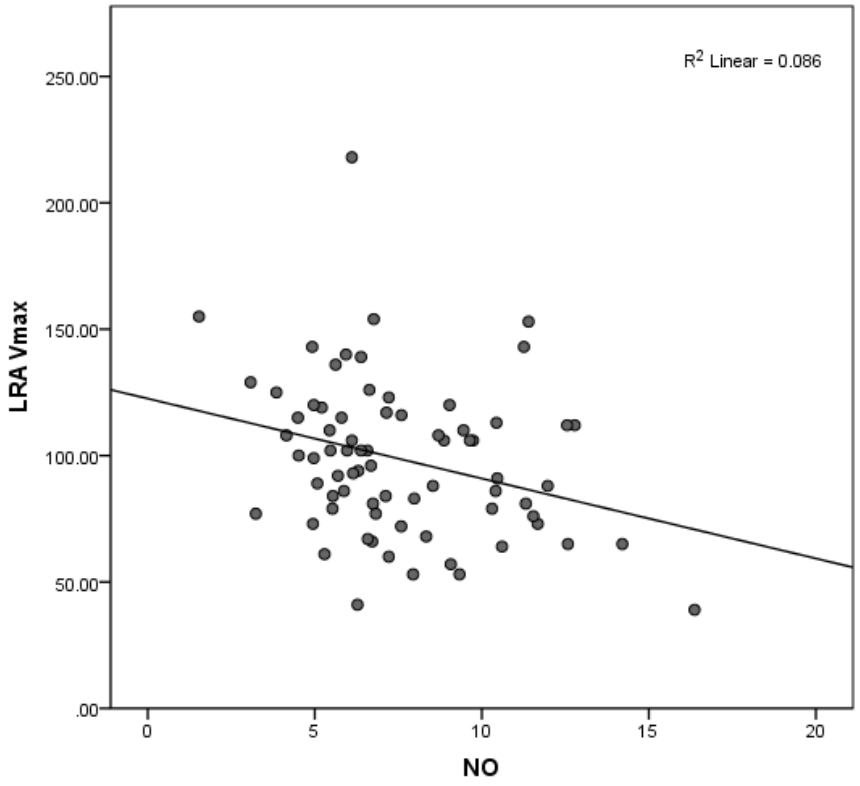

Figure 4. Correlation between nitric-oxide serum level and systolic blood flow velocity in left renal artery in patients with liver cirrhosis

Vmax – systolic blood flow veleocity, LRA – left renal artery, NO – nitric-oxide

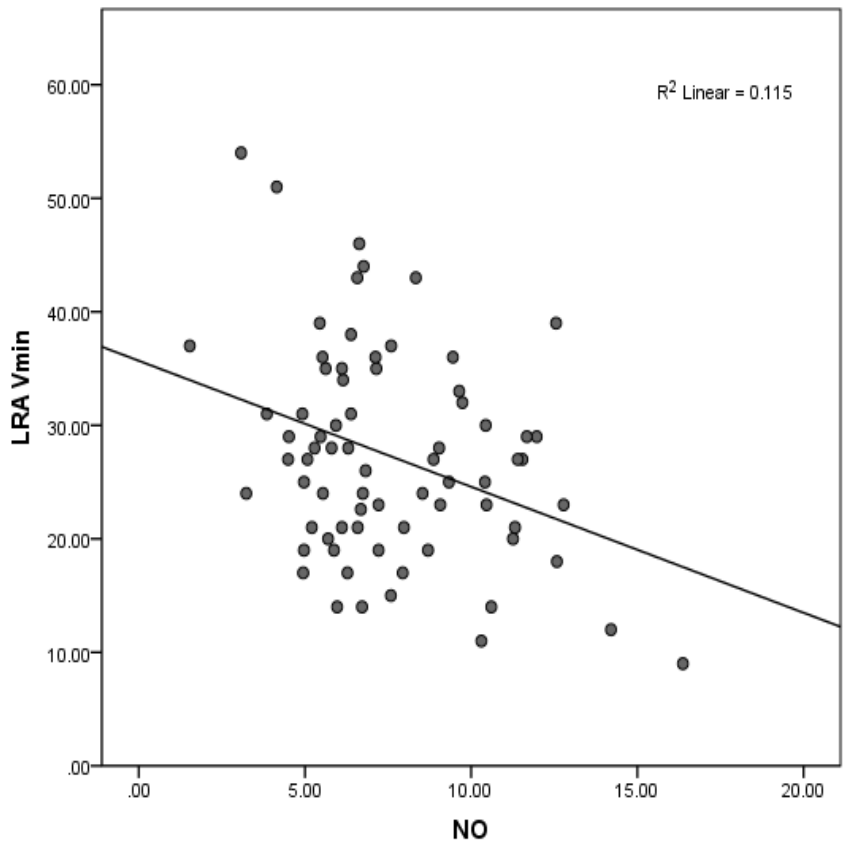

Figure 5. Correlation between nitric-oxide serum level and diastolic blood flow velocity in left renal artery in patients with liver cirrhosis

Vmin – dyastolic blood flow veleocity, LRA – left renal artery, NO – nitric-oxide

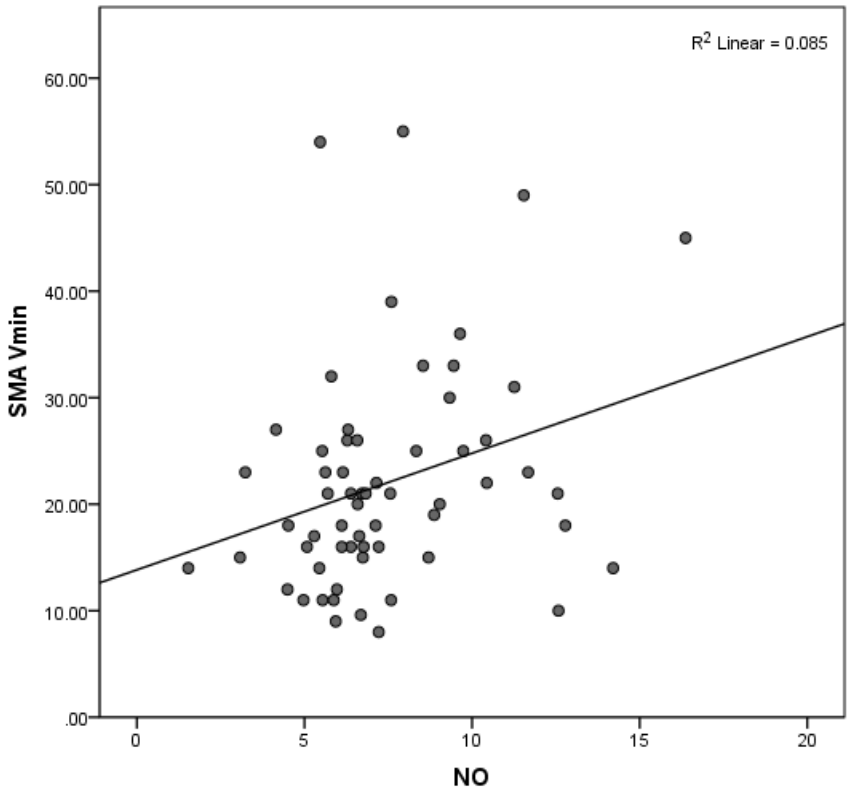

Figure 6. Correlation between nitric-oxide level and diastolic blood flow velocity in superior mesenteric artery in patients with cirrhosis of the liver

Vmin – diastolic blood flow velocity, SMA – superior mesenteric artery, NO – nitric-oxide

The average plasma level of NO in patients with cirrhosis is 7.7± 3.2 μmol/L. There is a statistically significant difference in median values for NO in the group of patients without ascites (Med=6.7 μmol/L) and in the group of patients with ascites (mild ascites Med=7.2 μmol/L; moderate/large ascites Med=8.3 μmol/L) ($p < 0.05$). Given the stage of cirrhosis according to the Child-Pugh classification there is a significant difference in median values for NO in the group of patients with stage A (Med=6.9 μmol/L) and stage C (Med=8.0 μmol/L) cirrhosis of the liver ($p < 0.05$).

There is a significant negative correlation between NO level and diameter of LRA **(Figure 3)**. There is a significant negative correlation between NO level and Vmax **(Figure 4)** and Vmin in LRA **(Figure 5)**. There is a significant positive correlation between NO level and Vmin in SMA **(Figure 6)**. There are no significant correlations between other variables.

DISCUSSION

Renal hemodynamic changes are commonly developed during the course of cirrhosis of the liver [22]. Duplex Doppler sonography can detect renal vasoconstriction in patients with cirrhosis [23].

In our study, the majority of patients with cirrhosis of the liver had normal levels of plasma nitric oxide, while NO value increased with the presence of cirrhosis and ascites, which is explained by the fact that the ascites result from hemodynamic changes. That matches with the results of other authors [24-26]. The Child-Pugh score is used to assess the prognosis of chronic liver disease, mainly cirrhosis. It consists of total bilirubin, serum albumin, prothrombin time, ascites and hepatic encephalopathy. We found that patients with stage C of liver cirrhosis had higher serum NO level, which means that in the advanced stages of cirrhosis there is an increased level of NO.

Arterial circulation in the kidneys is specific in patients with cirrhosis of the liver compared to other tested splanchnic artery and compared the left renal artery to the right.

There was a statistically significant negative correlation in median values of NO and the diameter of left renal artery (LRA). There was no correlation with the right renal artery. Increasing levels of NO lead to a decrease in LRA diameter. This intrarenal vasoconstriction is explained as a consequence of the complex impact of the portal and systemic circulation and with a fact that this nitrogen molecule has vasoconstrictive action in cirrhosis of the liver. These changes are described in the early pre-ascites phase of cirrhosis, and they precede the occurrence of hepatorenal syndrome [27, 28]. NO is vasodilator in visceral arteries. However, NO is vasoconstrictor in renal arteries. The phenomenon of renal vasoconstriction is very important in the study of hemodynamics in portal hypertension. In hepatorenal syndrome, renal vasoconstriction progresses, and the value of NO in serum grows.

We found statistically significant negative correlation between systolic and diastolic blood flow velocity and NO plasma levels in patients with cirrhosis. However, a specific flow only in the left renal artery can be the result of specific spleno-renal reflex, specific neural connections between the spleen and the left kidney, which participate in normal regulation of blood pressure and renal blood flow. They also participate in portal hypertension in renal and cardiovascular dysfunction. Study Jacobs-Kaufmann, Hamza et al, from 2003-2012, showed that NO, with other mediators, increases the release of fluid from the spleen vascular tree, increasing intrasplenic micro-vascular pressure, and the operation of the mediators in the afferent and efferent blood vessels of the spleen [29]. Changes in intrasplenic flow and the action of NO activate splenic afferent and efferent renal nerve fibers [29].

There was a statistically significant positive correlation between NO level and diastolic blood velocity flow in SMA that can be explained by vasodilator effect on this artery, like on the other splanchnic arteries. In addition to this explanation, increased release of NO by the SMA endothelium occurs before the development of hyperdynamic splanchnic circulation. Increased splanchnic iNOS occurs and persists in residual macrophages SMA. Elevated levels of NO and diastolic slower speed in SMA could open the way to the development of splanchnic hyperdynamic circulation and development of advanced liver disease.

CONCLUSION

In conclusion, patients with cirrhosis of the liver complicated with ascites and those with end-stage liver disease have greater NO levels. Specific blood velocity flow only in the LRA may be the result of specific spleno-renal reflex, and NO as a potent vasoactive molecule can have an influence on diameter of LRA, as well as on blood flow velocity in this artery. On the other hand, this nitric molecule has an opposite effect on the SMA leading to slower diastolic flow, but can be predictive data for the onset of splanchnic hyperdynamic circulation.

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None

Conflict of interest

None to declare.

Author Contributions

Glisic T, design of the work, collecting data, analysis, interpretation of data, preparing the draft of the manuscript, manuscript revision; Popovic D, collecting data,

interpretation of data, manuscript revision; Stojkovic-Lalosevic M, collecting data, interpretation of data, Martinov J, collecting data, interpretation of data; Stojanovic M, collecting data, interpretation of data; Jurisic V, conception and design of the work, collecting data, analysis.

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PROCENA SPLANHNIČNOG ARTERIJSKOG PROTOKA DOPPLER ULTRASONOGRAFIJOM KOD PACIJENATA SA CIROZOM JETRE: KORELACIJA SA NIVOOM AZOT OKSIDA

Tijana Glišić[1, 2], Dušan Đ. Popović[1, 2], Milica Stojković-Laloević[1, 2], Jelena Martinov[1, 2], Marija Stojanović[3], Vladimir Jurišić[4]

Sažetak

Uvod/cilj: Azot oksid (NO) je ključni medijator koji, paradoksalno, reguliše intrahepatičku i sistemsku/splanhničnu cirkulaciju. Glavni cilj ove studije bilo je merenje nivoa azot oksida, poređenje vrednosti serumskih vrednosti NO sa podacima o brzinama protoka krvi u visceralnim krvnim sudovima jetre, slezine, bubrega i creva kod pacijenata sa cirozom jetre.

Materijal i metode: U ovu prospektivnu studiju bilo je uključeno 80 pacijenata sa cirozom jetre. Doppler ultrasonografija je korišćena radi procene brzine protoka i rezistivnih indeksa (RI) u hepatičkoj (HA), desnoj (DRA) i levoj renalnoj (LRA), slezinskoj (SA) arteriji i gornjoj mezenteričnoj arteriji (SMA). Koncentracija NO je određivana primenom DetectX® Nitric Oxide kolorimetrijskog detekcionog kita.

Rezultati: Utvrđena je statistički značajna razlika u srednjoj vrednosti NO u grupi bolesnika bez ascitesa u odnosu na grupu sa ascitesom, kao i u grupi pacijenata sa A stadijumom u odnosu na C stadijum ciroze jetre ($p < 0,05$). Postoji statistički značajna negativna korelacija između vrednosti NO i dijametra i maksimalne i minimalne brzine u LRA. Prisutna je statistički značajna pozitivna korelacija između vrednosti NO i minimalne brzine u SMA.

Zaključak: Pacijenti sa cirozom jetre imaju signifikantno više RI u LRA, DRA, SA i HA. Kod pacijenata sa cirozom komplikovanom ascitesom i onima sa završnom fazom bolesti jetre, nivo NO je signifikantno viši. Koncentracija NO ima uticaja na dijametar i brzinu protoka u LRA, kao i na minimalnu brzinu protoka u SMA.

Ključne reči: ciroza jetre, azot oksid, Dopler ultrasonografija

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ORIGINAL ARTICLE

Prospective study of quality of life in patients with myotonic dystrophy type 2

Ivo Bozovic[1], Ivana Basta[1], Ana Cocic[1], Aleksa Palibrk[1], Ivana Kezic[2], Vukan Ivanovic[1], Jelena Lazovic[3], Stojan Peric[1]

[1] Neurology Clinic, University Clinical Centre of Serbia, Faculty of Medicine, University of Belgrade, Serbia;
[2] General hospital "Pljevlja", Montenegro;
[3] Neurology Clinic, Clinical Center of Montenegro, Montenegro.

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Correspondence to:
Ivo Bozovic
Neurology Clinic, Universuty Clinical Centre of Serbia
6, Dr Subotica Street
11 000 Belgrade, Serbia
Phone: +381 64 9443604
Fax: +381 11 2684577
E-mail: ivo.bozovic20@gmail.com

Summary

Introduction/aim: Although myotonic dystrophy type 2 (DM2) is generally milder than DM1, quality of life (QoL) seems to be similarly impaired in these two disorders. There are no studies that assessed QoL during DM2. Our aim was to assess QoL and disease outcome in patients with DM2 after a five-year follow-up period.

Material and Methods: Study originally comprised 49 DM2 patients at baseline. During the five-year period, seven patients died, eight were lost to follow-up, one patient moved, and one refused testing. The Short Form (36) Health Survey (SF-36) and Individualized Neuromuscular Quality of Life (INQoL) questionnaires were administered in 30 patients at baseline and at follow-up (47% males, 54±10 years old).

Results: Patients who were retested had better Role Physical (RP) and General Health (GH) scores of the SF-36 and better weakness score of the INQoL compared to non-retested ($p>0.05$). After the five-year follow-up, none of the SF-36 and INQoL scores differed compared to baseline ($p>0.05$).

Conclusion: QoL did not change in DM2 patients during a five-year period, as measured by both SF-36 and INQoL.

Key words: myotonic dystrophy type 2; quality of life; SF-36; INQoL; prospective study

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THE INTRODUCTION

Myotonic dystrophy type 2 (DM2) is an autosomal dominant inherited multisystem disease (1). Main characteristics of DM2 are proximal muscle weakness, variable myotonia, cataracts, cardiac disorders, endocrinological and metabolic disorders (diabetes mellitus type 2 and hyperlipidaemia), gastrointestinal symptoms and signs (constipation, diarrhoea) and the central nervous system manifestations.

DM2 is generally similar to myotonic dystrophy type 1 (DM1), but both muscular and non-muscular disease symptoms are less pronounced in DM2. Muscle weakness and affection of other tissues is known to be associated with poor quality of life (QoL) in patients with DM1 (2–6). Although DM2 is clinically milder than DM1, two studies that assessed QoL in DM2 showed that it was similarly affected in both diseases (7, 8). In addition, Tieleman and colleagues found a deterioration in QoL in 32 DM2 patients compared to general Dutch population (7). No prospective QoL study in patients with DM2 has been conducted so far. Also, there have not been much data on the natural history of DM2.

The aim of this study was to prospectively analyze QoL and disease outcome in patients with DM2 after a five-year follow-up period.

THE MATERIALS AND METHODS

All patients gave informed consent to participate in the study and the study was approved by the Ethical Board of the Neurology Clinic, University Clinical Centre of Serbia and the study was performed in compliance with the Declaration of Helsinki. In all patients, DM2 diagnosis was based on the clinical presentation and electromyography findings and further confirmed by genetic analysis using the repeat-primed polymerase chain reaction (RT-PCR) (9). Patients were initially consecutively tested between June 2013 and June 2015 during their regular Outpatient or Inpatient examination at the Neurology Clinic, University Clinical Centre of Serbia. They were invited and retested from September to December 2019.

Sociodemographic data and clinical data were obtained from the Serbian DM registry and patients themselves. Muscle strength was assessed according to the Medical Research Council (MRC) 0-5-point scale (0 = no movement, 5 = normal strength) by two raters. The following muscles were examined bilaterally: shoulder abductors and adductors, elbow flexors and extensors, thumb opponents, finger abductors and adductors, hip flexors, extensors, abductors and adductors, knee flexors and extensors, ankle plantar and dorsal flexors. Overall severity of motor impairment was analyzed as previously described (10). We added strength of the weakest muscle of the proximal/distal muscle groups of the upper limbs/lower limbs with maximum score being 20, where lower scores mean greater muscle impairment.

All patients completed the Serbian version of *The Short Form (36) Health Survey* (SF-36) questionnaire, as a measure of health-related QoL (11). This is a generic instrument that measures eight health concepts: physical functioning (PF), role physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role emotional (RE) and mental health (MH). Two main scores summarize these scales: physical composite score (PCS) and mental composite score (MCS), as well as total SF-36 score. All these scores fall within a 0-100 scale, with higher scores reflecting better QoL.

Patients also completed the *Individualized Neuromuscular Quality of Life questionnaire* (INQoL) version 1 (12). INQoL consists of 45 questions within 10 sections. Four sections measure the impact of common muscle disease symptoms (weakness, myotonia, pain and fatigue). Five sections measure the influence of the muscle disease on particular areas of life (activities, independence, social relationships, emotions and body image). The last section is related to the disease treatment, and it was not used in our study since it is not included in the total INQoL score. Total INQoL score is calculated from five sections assessing the influence of the muscle disease on particular areas of life (12). The final score for each of the nine sections and the total INQoL score are presented as a percentage of the maximum detrimental impact with a higher percentage indicating greater symptom impact or worse QoL.

Normality of data was assessed by the Kolmogorov-Smirnov test. Student t-test for paired samples was used to compare the results at baseline and at follow-up. The significance of all tests was two-sided, with $p<0.05$ for statistical significance and $p<0.01$ for high statistical significance.

THE RESULTS

At baseline, 49 patients were enrolled. During a five-year follow-up period, seven patients died, eight patients were lost to follow-up, one patient moved, and one refused testing. Two patients developed other serious diseases (epilepsy and laryngeal carcinoma) and were not retested because of the potential influence of these diseases on their QoL. Thus, 30 (61.2%) patients were retested at follow-up.

Retested patients compared to the non-retested were more commonly females and younger at baseline **(Table 1)**.

Patients who were retested had better RP and GH scores of the SF-36 and better weakness score of the INQoL compared to the non-retested ($p>0.05$) **(Table 2)**.

There were no differences in either SF-36 or in INQoL scores between baseline and follow-up testing **(Table 3)**.

Table 1. Baseline clinical features of DM2 patients

Features	Retested patients	Non-retested patients
N	30	19
Gender (% males) *	20.0	47.4
Age at testing (years, mean±SD)*	48.8±10.5	55.6±10.0
Education (years, mean±SD)	11.6±3.3	11.4±3.4
Age at onset (years, mean±SD)	36.0±8.7	35.6±13.0
Disease duration (years, mean±SD)	12.7±11.0	19.9±16.4
MRC score (mean±SD)	17.6±2.0	16.5±2.3

SD – standard deviation, MRC – Medical Research Council; * $p<0.05$

Table 2. Baseline quality of life scores in DM2 patients

QoL score	Retested patients	Non-retested patients
N	30	19
PF	53.5±30.2	43.2±23.6
RP *	53.8±44.5	23.5±33.6
BP	58.0±31.4	56.2±26.6
GH *	55.0±23.8	40.1±21.2
VT	50.8±24.4	39.1±28.5
SF	72.6±25.7	60.3±35.7
RE	60.3±46.2	47.1±42.6
MH	65.4±19.0	57.6±27.5
PCS	54.2±27.4	40.4±21.7
MCS	60.8±22.5	48.8±26.5
SF-36 total score	58.7±24.5	45.9±24.2
Weakness *	48.1±35.6	71.7±21.3
Myotonia	35.1±32.7	42.8±34.9
Pain	37.4±34.0	40.1±36.2
Fatigue	48.8±34.4	56.2±31.2
Activities	41.4±28.8	57.6±30.5
Independence	30.2±27.9	39.3±34.0
Social relations	19.3±21.5	19.6±23.2
Emotions	26.2±25.9	35.2±24.2
Body image	28.0±28.8	42.4±30.0
INQoL total score	35.6±25.0	45.7±22.5

physical functioning - PF, role physical - RP, bodily pain - BP, general health - GH, vitality - VT, social functioning - SF, role emotional – RE, mental health - MH, physical composite score - PCS, mental composite score - MCS; * $p<0.05$

Table 3. Quality of life scores at baseline and follow-up in DM2 patients (N=30)

QoL scores	First testing	Retest
PF	53.5±30.2	59.6±29.7
RP	53.8±44.5	60.6±49.1
BP	58.0±31.4	59.1±35.1
GH	55.0±23.8	51.5±24.2
VT	50.8±24.4	47.3±24.9
SF	72.6±25.7	74.5±31.3
RE	60.3±46.2	78.2±41.0
MH	65.4±19.0	67.2±21.5
PCS	54.2±27.4	55.6±27.3
MCS	60.8±22.5	63.7±24.1
SF-36 total score	58.7±24.5	62.2±26.5
Weakness	48.1±35.6	56.5±32.9
Myotonia	35.1±32.7	36.5±32.6
Pain	37.4±34.0	37.7±36.1
Fatigue	48.8±34.4	42.8±38.0
Activities	41.4±28.8	37.6±27.3
Independence	30.2±27.9	30.1±33.8
Social relations	19.3±21.5	12.0±18.8
Emotions	26.2±25.9	23.4±24.0
Body image	28.0±28.8	32.2±32.1
INQoL total score	35.6±25.0	33.7±25.0

physical functioning - PF, role physical - RP, bodily pain - BP, general health - GH, vitality - VT, social functioning - SF, role emotional – RE, mental health - MH, physical composite score - PCS, mental composite score - MCS

DISCUSSION

Understanding natural history of a certain disease has a great significance, not only for providing information about prognosis to patients, but also for designing possible clinical trials. These studies are lacking in patients with DM2. In order to fill in this gap, we prospectively monitored QoL in DM2 patients.

Our results showed no deterioration of QoL during a five-year follow-up period which, once again, confirms the previously known fact that DM2 is a slowly progressive disease (14, 15). On the other hand, the lack of changes in QoL measures may also indicate that used instruments are non-sensitive to detect changes in DM2. This probably means that these outcome measures are not a good choice for future clinical trials in DM2. If they cannot detect a change in QoL after five years, it is hard to believe that they would be able to do so in a shorter period of time, which is usual for clinical trials. The SF-36 is a generic questionnaire, which has to-date been widely applied in order to evaluate the QoL of patients suffering from different neurological and non-neurological chronic diseases (11, 15-17). One of the main advantages of this questionnaire is the ability of QoL analysis and comparison of different diseases. On the other hand, although widely used, this QoL measure has several limitations, including inability to capture disease-specific features of neuromuscular disorders. In order to overcome these limitations, Vincent and colleagues have created the INQoL questionnaire (12). It is a patient-reported outcome suggested to have advantages in the assessment of QoL in different neuromuscular diseases over widely used generic questionnaires (18-20). However, in our study neither SF-36 nor INQoL were able to detect a change in DM2 during a five-year follow-up period. Currently there are no QoL questionnaires specifically developed for DM2. All these facts indicate that it is necessary to

develop new, specific patient-reported outcome measures for DM2, which would have all the characteristics of modern instruments and which would be responsive.

The most significant limitation of our study is the small number of participants, although this is a pretty large cohort for such a rare disease. Another drawback of the study is the relatively small percentage (61%) of retested patients from the original cohort. In addition, retested patients were more often young and of female gender, which may indicate a selection bias. It is also possible that the patients who were retested are actually the ones who are generally better and therefore see their doctor more regularly. Anyway, our research gives important clues regarding the course and prognosis of DM2. Multicentric studies with a larger number of subjects from different cultural backgrounds are needed to definitely understand the natural course of DM2.

CONCLUSION

Quality of life in DM2 patients did not change significantly over a five-year follow-up period, which confirms a slowly progressive course of the disease and also suggests inability of currently available measures to detect changes in DM2.

Ethics approval

This research was approved by the Ethical Board of the Neurology Clinic, University Clinical Centre of Serbia.

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PROSPEKTIVNA STUDIJA KVALITETA ŽIVOTA KOD PACIJENATA SA MIOTONIČNOM DISTROFIJOM TIPA 2

Ivo Bozovic[1], Ivana Basta[1], Ana Cocic[1], Aleksa Palibrk[1], Ivana Kezic[2], Vukan Ivanovic[1], Jelena Lazovic[3], Stojan Peric[1]

Sažetak

Uvod/cilj: Uprkos činjenici da miotonična distrofija tipa 2 (DM2) ima generalno blažu fenotipsku prezentaciju od miotonične distrofije tipa 1 (DM1), postoje pretpostavke da je kvalitet života (KŽ) obe grupe pacijenata podjednako narušen. U dosadašnjoj literaturi nema prospektivnih istraživanja, koja su procenjivala KŽ tokom progresije DM2. Cilj ove studije je bila procena KŽ i ishoda bolesti kod pacijenata sa DM nakon petogodišnjeg perioda praćenja.

Materijal i metode: Inicijalna analiza je obuhvatila 49 DM2 pacijenata. Tokom petogodišnjeg perioda praćenja, sedam pacijenata je preminulo, osam pacijenata je izgubljeno iz praćenja, jedan pacijent se preselio, a jedan je odbio ponovno testiranje. Upitnici za procenu KŽ, „Mera zdravlja kratke forme"(engl. The Short-Form (SF-36) Health Survey - SF-36) i „Individualizovani upitnik o kvalitetu života kod neuromišićnih bolesti"(engl. Individualized Neuromuscular Quality of Life (INQoL)), primenjeni su kod 30 pacijenata nakon perioda praćenja.

Rezultati: Pacijenti koji su retestirani nakon pet godina imali su bolje skorove fizičkog funkcionisanja (engl. Role Physical (RP)) i opšteg zdravlja (engl. General Health (GH)) na SF-36 upitniku i bolji INQoL skor povezan sa slabošću u poređenju sa pacijentima koji nisu retestirani nakon pet godina ($p<0,05$). Posle petogodišnjeg perioda praćenja, nijedan od SF-36 i INQoL skorova se nije razlikovao u poređenju sa skorovima uočenim tokom inicijalne analize ($p>0,05$).

Zaključak: KŽ se nije promenio kod pacijenata sa DM2 tokom petogodišnjeg perioda praćenja, mereno generičkim SF-36 i individualizovanim INQoL upitnikom.

Ključne reči: Miotonična distrofija tip 2; kvalitet života; SF-36; INQoL; prospektivna studija

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ORIGINAL ARTICLE

Subacute sclerosing panencephalitis – changes in phenotype during the last decade

Ruzica Kravljanac [1,2], Ilija Palic [3], Biljana Vucetic Tadic [1,2]

[1]Neurology Department, Pediatric Clinic, Institute for Mother and Child Healthcare of Serbia „Dr Vukan Cupic", Belgrade, Serbia

[2]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[3]Institute of Neonatology, Belgrade, Serbia

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Correspondence to:
Ruzica Kravljanac MD, PhD, Head of Neurology Department, Pediatric Clinic, Associate Professor of Pediatrics, Faculty of Medicine University of Belgrade, Institute for Mother and Child Healthcare of Serbia „Dr Vukan Cupic", 6-8 Radoja Dakica Street, 11070 Belgrade, Serbia, Tel / Fax +381 11 3108 210, Cell + 381 64 261 22 10, e-mail: ruzica.kravljanac@gmail.com.

Summary

Introduction: Subacute sclerosing panencephalitis (SSPE) is a rare, progressive, neurodegenerative disease with poor outcome. Anti-measles vaccination contributed to a decreasing number of SSPE patients, but not to its eradication. The aim of our study is to evaluate the course of the disease in our SSPE patients with a focus on vaccinated children. The main goal is considering possibilities for improving prevention of the disease.

Methods: A retrospective study included the patients with SSPE treated in the period from December 2010 to December 2020 at the Pediatric Clinic of the Institute. The inclusion criteria were the patients with the diagnosis of SSPE based on clinical presentation, neuroimaging, electroencephalography and positive IgG anti-measles antibodies, both in serum and CSF.

Results: Five children with fulminant course of SSPE were included. All these patients were suffering from measles at an early age. Three of them had been vaccinated against measles and two had not. All of them had previously been healthy, immune-competent children, with normal general development. The course was extremely fulminant with lethal outcome within three months since the initial symptoms in four cases. Progressive motor and cognitive decline, behavior changes, movement disorders, myoclonic jerks and seizures were dominant in clinical presentation.

Conclusion: Despite vaccination, SSPE has not been eradicated. An increasing number of vaccinated immune-competent children with fulminant form of SSPE and history of measles infection at an early age were treated at our Clinic. As a measure for improving prevention, we suggest considering weaning of vaccine-derived immunity, and re-vaccination of girls at reproductive period.

Keywords: fulminant SSPE, vaccination, measles, children

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INTRODUCTION

Subacute sclerosing panencephalitis (SSPE) is a rare, progressive, neurodegenerative disease caused by a persistent defective measles viral infection. The incidence of SSPE is inversely related to Measles-Mumps-Rubella (MMR) vaccination coverage. It is endemic throughout the world, mainly in the countries where effective vaccination programs have not been completely realized (1,2). Pathogenesis of the disease is very complex. Numerous mutations in the M gene have been noted in the brain tissues of SSPE patients. Defects in the M protein result in the failure to form the virus particle facilitating the persistence of measles virus in neuronal cells and initiate inflammatory response that leads to extensive tissue damage. SSPE usually occurs 5-10 years after the measles virus infection, in otherwise healthy immune-competent children. The disease usually develops within 1-2 years, while about 10% of cases have fulminant form of SSPE with developing symptoms and signs within 3 months (3-5). Typical clinical presentation includes progressive motor and cognitive decline, behavior changes, myoclonic jerks and seizures. With disease progression, myoclonic jerks increase in frequency, followed by motor and cognitive deterioration. Brain magnetic resonance (MR) shows nonspecific periventricular white matter signal abnormalities, but at early stages of the disease there are no abnormalities in neuroimaging. Definitive diagnosis is based on the Dyken's criteria, which include two major and four minor criteria. Major criteria are as follows: 1) increased anti-measles antibody titers in cerebrospinal fluid (CSF) greater than or equal to 1:4 or ratio greater than or equal to 1:256 in serum, and 2) typical or atypical clinical presentation. Minor criteria include: 1) characteristic electroencepahlograhic (EEG) findings that include periodic, generalized, bilaterally synchronous and symmetrical high-amplitude slow waves that recur at regular intervals of 5–15 seconds called periodic slow-wave complexes also known as "Radermecker" complexes, 2) CSF globulin levels greater than 20% of the total CSF protein, 3) characteristic histopathological findings on brain biopsy and 4) specialized molecular diagnostic test to identify wild-type measles virus mutated genome. There is no specific treatment for SSPE, although oral Isoprinosine combined with intravenous infusion of Ribavirin and Intrathecal /intraventricular interferon-alpha has been recommended. Prognosis is poor with lethal outcome in 95% of the patients (2).

It was believed that prevention of SSPE by measles vaccination was crucial for eradicating this devastating disease (5-7). The annual incidence of SSPE in both adults and children after measles infection declined from 1 per 100,000 in the pre-immunization era to 0.06 per 100,000, after the introduction of immunization against measles (8). The aim of our study is to evaluate the course of disease in SSPE patients treated at our clinic during the past ten years with a focus on regularly vaccinated children against measles infection who were suffering from SSPE. The main goal is to consider possibilities for improving the prevention of the disease.

MATERIAL AND METHODS

A retrospective study included the patients with SSPE treated in the period from December 2010 to December 2020 at the Pediatric Clinic of the Institute for Mother and Child Healthcare of Serbia „Dr Vukan ČupiĆ", Belgrade. The inclusion criteria were patients with the diagnosis of SSPE based on clinical presentation, neuroimaging, EEG and positive IgG anti-measles antibodies, both in serum and CSF. All cases had clinical, neurological, neuroradiologic, EEG, microbiological and serological evaluation. In all the cases, serum and CSF were analyzed by Polymerase Chain Reaction (PCR) for Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), Human Immunodeficiency virus (HIV), West Nile virus (WNV) and Mycoplasma pneumoniae. Enzyme-linked immunosorbent assay (ELISA) test was used for anti-measles antibodies in serum and CSF. Concentration of immunoglobulin in serum and oligoclonal bands in CSF were evaluated. Medical charts were used for measles infection and vaccination status evaluation. The treatment included antiepileptic drugs, oral/parenteral corticosteroids, intravenous immunoglobulin, Isoprinosine, Ribavirin and interferon-alpha.

RESULTS

Five children with SSPE were treated at our Clinic during the past ten years, and all of them had a fulminant course of the disease. Four patients had measles during their childhood, and in one case (patient number 4) it is not clear whether the patient had measles infection or not, but since anti-measles IgG antibodies have been detected, it is supposed that the patient has had a subclinical form of measles. Two patients were not vaccinated, while three were vaccinated according to the regular schedule. The onset of SSPE varied and in two cases it was before the age of three. All the patients were previously healthy, immune-competent children, with normal general development. Progressive motor and cognitive decline, behavior changes, movement disorders, myoclonic jerks and different types of seizures were observed in clinical presentation of all patients. Two patients experienced *epilepsia partialis continua*, and one child had opsoclonus. In all patients, routine hematological, biochemical and acid-base profile, including renal function tests, liver enzymes, lactic acid, ammonia and creatine-kinase, and the concentration of immunoglobulin in blood were normal, so neurometabolic encephalopathy was excluded. Serological analyzes for HIV were negative. Interictal EEG showed slow background activity in all the cases, with epileptic discharges,

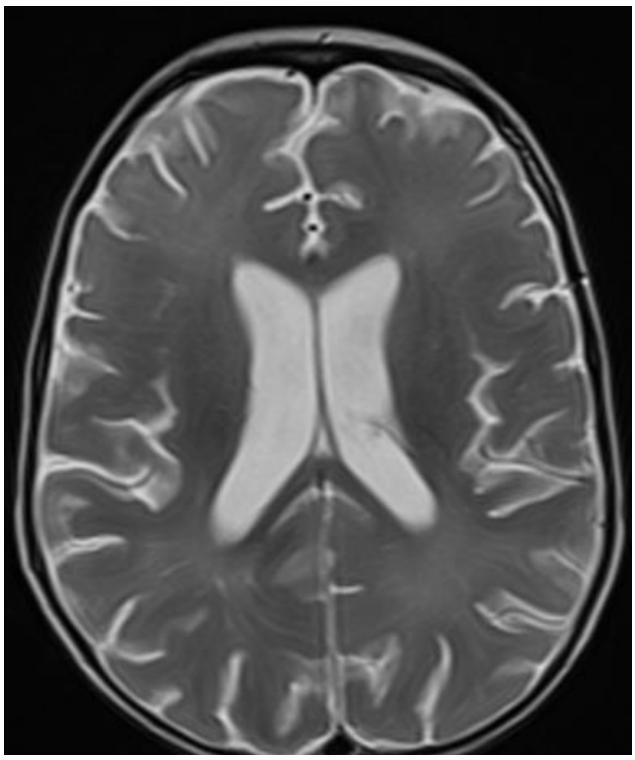

Figure 1. Brain MR in fulminant SSPE patient showed diffuse white matter T2W/FLAIR hypersignal abnormalites with supratentorial atrophy and ex vacuo ventriculomegaly

and periodic pattern in three patients. Brain MR revealed no abnormalities in two cases, diffuse white meter hypersignal in two cases, and brain MR was not available in one case. The clinical presentation and MR findings **(Figure 1)** were not consistent with the existence of leukodystrophy. In all the patients, IgG anti-measles antibodies in serum and CSF were detected by ELISA test, the index titer of IgG anti-measles antibodies in the serum vs. CSF is reduced, and oligoclonal bands were positive in CSF. IgM anti-measles antibodies were not detected in serum and CSF, and PCR test for measles was negative in serum and CSF. In all the patients, microbiological results were negative for bacterial agencies including tuberculosis, and PCR tests were negative for viruses (HSV, VZV, EBV, HIV) and Mycoplasma pneumoniae in both blood and CSF. Anti – NMDAR antibodies were negative in serum in two tested patients. The treatment of SSPE included:Isoprinosine 100mg/kg/day per os in all cases; Ribavirin at the dose of 10 mg/kg of body weight administered intravenously as a 30-min infusion three times a day for 7 days in three cases (patients 1, 2 and 3); intraventricular interferon IFN-α therapy (1 million IU three times a week (patients 4 and 5) and IFN-2α subcutaneous (70mcg/week) in patient 3. Despite the treatment, the course was extremely fulminant with lethal outcome within three months from the initial symptoms in four cases. Summarized results of all patients are presented in three tables. **Table 1** presents demographic and pre-morbidity features: sex (4 males, one female), perinatal history, and psychomotor development before SSPE onset, age and the course of measles infection, period from measles infection to SSPE onset, vaccination status, and immune-competence. The date from the table suggested that all the patients had normal perinatal history and early psychomotor development, with uncomplicated course of measles infection, and all of them were immune-competent. The age of measles infection was in range from 6 months to two years (mean age 12.5 months), while the onset of SSPE was in range from 2.5 to 16 years (mean age 8.1 years). Latency period from measles infection to SSPE onset was in range from 1.8 to 7 years (mean 4.32 years). **Table 2** shows that behavior changes, cognitive decline, dysarthria, ataxia, involuntary movements and seizures were the most common initial manifestations of the disease. The period from initial to full clinical presentation was very short, commonly about two months.

Table1. Demographic and pre-morbidity characteristics

Characteristics	Patient 1	Patient 2	Patient 3	Patient4	Patient 5
sex	male	female	male	male	male
age at admission (years)	2.6	8	3.2	16	7
calendar year at admission	2020.	2019.	2020.	2010.	2010.
perinatal history	normal	normal	normal	normal	normal
early psychomotor development	normal	normal	normal	normal	normal
age at measles infection (months)	8	12	6	no clear data	24
complicated course of measles infection	no	no	no	probably subclinical	no
age at SSPE onset (years)	2.5	8	3	16	7
latency period from measles infection to SSPE onset (years)	1.8	7	2.5	no clear data	6
age of MMR vaccination (months)	15	16	15	not vaccinated	not vaccinated
immune-competent	yes	yes	yes	yes	yes

Table 2. Clinical characteristics

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Initial manifestations	gait instability, speech difficulties, confusion, myoclonic jerks, seizures	behavior abnormalities, insomnia, ataxia, dysarthria	seizures, ataxia, weakness, lack of sphincters control, sleepiness, anxious when awake	ocular pain, cognitive dysfunctions, depression, behavioral problems	anxious, fears, language and behavior problems, drop attacks
Period from initial to full clinical presentation	2 months	3 months	2 months	2 months	2 months
Movement disorders	myoclonus, dystonia	myoclonus	myoclonus, dystonia, eyes deviation, opsoclonus	dystonia, decerebration, eyes deviation	tremor, myoclonus
Bulbar palsy development	within 3 months	within 2 months	within 1.5 months	within 2 months	within 6 weeks
Clinical finding on admission in our clinic	GCS 15, dysarthria, understanding the tasks, spastic hemiparesis, dystonia, tremor, Babinski's sign and clonus bilaterally, myoclonus, drop attacks, unable to walk	GCS 15, not following the gaze, horizontal nystagmus, mute, spastic quadriparesis, dystonic posture, unable to walk	GCS 15, not following the gaze, unable to sit and stand without support, dysarthria, spasticity, dystonia, flexion plantar response	GCS 8, no grimaces, spastic quadriparesis, decerebration position, no sitting and walking	bradykinesia, ataxia, tremor and myoclonic jerks
Types of seizures	myoclonus, atonic, atypical absence, GTC	myoclonic, atonic, GTC	myoclonic, atonic	episodes of focal onset clonic	atonic, focal onset nonmotor
Frequency of seizures	daily	daily	no seizure after admission	stopped by midazolam	stopped by midazolam
Continuous AED	valproate, levetiracetam, clonazepam	valproate, clobasm, clonazepam, topiramate	no	no	no

GTC – generalized tonic-clonic; AED – antiepileptic drugs

The table shows the type of movement disorders, the period within which bulbar palsy was developed, clinical findings at the admission to our clinic, the types and frequency of seizures and used antiepileptic drugs. **Table 3** presents brain MR finding, and the period from disease onset to brain MR, EEG features (background activity, epileptic discharges and periodical patterns), anti-measles antibodies in serum and CSF. All patients had positive oligoclonal bands and anti-measles IgG antibodies in serum and CSF, while both, IgM antibodies and PCR tests for measles were negative. There is no data about maternal vaccination status except for the mother of patient number three who had been vaccinated as a child, while none of the mothers had been revaccinated.

DISCUSSION

SSPE is a devastating disease and has been a big medical challenge since the disease has very poor prognosis and the treatment is of limited efficacy. The clinical course of typical SSPE is subdivided into four stages, unrecognized in fulminant forms (4). In fulminant SSPE cases, the course of disease is rapid with lethal outcome within a few months, even faster (8), as we showed in our group of patients. There is no data which prove that the children whose mothers have been vaccinated have more severe clinical presentation and earlier onset of disease, as we showed in our cases. Measles vaccination is supposed to be the best way of prevention and eradication of SSPE, and it is very important to increase awareness in population. In the post-vaccination era, measles virus infection in developed countries has drastically decreased, but anti-vaccination activity has led to measles epidemic, such as the one we experienced three years ago. The risk of SSPE is higher among persons infected with measles at an early age, especially in babyhood (9,10). According to the literature data, SSPE usually occurs 7–10 years after measles infection, but the latency varies from 1 month to 27 years, with shorter latency in children affected by measles at an earlier (<2 years) age (2). In our cases the range of latency was from 22 months to 7 years. All of our cases had a higher risk for SSPE since they suffered measles during babyhood, and in two of them, a fulminant SSPE started at a very early age, about three years of age.

Table 3. Brain magnetic resonance findings, laboratory results of blood and CSF analyzes

Analysis	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Brain MR	normal	normal	extensive white matter T2W/FLAIR hypersignal abnormalities with supratentorial atrophy and ex vacuo ventriculomegaly	diffuse white matter T2W/ FLAIR hypersignal abnormalities	not available
Period from initial symptoms to magnetic resonance	one month	two months	three months	one month	not available
EEG (from the onset to EEG)	one month	two months	one month	two months	one month
Background activity	slow	slow	slow	slow	slow
Epileptic discharges	generalized	generalized	no	no	yes
Periodic complexes	no	yes	yes	no	yes
Anti-measles antibodies					
Serum IgM	negative	negative	negative	negative	negative
CSF IgM	negative	negative	negative	negative	negative
Serum IgG	1:320	positive	1:320	1:320	1:640
CSF IgG	1:80	positive	1:5	1:40	1:32
PCR measles serum and CSF	negative	negative	negative	negative	negative
Oligoclonal bands	positive	positive	positive	positive	positive
Anti – NMDAR antibodies	negative	not done	negative	not done	not done

NMDAR - N-Methyl-D-Aspartate Receptor

Most infants are born immune to measles due to maternal antibodies transferred during pregnancy, regardless whether the mother had natural measles infection or was vaccinated. Measles antibody titers are lower in regularly vaccinated women than in those who had natural measles infection. So, the infants of vaccinated mothers have poorer protection, since they have lower titer and shorter duration of maternal anti-measles antibodies. In our last patient who got infected with measles at the age of six months, mother had the evidence that she had been regularly vaccinated. This contributes to the attitude that the infants of vaccinated mother are often left without transplacentally acquired measles antibodies before the age of one, even earlier, especially in preterm infants. Gestational age of the infant has an impact on the amount of transplacentally transferred antibodies, suggesting that premature infants receive lower titers of maternal antibodies (11,12). In none of our cases prematurity was a risk factor, and the mother of the last patient had data on regular anti-measles vaccination. A longitudinal prospective study reported progressive percentage decreasing of protected infants born to vaccinated mothers, from 69.6% at birth to 3.2% at six months and 0% at nine and twelve months (13). Low protection at the age of six months in our third case resulted by measles infection during measles epidemic in our country. According to these data, it is supposed that infants are vulnerable to measles infection in the period before regular vaccination schedule which included MMR vaccination between the age of 12 and 15 months. In addition to previous published SSPE cases, the data of our patients also support considering earlier measles vaccination compared to the current schedule, especially among premature infants and other infants of vaccinated mothers. There is a publication of SSPE in an HIV infected child, but none of our patients had inherited or acquired immunodeficiency (14).

The other observation from our practice and literature data is an increasing number of fulminant SSPE form in children. Retrospective evaluation of the total number of treated patients with SSPE at our Institute in the past ten years, suggested that all of them had fulminant course of the disease. Two of them, with *epilepsia partialis continua*, have already been published (4). Clinical presentation at the early stage of fulminant SSPE might be similar to the one in children with immune mediated encephalitis. Although immune mediated encephalitis is more frequent, SSPE has to be considered if the course is progressively deteriorating, in addition to the absence of certain antibodies and unresponsiveness to immune suppressive and immunomodulatory treatment. We suggest considering SSPE in all children with progressive neurological and cognitive deterioration, associated with movement disorders such as dystonia, insomnia, behavior problems and *de novo* epileptic events, even if the progression of deterioration is very fast. SSPE has to be evaluated in differential diagnosis of progressive deterioration in children, especially because valuable diagnostic procedures, such as serological CSF analyses, brain MR and EEG are non-invasive and available in most of the children hospitals. The observation in our cases is that brain MR in fulminant SSPE might be normal even in the stage when the patients are severely neurologically deteriorated. The other observation in our patients is that the stage with myoclonic jerks and atonic events associated with "Ra-

demecker's complexes", pathognomonic for SSPE, lasted only for a few weeks, and might be absent in some cases. We found pathognomonic EEG finding in three cases, in one short period of time. In all cases, EEG background activity was slow, even at the early stage of disease within the first month of the clinical onset. The treatment of epileptic seizures was challengeable in two of our patients and different antiepileptic drugs were given, especially at the onset of the disease.

CONCLUSION

In conclusion, five patients were reported, including three vaccinated children with fulminant SSPE associated with measles infection during babyhood. We pointed that scheduled measles vaccination of children contributed to a decrease in frequency but not to a complete eradication of SSPE. The explanation for this in our patients is that the children had measles before the scheduled vaccination.

The authors of this manuscript suggest two major issues. One is that in aiming to eradicate SSPE, we suggest considering weaning of vaccine-derived immunity, and re-vaccination of girls at reproductive period. The other issue is an increasing number of fulminant cases related to typical SSPE presentations and it might suggest upcoming changes in disease phenotype.

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SUBAKUTNI SKLEROZIRAJUĆI PANENCEFALITIS - PROMENE FENOTIPA TOKOM POSLEDNJE DECENIJE

Ruzica Kravljanac[1,2], Ilija Palic[3], Biljana Vucetic Tadic[1,2]

Sažetak

Uvod: Subakutni sklerozirajući panencefalitis (SSPE) je retka, progresivna, neurodegenerativna bolest sa lošim ishodom. Vakcinacija protiv malih boginja doprinela je smanjenju broja pacijenata sa SSPE, ali ne i eradikaciji. Cilj naše studije je da procenimo tok bolesti kod naših pacijenata sa SSPE sa fokusom na vakcinisanu decu. Osnovni cilj je sagledavanje mogućnosti za unapređenje prevencije bolesti.

Metod: Retrospektivnom studijom obuhvaćeni su pacijenti sa SSPE lečeni u periodu od decembra 2010. do decembra 2020. godine na Pedijatrijskoj klinici u Institutu. Kriterijumi za uključivanje su pacijenti sa dijagnozom SSPE na osnovu kliničke slike, neuromidžinga, elektroencefalografije i pozitivnih IgG antitela protiv morbila, kako u serumu, tako i u likvoru.

Rezultati: Uključeno je petoro dece sa fulminantnim tokom SSPE. Svi pacijenti su u detinjstvu bolovali od morbila. Troje ih je vakcinisano protiv morbila, dok dvoje nije vakcinisano. Radi se o zdravoj deci, bez poremećaja imuniteta i normalnog globalnog razvoja. Tok je bio izuzetno fulminantan, sa smrtnim ishodom u roku od tri meseca od početnih simptoma u četiri slučaja. U kliničkoj prezentaciji su dominirali progresivna regresija u motoričkom i kognitivnom razvoju, promene ponašanja, poremećaji pokreta, miokloničnі trzaji i napadi.

Zaključak: Uprkos sprovođenju vakcinacije, SSPE nije nestao. Sve veći broj vakcinisane imunokompetentne dece sa fulminantnim oblikom SSPE i anamnezom infekcije morbila u detinjstvu lečen je u našoj Klinici. Kao meru za poboljšanje prevencije, predlažemo da se razmotri mogućnost smanjenja imunskog odgovora nastalog vakcinom i revakcinacija devojčica u fertilnom periodu.

Ključne reči: fulminantni SSPE, vakcinacija, morbili, deca

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ORIGINAL ARTICLE

Trends in diagnostics and treatment of congenital adrenal hyperplasia

Jana Milenković[1], Tatjana Milenković[2], Katarina Sedlecki[2], Predrag Ilić[1,2], Vladimir Kojović[1,2], Jelena Martić[1,2], Katarina Mitrović[1,2], Slađana Todorović[2], Marko Marjanović[2], Jovana Tončev[2], Sanja Panić-Zarić[2], Danilo Pešić[1,3], Rade Vuković[1,2]

[1] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[2] Institute for Mother and Child Health Care of Serbia „Dr Vukan Cupic", Belgrade, Serbia

[3] Institute of Mental Health, Belgrade, Serbia

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Correspondence to:
Rade Vukovic, Institute for Mother and Child Health Care of Serbia „Dr Vukan Cupic", Belgrade, Serbia; email: radevukovic9@gmail.com.

Summary

Introduction: Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases caused by a deficiency of enzymes responsible for the steroidogenesis. There are three forms of CAH due to 21-hydroxylase deficiency: the classic form with salt loss, the classic virilizing and the non-classic form. The aim of the paper was to analyze the changes in the diagnosis and treatment of children with CAH during previous 15 years.

Material and methods: This retrospective cohort study includes patients who were diagnosed with CAH due to 21-hydroxylase deficiency in the period from 2007 to 2021 in endocrinology department of the Institute for Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic". Respondents were divided into two groups - a group whose diagnosis was made in the period between 2007 and 2014 and another group of those whose diagnosis was made in the period between 2015 and 2021. Statistical analysis using Hi-square and Mann Whitney U test was conducted using the software IBM SPSS ver. 22, and p values <0.05 were considered significant.

Results: Out of the total of 55 patients included in the study, 46 patients (83.6%) had 46, XX karyotype. The diagnosis was made in all patients on the basis of biochemical analyzes. In the second group the diagnosis was confirmed by genetic analysis in statistically significantly higher number of children (p <0.05). 49 patients (89.1%) received hydrocortisone and 16 patients received fludrocortisone. In patients from the second group a statistically significantly higher frequency of fludrocortisone therapy was noticed in patients with classic CAH.

Conclusion: Having in mind the constant advancement in the field, frequent improvements in clinical care of children with CAH are needed.

Keywords: congenital adrenal hyperplasia, recommendations, diagnosis, treatment

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive diseases caused by the disruption of enzymes responsible for cortisol synthesis (1). The most common cause is 21-hydroxylase deficiency (95-99% of patients), which is responsible for the conversion of 17-OH progesterone (17-OHP) into 11-deoxycortisol and progesterone into 11-deoxycorticosterone, which are precursors of cortisol and aldosterone (2). As a result of the block in steroidogenesis, there is an accumulation of 17-OHP, which is translated into androgens by three different enzymes, the most significant of which in patients with CAH is the alternative pathway, which is under control of 5α-reductase and 3α-reductase (3). There are three forms of the disease, the classic form with salt loss, the classic virilizing form and the non-classic form of CAH. In 75% of cases of the classic form of CAH, the enzyme 21-hydroxylase is completely inactive and there is a complete deficit of cortisol and aldosterone, which can lead to a salt-wasting crisis, and this is why this form was named salt wasting form -SW. In female newborns ambivalent genitalia are commonly noticed at birth, and in male newborns the disease is most often manifested by a salt-wasting crisis. This metabolic crisis most commonly occurs during the second or third week of life, presenting with vomiting, hyponatremia, hyperkalemia, dehydration, and shock. In the remaining 25% of patients the activity of the enzyme is reduced to 1-5%, enabling the synthesis of aldosterone and preventing salt wasting, which is why this is virilizing form of CAH (simple virilizing form -SV). The main manifestations of this form of CAH in female children are the ambivalent genitalia (in varying degrees), clitoromegaly, posterior labial fusion or hirsutism, and precocious puberty occurs in male children (4). The third form of the disease is non-classic KAH in which enzyme activity is reduced to 20-60%, which is why symptoms appear later, most often in adolescence. The disease is manifested by precocious pubarche, oligomenorrhea, hirsutism or reduced fertility (2).

What is common to all three forms of CAH is an elevated value of 17-OHP. In most patients with the classic form of CAH, the level of 17-OHP in the blood is over 30nmol/L, while the gold standard for diagnosis is The Short Synacthen Test. It is based on the application of synthetic ACTH and the measurement of the increase in the concentration of cortisol and 17-OHP after 30 and 60 minutes after the application of ACTH (3). The value of 17-OHP below 2.5 nmol/L excludes the diagnosis of CAH (5). It should be kept in mind that the level of 17OHP is high even in healthy newborns during the first 48 hours after birth, while after that the value drops in children with normal cortisol synthesis (6). Preterm children, children with infection or under stress have higher levels of 17OHP than healthy term children, therefore there are more false positive results (7).

In case of the classic form of CAH in female newborns, the diagnosis is usually made at birth due to the existence of ambivalent genitalia. The finding of ambivalent genitalia is described using the "External genitalia score" (EGS) or the Prader scale. EGS represents a modification "External masculinization score", which was introduced into literature in order to universally describe the finding of ambivalent genitalia in children with gender differentiation disorders. Within this score, 5 characteristics are described (labioscrotal fusion, length of the phallus, position of the opening of the urethra, positions of the right and left gonads) and the total score ranges from 0-12. The EGS value for the usual appearance of male genitalia is 12, while for female genitalia the score is 0. The Prader scale is used to describe the genitalia in female children with congenital adrenal hyperplasia and the values on this scale range from 1 (phenotypically female genitalia with clitoromegaly) to 5 (phenotypically male genitalia with nonpalpable gonads) (8).

In male newborns, the first symptom of the disease is often an adrenal crisis with salt loss. This metabolic disorder is most often seen in male infants with the most severe form of CAH, and at a later age it can be caused by infections or surgical interventions. It is manifested by hypotension, vomiting and electrolyte disturbances (hyponatremia and hyperkalemia). Unless this condition is promptly treated with parenteral administration of hydrocortisone and correction of hyponatremia and hypovolemia, it can lead to a fatal outcome. Due to the fact that infections are the most common causes of adrenal crisis, increasing hydrocortisone dose during intercurrent infections (''stress-dosing'') is recommended for all children with CAH.

The non-classic form of CAH has its own specificities in terms of clinical picture, diagnosis and therapy. The most common symptom in children under 10 years of age is premature pubarche and growth acceleration while in older girls irregular periods appear as the most common symptom (56% of patients), acne, hirsutism, clitoromegaly, irregular periods or primary amenorrhea, and later fertility problems. An important difference compared to the classical type of the disease is that there is no genital virilization in female newborns. The Short Synacthen Test is also used in the diagnosis of non-classic CAH, where a basal level of 17OHP above 7 nmol/L together with a level of 17-OHP above 30 nmol/L after ACTH administration is considered sufficient to make the diagnosis of non-classic CAH. The therapy of choice in pediatric age is hydrocortisone, most often in a dose of 10-15mg/m2 (5).

The therapeutic goal in patients with CAH is to compensate for cortisol, normalize androgen levels, preserve growth and fertility potential, and avoid complications (2). Hydrocortisone is the drug of choice for glucocorticoid replacement. The latest guidelines recommend hydrocortisone tablets at a dose of 10-15mg/m2 in growing children and 15-25mg in adults, divided into several doses during the day (10). In recent guidelines, fludrocorti-

sone therapy is recommended for all patients with classic CAH, including the simple virilizing (SV) form. Recommended doses are 0.05-0. 2mg per day. In addition to this, the use of sodium chloride is also recommended in infants for salt replacement, and the recommended dose is 1 mmol/kg per day. The guidelines do not recommend increasing the dose of therapy in case of daily emotional stress as well as minor infections (11).

Regarding the timing of genital surgery in CAH, there are still no sufficient data regarding the long term outcomes or randomized controlled studies of either the best age or the best methods for restoring functional female anatomy in virilized girls with CAH. However, during recent years there has been a shift towards later age at surgery in many centers, balancing the benefits and potential harms of early surgery.

Congenital adrenal hyperplasia is a complex disease with evolving diagnostic and management protocols. The aim of the present paper is to analyze the changes in the diagnostic and therapeutic approach to children with CAH in a tertiary center during the previous 15 years.

THE MATERIAL AND METHODS

In this retrospective cohort study, a total of 55 children diagnosed with congenital adrenal hyperplasia due to 21-hydroxylase deficiency were examined. The data were collected from the database of the endocrinology department of the Institute for Mother and Child Health Care of Serbia, "Dr. Vukan Čupić", and the study included patients who were diagnosed in the period from 2007 to 2021. The first group of respondents includes patients whose diagnosis was made in the period between 2007 and 2014, and the second group of respondents includes patients who were diagnosed with CAH in the period between 2015 and 2021.

The data that were analyzed included the diagnosis, the first symptoms, biochemical analyses at the time of diagnosis, the data regarding genetic findings and the timing of the first genital surgery in patients who had undergone the intervention, the medical treatment that was applied and the occurrence of adverse disease outcomes, including salt-wasting crisis and death. Statistical analysis of variables between the two groups of subjects was performed using Chi-square and the Mann Whitney U test was performed using the software *IBM SPSS ver. 22*, and p values <0.05 were considered significant.

RESULTS

In total, 55 patients were included in this study, of which 38 (69%) were patients from the first group, and 17 (31%) belonged to the second group. The most common type of disease was the non-classic form (NC) of CAH in 32 patients (58.2%), while the classic form with salt loss (SW) was diagnosed in 15 patients (27.3%), whereas the classic virilizing form (SV) was found in 8 patients (14.5%). A total of 46 (83.6%) of all CAH patients had the female karyotype (46,XX), and 9 (16.4%) had the male karyotype (46,XY), with a decreasing proportion of 46,XY karyotype in SV and NC forms compared to subjects with SW form **(Figure 1)**. The karyotype was determined by the G-bending technique, at 32 metaphases. The average age at which the diagnosis was made was 7.1 years, and

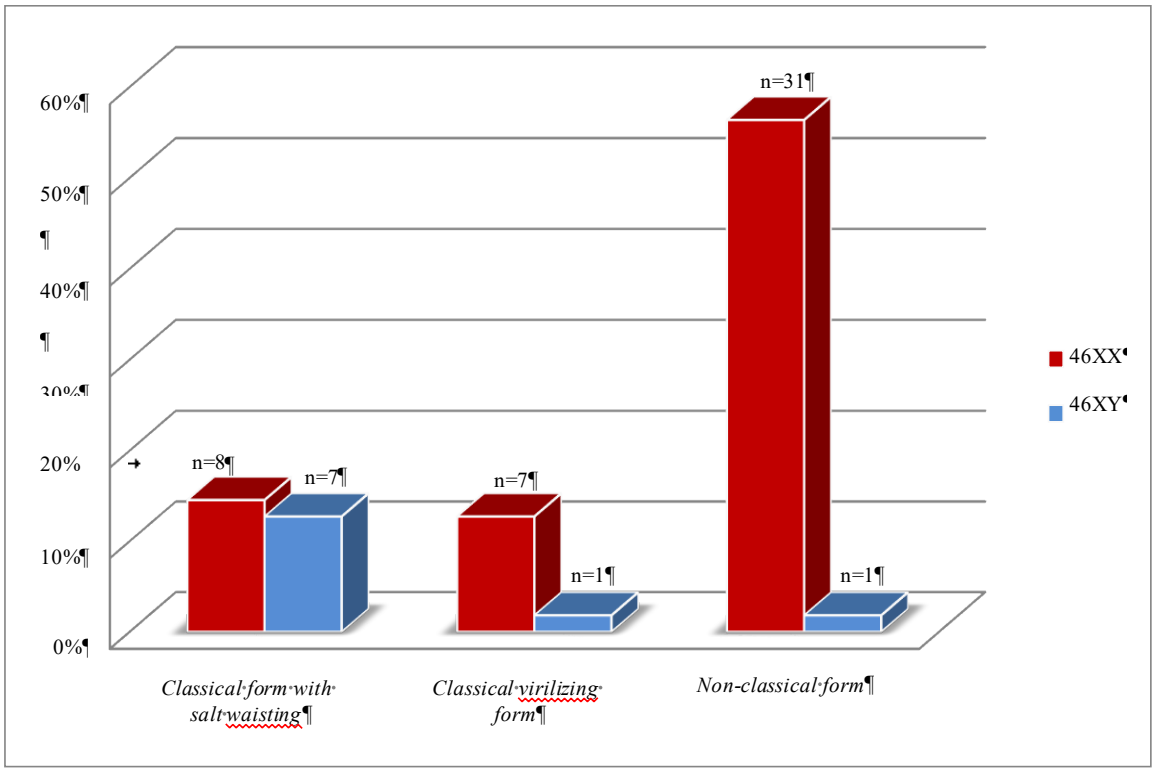

Figure 1. Frequency of CAH forms in children with female and male karyotype

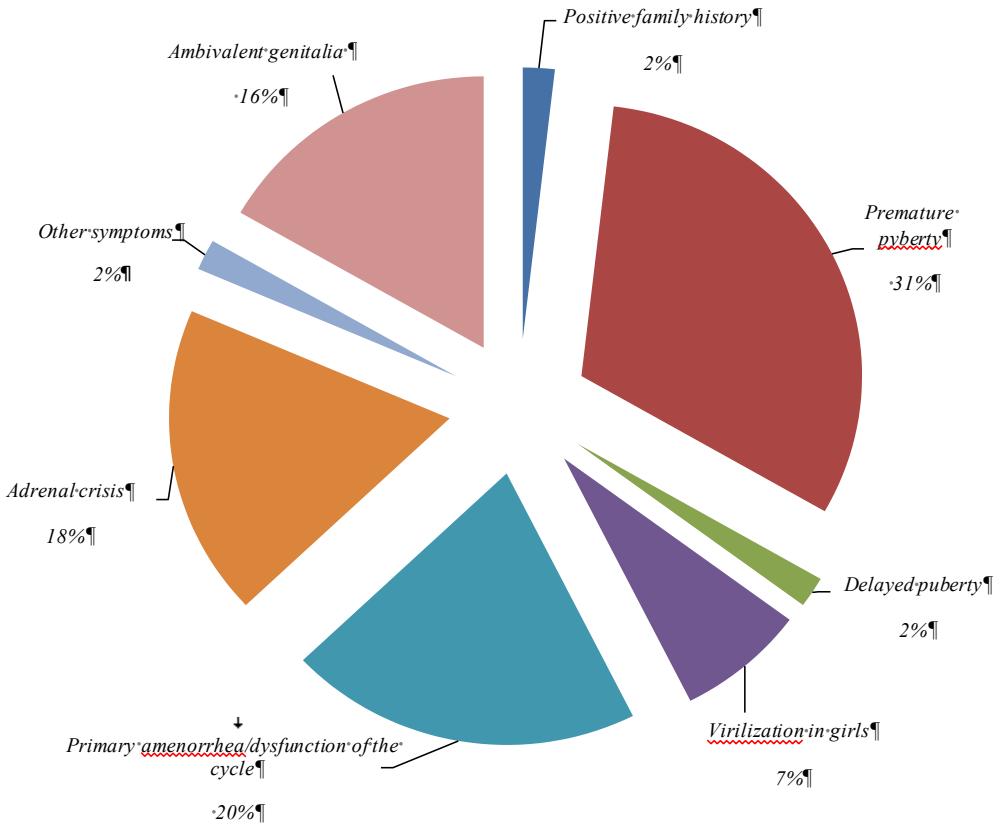

Figure 2. The first symptoms of the disease in children with congenital adrenal hyperplasia

in 22 patients (40%) the diagnosis was made during the first year of life. All patients were diagnosed on the basis of biochemical findings of elevated 17-OHP, while in 4 patients (7.3%) the diagnosis was confirmed by genetic analyses. A statistically significant difference ($p<0.05$) was observed regarding the percentage of patients from the first group whose diagnosis was confirmed by genetic analyzes (2.6%, n=1) compared to the number of patients from the second group whose diagnosis was confirmed by the existence of genetic mutations (17.6%, n=3).

In 10 patients, a salt loss crisis was the first recognized manifestation of the disease, and in the total of 15 patients (65.2% of all children with classic CAH) the salt loss syndrome developed before the definitive diagnosis of CAH. In addition, the most common first symptom of the disease was the appearance of signs of premature puberty in 17 patients, primary amenorrhea (or other disorders of the menstrual cycle) in 11 patients and ambivalent appearance of the external genitalia in 9 patients (**Figure 2**). The Prader scale and the External Genitalia Score (EGS) were used to describe genital masculinization in girls with classic CAH. The EGS values ranged from 0.5 to 11, and the values on the Prader scale from 0 to 4. The mean EGS value was 5.43 ± 4.48 and the Prader virilization score was 2.47 ± 1.73.

Of all patients, 6 patients with non-classic form of CAH did not receive any corticosteroid therapy. 49 patients (89.1%) received hydrocortisone, while 16 patients (29.1%) also received fludrocortisone. A statistically significant difference was noticed in the number of patients diagnosed with classic CAH (including both SW and SV forms) who received hydrocortisone and fludrocortisone when comparing the two groups of subjects ($p<0.05$). While 56.3% of children from the first group with classic CAH received fludrocortisone, all children from the second group with classic CAH received fludrocortisone.

Eight patients (14.5%) underwent genital surgery, with five patients belonging to the first group of subjects. Although the difference was not statistically significant, the average age at which genital surgery was performed was 9.6 months, while in the second group, the average age at the time of the first genital surgery was 2.2 years.

DISCUSSION

In the present study, a significantly higher proportion of subjects with CAH had 46,XX karyotype compared to the number of subjects with 46,XY karyotype. Considering that it is an autosomal recessive disease, the same risk for CAH is expected in children of both sexes. One of the possible explanations for this predominance of girls in children diagnosed with CAH is that in girls the early diagnosis is facilitated by the clinical finding of ambiguous genitalia at birth, while in boys the disease often manifests itself as an adrenal crisis which can have a fatal outcome before the diagnosis has even been made. This is exactly what reflects the importance of newborn screening. Currently neonatal screening for CAH is performed in more than 35 countries worldwide. The latest guidelines suggest double-check screening with an upper limit of 17 OHP adjusted for ges-

tational age along with birth weight. The first method used in screening for CAH is measuring the level of 17OHP by immunoassay method from blood on filter paper, which is used for screening other diseases. This method has a lot of false positive results, so liquid chromatography with mass spectrometry is used for the second screening method (7).

Genetic analyses represent an important factor when the next pregnancy is being planned as well as a possible prenatal therapy in the next pregnancy. Ten patients (18.2%) in our study had a positive family history. In patients belonging to the second group, a statistically significantly higher number of children whose analysis was confirmed by genetic analyses was observed compared to the number of patients from the first group who underwent genetic analyses, which is in accordance with the latest global recommendations. 21 hydroxylase deficiency is caused by mutations in the CYP21A2 gene, most often by intergenic recombination. 65-75% of patients with CAH are compound heterozygotes. Mutations are divided into 4 groups and each group is typical for a certain form of CAH. Group 0 is associated with the classic salt-wasting form, group A occurs in both classic types of the disease, group B in the classic virilizing form and group C in the non-classic form of CAH. The phenotype depends on the milder mutation (10).

Genital surgery is commonly used for restoring functional anatomy in female children with pronounced virilization of genitalia. Feminizing genitoplasty includes clitoroplasty, opening of the vaginal meatus if there is only an opening of the urogenital sinus, and labiaplasty. In our study, genital reconstructive surgery was performed in eight virilized female patients with CAH. There are still no clear recommendations about the ideal age for surgical intervention. In a study that processed data on patients who had been treated at the Institute for Mother and Child Health Care of Serbia with disorders of sexual differentiation of various causes, an older age at which the operation was performed was noticed, as well as a lower frequency of genital surgery as a treatment method in the last period (12). In patients who do not have a malformation in the form of the urogenital sinus but the vagina and bladder are normally developed, there are two approaches, earlier and later surgery. Earlier guidelines recommend surgery shortly after birth for tissue elasticity, reduction of parental stress due to ambivalent genitalia, and prevention of hydrometrocolpos (10). On the other side, the benefits of later surgery are the formation of the child's own opinion about the decision related to the surgery and the formation of gender identity. The most common complication that occurs after surgery is vaginal stenosis, while fistulas, urinary incontinence and infections occur less often (11). In our research, although statistical significance was not confirmed, a clear trend towards postponing genital surgery to a later age can be observed, in accordance with most world guidelines and trends in clinical practice.

Newer guidelines recommend the use of fludrocortisone in addition to hydrocortisone in all patients with the classic form, including SV without salt loss, because aldosterone replacement enables therapy with lower doses of hydrocortisone and thus reducing the side effects of corticosteroid use. Also, although the aldosterone deficit is clinically manifest only in the classic form of CAH with salt loss, even in the classic virilizing form of CAH there is a subclinical aldosterone deficit. Maintenance of electrolyte concentration is important due to euvolemia and reduction of vasopressin and ACTH secretion, which allows for lower doses of hydrocortisone. Therefore, fludrocortisone along with hydrocortisone is introduced as therapy in all patients with the classic form of CAH (11). In our study, a statistically significantly higher frequency of fludrocortisone administration was observed in patients from the second group with the classic form of CAH, in accordance with the latest international guidelines for the treatment of children with CAH.

CONCLUSION

Congenital adrenal hyperplasia is a condition that can be life-threating, and the outcome of which significantly depends on the moment of diagnosis. In present study, a significant difference was observed in the number of female and male children with CAH, which indicates a probable failure to establish a diagnosis in a significant number of boys with CAH. Timely diagnosis of CAH by means of newborn screening and early replacement therapy could prevent the occurrence of adrenal crisis, which can have a fatal outcome. Also, replacement therapy would allow for reduced testosterone production, which would suppress further masculinization of the genitals in female children. The importance of confirming the diagnosis with genetic analyses is observed when planning further offspring.

Performing genital surgery interventions at an older age allows parents, together with doctors, to monitor the child's psychological and gender development.

The observed changes in medical and surgical practice in our center during previous 15 years highlight the constant advancements in the field as well as the need for regular update of clinical practice protocols in the management of CAH.

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PROMENE U DIJAGNOSTICI I LEČENJU KONGENITALNE ADRENALNE HIPERPLAZIJE - ISKUSTVO TERCIJERNOG CENTRA

Jana Milenković[1], Tatjana Milenković[2], Katarina Sedlecki[2], Predrag Ilić[1,2], Vladimir Kojović[1,2], Jelena Martić[1,2], Katarina Mitrović[1,2], Slađana Todorović[2], Marko Marjanović[2], Jovana Tončev[2], Sanja Panić-Zarić[2], Danilo Pešić[1,3], Rade Vuković [1,2]

Sažetak

Uvod: Kongenitalna adrenalna hiperplazija (KAH) predstavlja skup autozomno recesivnih bolesti koje su prouzrokovane deficitom enzima odgovornih za sintezu hormona nadbubrežne žlezde. Postoje tri oblika KAH usled deficita 21-hidroksilaze: klasičan oblik sa gubitkom soli, klasičan virilizujući i neklasični oblik. Cilj ovog rada je analiza izmena u dijagnostici i terapiji dece sa KAH tokom prethodnih 15 godina.

Materijal i metode: U ovoj retrospektivnoj kohortnoj studiji izdvojeni su pacijenti kojima je u okviru rada službe za endokrinologiju Instituta za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić" utvrđena dijagnoza KAH usled deficita 21-hidroksilaze u periodu od 2007. do 2021. godine. Ispitanici su podeljeni u grupu čija je dijagnoza postavljena u periodu od 2007. do 2014. godine i drugu grupu onih kojima je dijagnoza postavljena od 2015. do 2021. godine. Statistička analiza upotrebom Hi-kvadrat i Mann Whitney U testa je sprovedena upotrebom softvera IBM SPSS ver. 22, a značajnim su smatrane p vrednosti <0,05.

Rezultati: Od ukupno 55 pacijenata uključenih u istraživanje, 46 pacijenata (83,6%) imalo je 46, XX kariotip. Dijagnoza je kod svih pacijenata postavljena na osnovu biohemijskih analiza, a u drugoj grupi pacijenata je dijagnoza potvrđena genetičkim analizama kod statistički značajno većeg broja dece (p<0,05). 49 pacijenata (89,1%) je primalo terapiju hidrokortizonom, a 16 pacijenata je dobijalo fludrokortizon. Kod pacijenata iz druge grupe uočena je statistički značajno češća učestalost primene fludrokortizona kod pacijenata sa klasičnim oblikom KAH.

Zaključak: Imajući u vidu konstantan napredak na ovom polju, potrebna su stalna unapređenja u dijagnostici i lečenju dece sa KAH.

Ključne reči: kongenitalna adrenalna hiperplazija, preporuke, dijagnostika, lečenje

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ORIGINAL ARTICLE

The impact of the covid-19 pandemic and social isolation on behavior and mental health of medical and non-medical staff – experience from a gynecology and obstetrics clinic

Radomir Aničić[1,2], Aleksandar Rakić[2], Dragutin Sretenović[2], Jovana Kocić[2], Jelena Štulić[2], Aleksandar Ristić[1,2], Dejan Dimitrijević[1,2], Lazar Nejković[1,2]

[1] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[2] The Obstetrics and Gynecology Clinic Narodni Front, Belgrade, Serbia

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Correspondence to:
Radomir Aničić; University of Belgrade, Faculty of Medicine, Belgrade, Serbia; The Obstetrics and Gynecology Clinic Narodni Front, 62, Kraljice Natalije Street, 11000 Belgrade, Serbia

radomir.anicic@gmail.com

Summary

Introduction/Aim: Occupational exposure makes health workers m vulnerable and at high-risk for COVID-19 infection and major psychological disturbance. Fear from the unknown, anxiety for close family and friends, rapid exhaustion of protective equipment, direct contact with infected patients, in combination with media-provoked panic, create a considerable psychological burden in healthcare workers. The aim of this study was to assess mental health of medical and non-medical staff of a university gynecology and obstetrics clinic during COVID-19 epidemic in Serbia.

Methods: The study was conducted from 1st to 31st of May 2020 through 160 online questionnaires distributed among the staff of Obstetrics and Gynecology Clinic Narodni front. This online survey consisted of two sections: one included questions related to demographic characteristics, medical history, behavior and habits during the COVID-19 pandemic, while the other comprised questions included in Depression, Anxiety and Stress Scale 21 (DASS-21).

Results: Among 118 employees who had participated in the study, depression, anxiety, and stress were present in 35.6%, 40.7%, and 27.1% participants. Participants with lower education had higher total DASS, depression, anxiety, and stress scores compared to participants with higher education. Non-medical staff had significantly higher total DASS and anxiety scores than medical staff. Participants with lower education and married subjects were more likely to have anxiety and depression symptoms.

Conclusion: Apart from medical staff, non-medical personnel and their mental status should not be neglected, and we believe that future studies related to the psychological impact of public health emergencies, should include this group.

Key words: COVID-19, medical staff, non-medical staff, obstetrics and gynecology, psychological impact

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INTRODUCTION

Back in December 2019, the history of the world as we know it was rewritten. What started as a cluster of unknown-cause pneumonia cases in Wuhan, Hubei Province, People's Republic of China, led to the World Health Organization (WHO) declaration of the worldwide coronavirus disease 19 (COVID-19) pandemic caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on March 11th, 2020 (1). The first COVID-19 positive patient in The Republic of Serbia was registered on March 6th, 2020. This was one of the reasons why the national borders of the Republic of Serbia were among the last to close. Many Serbian citizens, working and staying abroad, had to return to their home country. According to national news reports, by Easter holidays, over 340 000 people had arrived in Serbia in a short period of time, most of them coming from countries which were already experiencing major COVID-19 outbreaks. This, along with sparse knowledge of the illness itself, represented a challenge for healthcare institutions. One year later, by March 20th, 2021, there were 546 986 registered COVID-19 cases, with 4900 deaths in the Republic of Serbia (2). The Ministry of Health of the Republic of Serbia designated certain institutions to become COVID-19 centers. In the original decision of the Ministry of Health, two university clinics were envisaged to treat all gynecological and obstetric patients with suspicion of COVID-19 or confirmed COVID-19 disease. From the beginning, recommended epidemiological measures were introduced to protect patients, along with medical and non-medical staff. The regular program was put on hold and only the patients with urgent obstetrical and gynecological conditions were treated surgically. Admission was limited exclusively to emergency cases. Even with the implementation of the prescribed measures, many cases of infection were registered among the staff.

Since the beginning of the pandemic, much effort has been put into achieving the optimal number of health workers, medical devices, and safety equipment supplies in institutions and departments for the treatment of patients with confirmed COVID 19 disease (3). Apart from comprehensive research focused on disease transmission and the mechanisms underlying its short and long-term effects, there has been an urge for a detailed investigation of its psychological effects, especially on healthcare workers (4). Substantial symptoms of anxiety, stress, and depression in healthcare workers had been previously reported in similar viral outbreaks (5,6)and 20 of 218 health care workers (9 percent. Based on previous experience, there was a prompt reaction in conducting similar studies in the current COVID-19 pandemic (7)anxiety, depression, and stress during the initial stage of the COVID-19 outbreak. The data will be used for future reference. Methods: From 31 January to 2 February 2020, we conducted an online survey using snowball sampling techniques. The online survey collected information on demographic data, physical symptoms in the past 14 days, contact history with COVID-19, knowledge and concerns about COVID-19, precautionary measures against COVID-19, and additional information required with respect to COVID-19. Psychological impact was assessed by the Impact of Event Scale-Revised (IES-R. Occupational exposure makes health workers especially vulnerable and at high risk, not only for COVID 19 infection but for major psychological disturbance as well. Reports indicate a more frequent occurrence of anxiety, sleep disturbance, and somatic symptoms manifested during the COVID 19 pandemic among healthcare workers compared to other professions (8)as declared on March 11th 2020 by the World Health Organization, with respect to which institutional variables might distinguish the impact of COVID-19 in medical and non-medical professionals. Methods: A cross-sectional study was performed nationwide between 16th March and the 26th April 2020 in Poland. A total of 2039 respondents representing all healthcare providers (59.8%. Fear of the unknown, fear for close family and friends, rapid exhaustion of personnel protection equipment, direct contact with infected patients, combined with newly created, media-encouraged panic, caused an immersive psychological burden for healthcare workers.

On the other hand, the relationship between SARS Cov-2 and pregnancy remains a highly controversial subject. Firstly, there were concerns regarding prognoses of pregnant patients infected with the virus. Secondly, an increasing number of studies investigate possible vertical transmission of the novel coronavirus and its prospective effects on fetal growth, malformations, and miscarriages (9,10). Since there is a high probability that the answers to these questions will not get a universal consensus, gynecologists and obstetricians remain medical specialists with even more responsibility and, consequently, more psychological pressure.

Understanding the importance of psychological well-being of both medical and non-medical personnel, we tried to assess the impact of the ongoing pandemic and social isolation on mental health of the employees of a University Obstetrics and Gynecology Clinic in Serbia.

MATERIALS AND METHODS

Participants and questionnaire

This cross-sectional study was conducted in the period from 1st to 31st of May 2020. 160 online questionnaires were distributed to the employees of the University Obstetrics and Gynecology Clinic. The survey was distributed after the first peak of the outbreak had ended and after the majority of staff who had been affected by the virus had recovered.

This study was approved by the Ethics Committee of the Obstetrics and Gynecology Clinic Narodni Front (number 05006-2020-8351). The Ethics Committee of

the Clinic decided that the question "Have you had the SARS CoV-2 infection?" was to be retracted.

The questionnaire contained two parts: the first part had 35 questions regarding demographic characteristics (age, sex, marital status, educational level, being a member of medical or non-medical staff), medical history (presence of any chronic diseases), behavior and habits during the COVID 19 pandemic; the second part comprised questions included in Depression, Anxiety and Stress Scale 21 (DASS 21).

Depression, anxiety, and stress assessment

Depression, anxiety, and stress were assessed using DASS-21. This scale contains 21 questions divided into 3 subscales (subscale of depression, anxiety, and stress), each composed of 7 items. In every question, the participants were asked to assess how well they agreed with the statement during the first peak of the COVID 19 pandemic, by choosing one number on an ordinal scale (0 – "I never felt that way", 3 – "I felt that way almost all the time"). Depression, anxiety, and stress scores were calculated as a sum of the value of the questions in each subscale, and multiplied by two. Based on the scores, the participants were divided into five categories („normal", „mild", „moderate", „severe", „extremely severe") in each subscale. DASS-21 was already used to assess the psychological disturbance among medical staff during COVID 19 and previous pandemics (11,12). Moreover, this scale was also used to assess the mental health status of the adult population in Serbia during this pandemic (13).

Statistical analysis

Numerical data were presented as means with standard deviations or as median with ranges. Categorical variables were summarized by absolute numbers with percentages. Differences in DASS-21 scores among different demographic groups and between medical and non-medical staff were calculated using the Student's t-test or Mann-Whitney U test, based on the normality of the distribution. The normality was assessed using the Shapiro-Wilk test. Pearson's chi-squared test was used to determine the differences in frequency distributions for categorical variables between different groups. Regression models were used to assess predictors of depression, anxiety, and stress, such as sociodemographic characteristics, medical and non-medical staff, and behavior and habits. p-value < 0.05 was considered statistically significant. Statistical analysis was performed using jamovi version 1.6 (13).

RESULTS

Out of 160 initially randomly selected employees, 43 respondents either refused to participate in the study or completed their questionnaire incorrectly or incompletely. A total of 118 employees participated in the study: 81 were medical staff (doctors and nurses) and 36 were non-medical staff (human recourses department, janitors, cleaners). More than 80% of participants were female. 55% of the participants were older than 40. Most of the younger employees belonged to the medical staff. More than half of the respondents were married. 30% of the participants were smokers and 48 participants were diagnosed with some chronic disease. The demographic and medical characteristics of the participants are presented in **Table 1**.

Behavior and habits of medical and non-medical staff during the COVID-19 pandemic are presented in Table 2. Overall, more participants had fear for their family and close friends (79.7%) than for themselves (51.7%). Difficulty with concentrating and sleeping were present in 28% and 40.7% of the participants, respectively. There were no statistically significant differences between behavior and habits between males and females, older and younger participants, participants with lower and higher educational levels, and medical and non-medical staff.

More than half of the participants reported avoiding information about COVID 19, while an increase in activities such as watching TV, reading books or magazines, and using social networks was reported in 57.6%, 59.3%, and 59.3% of the participants, respectively. There were no significant differences in behavior and habits between groups **(Table 2)**.

Based on DASS-21 scores, depression, anxiety, and stress were present in 42 (35.6%), 48 (40.7%), and 32 (27.1%) participants, respectively **(Table 3)**.

Anxiety and depression were significantly more frequent in participants with lower education compared to the participants with higher education (p = 0.01; p = 0.03; **Table 3**).

Participants with lower education had significantly higher total DAS-21, depression, anxiety, and stress scores compared to participants with higher education **(Table 4)**. On the other hand, non-medical staff had significantly higher total DASS-21 and anxiety scores than medical staff **(Table 4)**.

There were no significant differences in depression, anxiety, and stress types between genders, age groups, married and unmarried participants, participants with higher and lower education, and medical and non-medical staff.

Regression analysis revealed that participants with lower education were more than twice as likely to have anxiety symptoms (Odds Ratio (OR): 2.58, 95% Confidence Interval (CI): 1.21 – 5.4; p = 0.01; **Table 5**.) than participants with higher education. Participants with lower education were also more than twice as likely to have depression symptoms compared to particpants with higher education (OR: 2.25, 95% CI: 1.04 – 4.86; p = 0.03; **Table 5**.). Married subjects were more likely

Table 1. Demographic and medical characteristics of the respondents

		n (%)	Medical staff n=82 (%)	Non-medical staff n=36 (%)
Gender	Male	25 (21.2%)	13 (15.9%)	12 (33,3%)
	Female	93 (78.8)	69 (84,1%)	24 (66,7%)
Age	younger (18-39)	53 (44.9%)	40 (48.8%)	13 (36.1%)
	older (40+)	65 (55.1%)	42 (51.2%)	23 (63.9%)
Marital status	not married (single/widowed/	59 (50%)	42 (51.2%)	17 (47.2%)
	extramarital union)			
	married	59 (50%)	40 (48.8%)	19 (52.8%)
Level of education	elementary/high school	55 (46.6%)	29 (35.4%)	26(72.2%)
	higher education	63 (53.4%)	53 (64.6%)	10 (27.8%)
Smoking		38 (32.2%)	26 (31.7%)	12 (32.3%)
Previous chronic condition		48 (40.7%)	31 (37.8%)	17 (47.2%)
Hypertension		27 (23.1%)		
Hyperlipidemia		4 (3.4%)		
Diabetes Mellitus		3 (2.6%)		
Asthma		3 (2.6%)		
Eczema		4 (3.4%)		
Migraine		25 (21.4%)		
Cerebrovascular insults		4 (3.4%)		
Psychiatric diseases		2 (1.7%)		
		Total 118 (100%)		

Table 2. Behavior and habits of medical and non-medical staff during the COVID-19 pandemic

	All n=118 (%)	Medical staff n=82 (%)	Non-medical staff n=36 (%)	*p* value
Fear for self	61 (51.7%)	39 (48.1%)	21 (58.3%)	0.309
Fear for family and friends	94 (79.7%)	66 (81.5%)	28 (77.8%)	0.642
Difficulties with concentration	33 (28.0%)	20 (24.7%)	13 (36.1%)	0.205
Sleep disturbances	48 (40.7%)	31 (38.3%)	17 (47.2%)	0.364
Change of appetite	23 (19.5%)	18 (22.2%)	6 (16.7%)	0.492
Weight loss	25 (21.2%)	20 (24.7%)	5 (13.9%)	0.188
Weight gain	35 (29.7%)	24 (29.6%)	10 (27.8%)	0.839
Increased use of alcohol	10 (8.5%)	6 (7.4%)	4 (11.1%)	0.722
Increased use of drugs	15 (12.7%)	10 (12.3%)	5 (13.9%)	0.818
Aggravation of chronic conditions	5 (4.2%)	4 (4.9%)	1 (2.8%)	0.594
Aggravation of mental health	12 (10.2%)	10 (12.3%)	3 (8.3%)	0.524
Avoiding Information about COVID-19	62 (52.5%)	46 (56.8)	16 (44.4)	0.217
Watching TV	68 (57.6%)	48 (59.3)	20 (55.6)	0.708
Reading books, magazines, comic books	70 (59.3%)	53 (65.4)	17 (47.2)	0.064
Spent more time on social networks	70 (59.3%)	52 (64.2)	18 (50)	0.148
Physical exercises (including breathing, mediation)	48 (40.7%)	33 (40.7)	15 (41.7)	0.925

to have anxiety symptoms than the unmarried subject (OR: 2.53, 95% CI: 1.07 – 5.98; $p = 0.03$; **Table 5**.). Participants who feared for themselves and their family and friends were more likely to have depression, anxiety, and stress symptoms **(Table 5)**.

Table 3. Differences in stress, anxiety, and depression symptoms and types between medical and non-medical staff and between subjects with higher and lower education (* - indicates statistically significant difference)

		All n=118	Medical staff n=82	Non-medical staff n=36	Higher education n= 63	Lower education n= 55
Stress type	Normal	85	63	23	49	37
	Mild	8	4	4	4	4
	Moderate	6	4	2	3	3
	Severe	13	8	5	4	9
	Extremely severe	5	3	2	3	2
No. of subjects with stress symptoms (%)	32 (27.1%)	19 (23.2%)	13 (36.1%)	14 (22%)	18 (32.7)	
Anxiety type	Normal	52	42	10	44	26
	Mild	11	7	4	1	5
	Moderate	22	12	10	9	10
	Severe	8	5	3	2	4
	Extremely severe	24	15	9	7	10
No. of subjects with anxiety symptoms (%)	48 (40.7%)	29 (35.4%)	19 (52.8%)	19(30%)	29(53%)*	
Depression type	Normal	64	57	19	46	30
	Mild	12	9	3	6	6
	Moderate	17	10	7	7	10
	Severe	6	5	1	1	5
	Extremely severe	7	1	6	3	4
No. of subjects with depression symptoms (%)	42 (35.6%)	25 (30.5%)	17 (47.2%)	17(27%)	25(45.5%)*	

Table 4. DASS-21* scores in medical and non-medical staff and participants with higher and lower education (SD – standard deviation; * - Depression, Anxiety and Stress Scale 21; ** - indicates statistically significant difference)

	All n=118	Medical staff n=82	Non-medical staff n=36	p value	Higher education n=63	Lower education n=55	*p* value
Total DASS-21 score (mean ± SD)	28.2±27.9	24.2±23.40	37.2±33.20	0.020**	22.5±21.59	34.7±29.20	0.017**
Total depression score (mean ± SD)	8.71±7.91	7.0±6.41	12.6±12.4	0.064	6.73±5.83	10.9±10.7	0.013**
Total anxiety score (mean ± SD)	8.15±7.21	7.1±6.59	10.6±10.2	0.048**	6.44±5.53	10.1±9.64	0.020**
Total stress score (mean ± SD)	11.3±10.4	10.1±9.90	14.0±11.0	0.050	9.24±8.97	13.7±10.4	0.008**

Table 5. Factors associated with the presence of anxiety, depression, and stress (* - Reference value)

Factors associated with the presence of anxiety symptoms			
Factor	OR	95% CI	*p* value
Lower education (Ref.* Higher education)	2.58	1.21 – 5.49	0.01
Married subject (Ref. Unmarried subject)	2.53	1.07 – 5.98	0.03
Fear for self (Ref. Without fear)	9.45	3.90 – 22.86	< 0.01
Fear for family and friends (Ref. Without fear)	3.2	1.10 – 9.29	0.03
Factors associated with the presence of depression symptoms			
Lower education (Ref. Higher education)	2.25	1.04 – 4.86	0.03
Fear for self (Ref. Without fear)	5.186	2.22 – 12.10	< 0.01
Fear for family and friends (Ref. Without fear)	4.96	1.38 – 17.80	0.01
Factors associated with the presence of stress symptoms			
Fear for self (Ref. Without fear)	6.31	2.35 – 16.92	< 0.01
Fear for family and friends (Ref. Without fear)	5.15	1.13 – 23.36	< 0.01

DISCUSSION

To the best of our knowledge, this is the first study regarding behavior and mental status of medical and non-medical staff in an obstetrics and gynecology clinic in the Balkan region. Some studies investigated psychological burden in obstetricians and gynecologists (14–16) p=0.023. but non-medical staff were not included in these studies. Lu et al. showed that medical staff had significantly higher levels of fear, anxiety, and depression than administrative staff (17). Another study that compared mental health of medical and non-medical professionals during the pandemic revealed that medical staff presented more often with anxiety and other psychopathological symptoms, while male sex and older age were associated with lower General Health Questionnaire-28 (GHQ-28) scores among medical personnel (8)as declared on March 11th 2020 by the World Health Organization, with respect to which institutional variables might distinguish the impact of COVID-19 in medical and non-medical professionals. Methods: A cross-sectional study was performed nationwide between 16th March and the 26th April 2020 in Poland. A total of 2039 respondents representing all healthcare providers (59.8%.

In our study, 35.6%, 40.7%, and 27.1% of medical staff had symptoms of depression, anxiety, and stress, respectively. There are several meta-analyses regarding psychological disturbances among healthcare workers during the COVID-19 pandemic. Pappa et al. included 13 studies in their meta-analysis, of which 12 were from China (18). The prevalence of depression and anxiety was 22.8% and 23.21%, respectively, while this prevalence in doctors was 25.23% for depression and 21.73% for anxiety. They also showed that the prevalence of depression and anxiety in nurses was 30.3% and 25.80%, respectively. Another, more recent (19), meta-analysis included 62 studies and compared the prevalence of anxiety, depression, and stress among healthcare workers and general public. This study showed that the overall anxiety prevalence was 33% and that it was similar between general public and healthcare workers. The prevalence of anxiety among healthcare workers ranged between 7 and 57%, the highest being in studies from Italy. A depression prevalence followed a similar pattern, and it ranged from 9 to 51% of healthcare workers, with studies from China being the ones with highest percentages. Finally, Batra et al. concluded that the prevalence of anxiety, depression, and stress in healthcare workers was 34.4%, 31.8%, 40.3%, respectively (20).

Interestingly, there were no significant differences in behavior and habits, depression, anxiety, and stress symptoms between these groups in our study. Non-medical staff had higher total DASS-21 scores, along with higher anxiety scores than medical staff.

A possible explanation could be that the level of education plays a part in determining the amount of fear an individual feels, or, more specifically, that medical staff have prior knowledge of illnesses, possibilities of treatment, preventative measures, and therapies available (21). In our study, participants with lower education had higher total DASS-21, depression, anxiety, and stress scores. Moreover, based on DASS scores, anxiety and depression were more frequent in these participants. Naturally, the level of education was higher in medical staff. The age of the respondents should also be taken into consideration, as well as the presence of chronic illnesses, since the majority of younger participants belonged to medical staff, and, while there was no statistical significance, chronic conditions were more prevalent in non-medical staff. There are strong suggestions that elderly people with chronic diseases (especially hypertension) belonged to the group of those who had a higher risk of having more serious forms of COVID 19 (22,23).

There were no significant differences in behavior and habits between healthcare and non-health-care workers. Moreover, none of the habits of the participants were associated with either higher or lower probability of depression, anxiety, or stress manifestations, except for the fear for self and fear for family and friends. On the other hand, more participants feared for the health of their families and close friends than for their own health. This is in accordance with other studies which showed that these were the most common concerns and that they were associated with a higher probability of meeting the criteria for significant mental deterioration (21,24). Many studies have reported that increased physical activity not only was one of the most common coping mechanisms of healthcare workers during the pandemic (25), but it was one of the protective factors for health-related quality of life, anxiety, and depression, in both healthcare workers and other subgroups of general population (26). However, increased physical activity was observed in less than half of the participants in our study, among both healthcare and non-healthcare workers.

Participants with lower education in our study were more likely to meet the criteria for depression and anxiety than participants with higher education. Age, gender, being a member of healthcare or non-health-care staff, and having a chronic illness were not associated with a higher probability of developing the symptoms of depression, anxiety, and/or stress. Chen et al. reported that subjects with higher educational degree were more likely to have anxiety symptoms (27). We think that lower educational levels are undoubtedly associated with a sparse knowledge of pathophysiological mechanisms of the novel coronavirus and available treatment options, so the fear of the unknown, while also being present in the population with higher education, is one of the main contributors to this result of our study.

According to literature, one of the main risk factors for serious mental deterioration was being employed as a front-line health worker. Many studies confirmed that

front-line health workers were more likely to develop the symptoms of depression, stress, and anxiety (28,29). Moreover, some studies suggested that second-line healthcare workers were less likely to have symptoms of depression and anxiety and that they had significantly lower scores in various scales used to determine the presence of previously mentioned psychological disturbances (29). In our questionnaire, which was designed by the Ministry of Health, there were no questions about front- and second-line workers, so we could not determine the exact impact of these positions on mental health of the caregivers.

Gender had a substantial impact on mental deterioration of healthcare workers during the COVID-19 pandemic. The results of many studies indicate that females are more likely to develop anxiety, depression, and stress symptoms (28–30). Moreover, other studies concluded that nurses were more likely to present with symptoms of anxiety compared to other healthcare workers (28)survey-based, region-stratified study collected demographic data and mental health measurements from 1257 health care workers in 34 hospitals from January 29, 2020, to February 3, 2020, in China. Health care workers in hospitals equipped with fever clinics or wards for patients with COVID-19 were eligible.The degree of symptoms of depression, anxiety, insomnia, and distress was assessed by the Chinese versions of the 9-item Patient Health Questionnaire, the 7-item Generalized Anxiety Disorder scale, the 7-item Insomnia Severity Index, and the 22-item Impact of Event Scale–Revised, respectively. Multivariable logistic regression analysis was performed to identify factors associated with mental health outcomes.A total of 1257 of 1830 contacted individuals completed the survey, with a participation rate of 68.7%. A total of 813 (64.7%.

Married subjects in our study were more likely to develop symptoms of anxiety compared to unmarried subjects. This is in accordance with the results of other studies (27). This could be explained by greater fear for the members of the close family, which was, as it has been mentioned, one of the most common concerns of healthcare workers during the pandemics.

While there are fewer studies that included non-healthcare workers in their investigations, Zhang et al. reported that in this population, having an organic disease was a major risk factor for depression symptoms (30).

We have previously mentioned that many healthcare workers were transferred to other facilities or institutions or were doing assignments which were not in their primary field of work. Keihanian et al. reported that even in the group of non-physician healthcare workers, the COVID-19 pandemic had a great impact on work schedule, reassignments, and greater concerns about job security (31).

There are several limitations to our study. Firstly, we think that obtaining the data about personal habits and psychological disturbances before and during the COVID 19 pandemic would be more representative in terms of the actual impact of the ongoing pandemic on mental status in medical and non-medical personnel. We think that the study would be more informative if there were questions regarding infection with SARS CoV-2 and more detailed questions regarding the actual position of the medical staff (i.e., obstetrics and gynecology specialist, anesthesiologist, nurse, etc). Questions regarding the treatment of infected patients (front-line positions) and transfer to other institutions or job positions would give more specific information regarding the mental health of these participants. Since the questionnaire was designed by the Ministry of Health of the Republic of Serbia, we did not have any influence on the questions. Thus, with more informative data and a multicenter study, along with other obstetrics and gynecology centers in Serbia, we would try to conduct a similar, and more illustrative investigation regarding the psychological impact of the COVID 19 pandemic on medical and non-medical staff in obstetrics and gynecology centers.

CONCLUSION

While there are unquestionable catastrophic consequences of the COVID 19 pandemic on health and healthcare sectors, we think that the psychological impact of the pandemic on healthcare workers will be perceived in the coming months or even years. Apart from medical staff, non-medical personnel and their mental status should not be neglected, and it is our aim in future studies, regarding the psychological impact of public health emergencies, to include this group in research.

Conflict of interest:

None to declare

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UTICAJ PANDEMIJE KOVIDA 19 I SOCIJALNE IZOLACIJE NA MENTALNO ZDRAVLJE MEDICINSKOG I NEMEDICINSKOG OSOBLJA – ISKUSTVO IZ JEDNE GINEKOLOŠKO-AKUŠERSKE KLINIKE

Radomir Aničić[1,2], Aleksandar Rakić[2], Dragutin Sretenović[2], Jovana Kocić[2], Jelena Štulić[2], Aleksandar Ristić[1,2], Dejan Dimitrijević[1,2], Lazar Nejković[1,2]

Sažetak

Uvod: Profesionalna izloženost zdravstvene radnike čini posebno osetljivim i pod visokim rizikom za oboljevanje od infekcije KOVID-19 i nastanak velikih psiholoških poremećaja. Strah od nepoznatog, strah za bližnje, brza potrošnja i manjak lične zaštitne opreme, u kombinaciji sa medijski podstaknutom panikom, stvaraju značajno psihološko opterećenje zdravstvenih radnika. Cilj ove studije je bio da se proceni mentalno zdravlje medicinskog i nemedicinskog osoblja u jednoj univezitetskoj ginekološko-akušerskoj klinici tokom pandemije kovida 9 u Srbiji.

Metode: Studija je sprovedena u period od 1. do 31. maja 2020. putem 160 onlajn upitnika podeljenih osoblju Klinike za ginekologiju i akušerstvo. Onlajn anketa se sastojala od dva odeljka: prvi je sadržao pitanja koja se odnose na demografske karakteristike, istoriju bolesti, ponašanja i navike tokom pandemije kovida 19; drugi su činila pitanja u sklopu skale depresije, anksioznosti i stresa 21 (engl. Depression, Anxiety and Stress Scale 21, DASS-21).

Rezultati: Među 118 zaposlenih koji su učestvovali u istraživanju, depresija, anksioznost i stres su bili prisutni kod 35,6%, 40,7% i 27,1% učesnika. Učesnici sa nižim obrazovanjem imali su veći ukupni DASS skor, depresiju, anksioznost i stres u poređenju sa učesnicima sa visokim obrazovanjem. Nemedicinsko osoblje imalo je značajno više ukupne rezultate DASS-a i anksioznosti od medicinskog osoblja. Učesnici sa nižim obrazovanjem i oženjeni ispitanici su češće imali simptome anksioznosti i depresije.

Zaključak: Osim medicinskog, ne smemo zanemariti nemedicinsko osoblje i njihovo mentalno zdravlje. Buduća istraživanja o psihološkom uticaju vanrednih situacija na javno zdravlje bi nesumnjivo trebalo da uključe i istraže ovu grupu.

Ključne reči: kovid 19, medicinsko osoblje, nemedicinsko osoblje, ginekologija i akušerstvo, psihološki uticaj

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REVIEW

The relevance of cholesterol and triglycerides in pregnancy

Milos Milincic [1], Stefan Dugalic [1, 2], Miroslava Gojnic [1,2]

[1] Department of Gynecology and Obstetrics, University Clinical Centre of Serbia, Belgrade, Serbia
[2] University of Belgrade, Faculty of Medicine, Belgrade, Serbia

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Correspondence to:
Miloš Milinčić, University of Belgrade, Faculty of Medicine, Belgrade, Serbia, 8, Dr Subotića Street, 11000 Belgrade, Serbia, +381658720461
milosmilincic@gmail.com

Summary

Cholesterol participates in the construction of all body cells, steroid hormones, and bile acids. Its role in pregnancy is of key importance in the form of the synthesis of hormones without which conception and pregnancy would not be possible, and later in developing the physiological functions of the fetus as well. Triglycerides as the main energy substrate serve in the normal growth and development of the fetus. Normal reference values for lipid status in pregnancy have not been established yet. It has been proven that elevated, as well as reduced values of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) participate in pathological conditions of pregnancy – gestational diabetes mellitus, preeclampsia, macrosomia, restricted fetal growth, etc. The treatment of dyslipidemia is limited due to the lack of pharmacological studies on pregnant women, as well as the teratogenic effect of anti-lipid drugs. This review article deals with the effect of cholesterol and triglycerides on pregnancy, pregnancy outcomes, prenatal and postnatal effects on the fetus, as well as current and future treatment options.

Keywords: Pregnancy; Hypercholesterolemia in pregnancy; Dyslipidemia in pregnancy; LDL-C in pregnancy, TG in pregnancy.

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INTRODUCTION

Cholesterol takes part in the development of all cells and serves as a starting compound for the synthesis of steroid hormones and bile acids (1). Tissues use exogenous cholesterol (intake from food) and endogenous cholesterol (synthesized in tissues, primarily in the liver). Cholesterol ingested with food is resorbed into the epithelial cells of the small intestine, where it is packaged together with other lipids and proteins into chylomicrons (1). The protein part of chylomicrons and other lipoproteins is called apolipoprotein (APO). Chylomicrons travel from the intestines through the thoracic lymphatic duct into the bloodstream and to the liver (1). Cholesterol stored in the body is transported from the liver in the composition of VLDL-C lipoproteins to peripheral tissues. In plasma, cholesterol, like other lipids, is found primarily in the composition of lipoproteins, and to a lesser extent as free cholesterol bound to plasma proteins (2). Cholesterol is monitored in a routine laboratory analysis together with triglycerides as a factor in many diseases, primarily cardiovascular, metabolic and endocrine. Cholesterol is a precursor to steroid hormones critical to pregnancy, including estrogens, progesterone, androgens, corticosteroids, and vitamin D (1,2). Many of these hormones are produced by the placenta (3).

Triglycerides are the most abundant fats in the human diet because they are the main form of fat storage in plants and animals (1,2). They consist of glycerol that is esterified with three fatty acids. They are broken down by a series of lipases in the intestinal tract and then absorbed in the small intestine, where they have condensed again into triacylglycerols, which combine into lipoprotein particles - chylomicrons (which contain both cholesterol and liposoluble vitamins (1,2). Chylomicrons in the peripheral blood are broken down by lipoprotein lipase stimulated by insulin. When the triglyceride level increases after a meal, insulin is released, which additionally activates LPL and accelerates the release of fatty acids from TG by hydrolysis which are then stored in adipose tissue. The rest of the chylomicrons are transported to the liver and absorbed into hepatocytes (4).

Lipids in pregnancy

There is indisputable evidence that changes in lipid status occur during pregnancy. These changes are necessary for the normal growth and development of the fetus (5). During pregnancy, there is a physiological increase of 30-50% in triglycerides and total cholesterol in the plasma, especially in the second and third trimester. The reason for such a significant change in lipid status during pregnancy is found in changes in hormones such as estrogens, progesterone, HPL, and insulin, as well as altered general metabolism of the liver and fat tissue (5). In the first trimester, under the influence of insulin, cholesterol, and triglycerides increase based on increased lipid synthesis (lipogenesis) and inhibition of lipid degradation (lipolysis) (6). As pregnancy progresses and the fetus develops, more and more energy is needed to perform normal metabolic functions. For this reason, there is a shift of the mother's lipid metabolism towards increased lipolysis, compared to its inhibition during early pregnancy (7). The consequences of these changes lead to an increased deposition of lipids in adipose tissue during the first trimester, and in the second and third trimesters to a marked mobilization of lipids in the form of an increased level of free fatty acids, triglycerides and cholesterol, which will be delivered to the fetus via the placenta and serve it for general metabolic functions, and the synthesis of necessary steroid hormones (5,7).

Recommended reference values by WHO (World Health Organization) lipid profile in the normal population are as follows: total cholesterol <5.20, HDL-C >1.53, LDL-C <2.6, Triglycerides <1.69. When it comes to pregnant women, there are still no recommended values. The reason for this practice is the insufficiently researched influence of hyperlipidemia and hypercholesterolemia during normal pregnancy. Although the reference values of the lipid profile in pregnant women are not established, we can see the values obtained in the latest research. Wang et al. showed the values of TC, LDL-C, HDL-C, TG, and the TG/HDL-C ratio in a large population of pregnant women of Asian origin, as well as the changes in these values through the trimesters of pregnancy. The values of TC (from 4.04 to 6.16 mmol/L), HDL-C (from 1.42 to 1.71 mmol/L), LDL-C (from 2.15 to 3.30 mmol/L), TG (from 0.94 to 3.14 mmol/L), TG/HDL-C (from 0.70 to 1.96) (8). Given the physiological differences in lipid status between different ethnic populations (9), in a similar study by Bever AM et al., the following values were obtained from the Caucasian population – TC 6.28 mmol/L, LDL-C 3.38 mmol/L, HDL-C 1.67 mmol/L, TG 2.51 mmol/l (10). What is of particular importance is that molecularly smaller LDL-C particles are created during pregnancy, which have been proven to be more atherogenic than those outside of pregnancy (5). As there has been a general epidemic of metabolic syndrome, heart disease, high blood pressure, atherosclerosis, and diabetes, more attention has been paid to the lipid status of pregnant women. Pregnancies burdened with gestational diabetes, gestational hypertension, and preeclampsia showed higher than normal lipid values. These changes have also shown an impact on the growth and development of the fetus, as well as on the outcome of the pregnancy. This created a wide and unexplored field for further research (5,6).

Role of the placenta in lipid synthesis and transport

The placenta is the link between the mother and the fetus. Through it, elevated lipid concentrations can affect the

growth and development of the fetus (11). The placenta participates in the biosynthesis, regulation, and transport of cholesterol between mother and fetus. Approximately 20% of the total cholesterol requirement of the fetus is transported across the placenta and this percentage increases in states of maternal hypercholesterolemia (12). Evidence for this transport can be found in Smith-Lemli-Opitz syndrome, a congenital defect in cholesterol synthesis (13). Given that there is no synthesis by the fetus, and fetuses are born with a certain amount of cholesterol, this confirms the transfer pathways between the placenta and the fetus (13). The placenta is also equipped with the necessary enzymes for the production of many steroid hormones for the maintenance of pregnancy and the development of the fetus - estrogens, progesterone, androgens, and glucocorticoids. Given that all cells require cholesterol, it is clear why large amounts of this lipid are needed in a fast-growing organism. In the syncytiotrophoblast, there are receptors for uptaking cholesterol from the mother's bloodstream through receptors similar to those in hepatocytes - low-density lipoprotein receptor (LDL-CR) and scavenger receptor class B type I (SRB1) (14). ApoB in the trophoblast controls lipoprotein uptake. Also, VLDL-C metabolism is changed in pregnancy due to reduced activity of lipoprotein lipase in the liver and adipose tissue, and increased activity in the placenta. In this way, degraded lipoproteins, i.e., products of enzymatic degradation, can effectively reach the fetus and fulfill its increased metabolic requirements (10).

The impact of dyslipidemia on pregnancy

Dyslipidemia in pregnancy is associated with gestational diabetes mellitus (GDM), preeclampsia, macrosomia, preterm birth, and other pregnancy complications. Women with a rich-in-cholesterol diet have a higher incidence of developing gestational diabetes (15). A low level of HDL-C and an elevated BMI have a particular impact on the development of GDM (13). On the other hand, women with gestational diabetes show elevated levels of LDL-C and apoB in their laboratory lipid values. A peculiarity in pregnancy is that LDL-C particles are smaller in size than those outside pregnancy (5). It has been proven that these LDL-C particles are more atherogenic and can lead to faster clogging of blood vessels, especially coronary arteries. A protective factor, if we can say so, is the length of pregnancy of 40 weeks, during which so many extensive changes in the myocardium cannot occur. Whether any reversible changes occur remains to be seen (5).

Elevated LDL-C is associated with preeclampsia due to its endothelial damaging properties via its oxidized form. In pregnant women with preeclampsia, there were elevated LDL-C values in laboratory values, which were maintained up to three years after delivery. In a large study by Jin, Wy et al. it has been proven that an increase in each unit of triglycerides and total cholesterol, especially LDL-C, during pregnancy leads to an increased risk of developing gestational diabetes, preeclampsia, macrosomia, and premature birth. On the other hand, with an increase in HDL-C concentration in the total ratio with LDL-C, the percentage of occurrence of pathological conditions and poor pregnancy outcomes was lower (16).

On the other hand, reduced cholesterol values have a negative impact on pregnancy and may contribute to the risk of premature birth ($p=0.001$) (17). Newborns of mothers with lower total cholesterol values had 150g lower body weight at birth than healthy controls. The authors also suggested that the risk was somewhat increased in mothers with hypercholesterolemia.

In a study conducted by Sharami S. et al. pregnant women with dyslipidemia in the form of elevated triglycerides, cholesterol, and LDL-C had increased incidences of gestational diabetes ($p<0.001$), preeclampsia ($p<0.001$), cholestasis ($p=0.041$) and macrosomia ($p<0.001$) (18).

The effect of dyslipidemia on the fetus

Disturbed maternal lipid values during pregnancy have a negative outcome for the fetus. The consequences are visible in all parts of fetal development and later in adulthood. The influence of elevated lipid values in utero leads to LGA (large for gestational age) and fetal macrosomia. It affects the term of delivery in the form of preterm birth, as well as the impact on postpartum life in the form of metabolic syndrome later in life. Recent studies have shown the influence of reduced triglyceride values on intrauterine fetal growth (17).

This raises the question of the importance of lipid control in pregnancy and it focuses on the aspect of fetal programming that can prevent these metabolic changes later in adult life.

A meta-analysis by Wang Y. et al. investigated the relationship between maternal dyslipidemia and intrauterine growth retardation. They monitored the values of total cholesterol, triglycerides, LDL-C, and HDL-C. A total of eight studies (over 14,000 pregnant women) were included in the analysis. The result of the research indicated that reduced values of total cholesterol, triglycerides, and LDL-C were risk factors for SGA (small for gestational age) fetuses (20).

The results so far show that the lipid status of the mother during pregnancy has a direct effect on the growth of the fetus and its metabolism later in life. In a physiological pregnancy, there is an increase in the level of lipids in the mother's blood and fat tissue, but if malnutrition occurs, or a pathologically high accumulation of total cholesterol and triglycerides, then there are consequences for the fetus in the form of decreased or increased body weight at birth (21). When in question, otherwise protective in the cardiovascular sense, HDL-C in the study by Kramer et al. showed that higher mean

HDL-C values in the second trimester of pregnancy were found in fetuses with a lower than average body weight, while elevated triglyceride values were protective and associated with a lower risk of SGA (22).

Treatment

Since reference values for lipids in pregnant women have not been established yet, it is difficult to talk about specific treatment of hyperlipidemia. Statins (HMG CoA-reductase inhibitors) that are widely used in the treatment of hypercholesterolemia are not used in pregnancies (23). In animal studies, statin use resulted in various skeletal abnormalities and increased fetal morbidity and mortality. The impact of statins that cross the blood-brain barrier in the form of neurological damage has also been confirmed. In addition, in the majority of studies dealing with the treatment of dyslipidemia, pregnant women were excluded from research. The result is a huge gap in the control and treatment of this condition in pregnancy.

Omega-3 fatty acids are used as a supplement to regulate elevated lipid levels. They have been proven to reduce triglyceride levels. They have a direct impact on increasing "good" HDL-C and reducing "bad" LDL-C (24). Williams M. et al. showed that supplementation with fish oil led to a decrease in triglycerides and an increase in HDL-C cholesterol in maternal plasma (22).

For now, a successful type of therapy for both reduced and elevated maternal plasma lipid values is performed through a dietary hygiene regimen. One of the drastic types of therapy in pregnancy is used in the treatment of the rare condition of acute pancreatitis and familial hypercholesterolemia. Due to extremely elevated cholesterol values in this condition, the process of LDL-C aphaeresis is used, which reduces the concentration of LDL-C cholesterol in a targeted manner, while HDL-C cholesterol remains unchanged (25).

CONCLUSION

The influence of cholesterol and triglycerides on the mother and fetus is complex. Dyslipidemia contributes to the development of cardiovascular diseases, gestational diabetes, hypertension in pregnancy, preeclampsia, and other pathological conditions. Elevated lipid levels contribute to premature birth, macrosomia, and the development of metabolic syndrome later in life. The lack of cholesterol and triglycerides during pregnancy negatively affects the growth and development of the fetus. Further studies are necessary to define lipid reference values in pregnancy, as well as numerous scientific contributions and pharmacological studies for establishing adequate therapy.

Conflict of interest

None to declare

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RELEVANTNOST HOLESTEROLA I TRIGLICERIDA U TRUDNOĆI

Milos Milincic[1], Stefan Dugalic[1, 2], Miroslava Gojnic[1,2]

Sažetak

Holesterol učestvuje u izgradnji svih ćelija organizma, steroidnih hormona i žučnih kiselina. Njegova uloga u trudnoći je od ključnog značaja u vidu sinteze hormona bez kojih začeće i trudnoća ne bi bili mogući, a kasnije i u razvijanju fizioloških funkcija fetusa. Trigliceridi kao glavni energetski supstrat služe normalnom rastu i razvoju ploda. Normalne referentne vrednosti lipidnog statusa u trudnoći još uvek nisu uspostavljene. Dokazano je da povišene, kao i smanjene vrednosti ukupnog holesterola, holesterola lipoproteina velike gustine (HDL-C), holesterola lipoproteina male gustine (LDL-C) i triglicerida (TCG) učestvuju u patološkim stanjima trudnoće – gestacioni dijabetes melitus, preeklampsija, makrozomija, restrikcija rasta, itd. Lečenje dislipidemije je ograničeno zbog nedostatka farmakoloških studija na trudnicama, kao i teratogenog efekta antilipidnih lekova. Ovaj pregledni rad se bavi uticajem holesterola i triglicerida na trudnoću, ishod trudnoće, prenatalnim i postnatalnim uticajem na plod, kao i mogućnostima trenutnog i budućeg lečenja.

Ključne reči: Trudnoća; Hiperholesterolemija u trudnoći; Dislipidemija u trudnoći; LDL holesterol u trudnoći, Trigliceridi u trudnoći.

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REVIEW

Pathology of sellar tumors: a contemporary diagnostic approach

Emilija Manojlović Gačić[1], Martina Bosić[1], Ivan Zaletel[2], Marko Stojanović[3,4], Mirjana Doknić[3,4], Sandra Pekić Đurđević[3,4], Dragana Miljić[3,4], Dejan Rašić[4,5], Sofija Glumac[1]

[1]Institute of Pathology, University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[2]Institute of Histology, University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[3]Neuroendocrine Department, Clinic for Endocrinology, Diabetes and Metabolic Diseases, University Clinical Center of Serbia, Belgrade, Serbia

[4]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

[5]Clinic for Ophthalmology, University Clinical Center of Serbia, Belgrade, Serbia

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Correspondence to:
Emilija Manojlović Gačić, Institute of Pathology, University of Belgrade, Faculty of Medicine,
1, Dr Subotića Street, Belgrade, Serbia,
emilija.manojlovic-gacic@med.bg.ac.rs
emanojlovicgacic@gmail.com
Phone: +381 11 364 34 26

Summary

The sellar region is a complex area situated in the middle of the cranial base, with the pituitary gland in central position and anatomically close to the crossroads of vital structures, which makes the basis for the development of numerous endocrinological and neurological conditions caused by the expansion or secretory activity of the tumor tissue. In this article, we will summarize the literature enclosing histopathological and immunohistochemical aspects of sellar tumors, together with clinical characteristics, being the key elements in making a proper diagnosis. A histopathological diagnostic algorithm will be presented for the most frequent tumors of this region, pituitary neuroendocrine tumors, as well as tumors of the posterior pituitary, meningiomas, craniopharyngiomas, chordomas, germ cell tumors hematological malignancies, Rathke cleft cyst and metastases. Contemporary histopathological diagnostic approach to sellar tumors strongly depends on the routine use of immunohistochemistry for a broad spectrum of antibodies, as well as a detailed correlation with endocrinological, neurological, neurosurgical and neuroradiological aspects, which are mandatory for establishing an accurate diagnosis, reducing dilemmas, and offering the best options for further treatment of patients with sellar tumors.

Key words: sellar tumor, pituitary neuroendocrine tumor, immunohistochemistry

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INTRODUCTION

The sellar region is a complex area located in the middle of the cranial base, with the pituitary gland in central position and anatomically closely connected with the crossroads of vital structures, including cavernous sinuses, sphenoid sinus and optic chiasm. The superposition of important endocrinological, neural and vascular structures of the sellar area represents the basis for the generation of numerous endocrinological and neurological conditions caused by the expansion of tumor tissue. Their type and severity depend on endocrinological activity, size, location, propagation, and biological behavior (1,2).

The most frequent neoplasms in the sellar region are pituitary neuroendocrine tumors (PitNETs), comprising up to 90% of neoplasms in this region (3). The remaining 9% are rare neoplasms originating from different cells and grades of malignancy. The heterogeneity of sellar tumors requires the use of several WHO classifications of tumors, presumably the classification of tumors of endocrine organs (4), followed by the classification of tumors of the central nervous system (5) and the classification of tumors of bone and soft tissues (6). Contemporary histopathological diagnostic approach to sellar tumors strongly depends on a routine use of immunohistochemistry for a broad spectrum of antibodies, as well as a detailed correlation with endocrinological, neurological, neurosurgical and neuroradiological aspects.

In this article, we will summarize the literature enclosing histopathological and immunohistochemical aspects of sellar tumors, together with clinical characteristics, being the key elements in making a proper diagnosis.

Pituitary neuroendocrine tumor (PitNET)

Pituitary neuroendocrine tumors, previously named pituitary adenomas (7), are the most frequent primary tumors of the pituitary gland (3). A contemporary diagnostic approach for PitNETs relies on the application of immunohistochemistry and the correlation of its results with clinical data (functioning vs non-functioning PitNET, the size and invasiveness of the tumor) (4). The diagnostic immunohistochemical panel consists of antibodies against anterior pituitary transcription factors – steroidogenic factor-1 (SF1), pituitary-specific transcription factor 1 (PIT1) and T-box family member TBX19 (TPIT), followed by antibodies against anterior pituitary hormones: growth hormone (GH), prolactin (PRL), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH). The use of the antibody against low molecular weight cytokeratin (LMWCK) is not mandatory; it is of great importance for the recognition of granulation patterns of somatotroph tumors (4,8). PitNETs are classified according to their lineage of differentiation as follows:

1. PIT1-lineage PitNETs
   - Somatotroph tumors
     - Densely granulated somatotroph tumor
     - Sparsely granulated somatotroph tumor
   - Lactotroph tumors
     - Sparsely granulated lactotroph tumor
     - Densely granulated lactotroph tumor
   - Mammosomatotroph tumors
   - Thyrotroph tumors
   - Mature plurihormonal PIT1-lineage tumors
   - Immature PIT1-lineage tumors
   - Acidophil stem-cell tumor
   - Mixed somatotroph and lactotroph tumor
2. SF1-lineage PitNETs
   - Gonadotroph tumor
3. TPIT-lineage PitNETs
   - Corticotroph tumors
     - Densely granulated corticotroph tumor
     - Sparsely granulated corticotroph tumor
     - Crooke cell tumor
4. PitNETs with no distinct cell lineage
   - Null-cell tumor
   - Plurihormonal tumor (4,8)

The group of PIT1-lineage PitNETs is the most complex one, with numerous sub-categories whose precise diagnosis is of great importance since some of them (i.e., sparsely granulated somatotroph tumor, mature plurihormonal PIT1-lineage tumors and immature plurihormonal PIT1-lineage tumors) frequently show aggressive biological behavior and need a detailed and frequent follow-up.

Somatotroph tumors are most frequently functional, causing acromegaly. They are sub-classified according to their granulation pattern into densely and sparsely granulated somatotroph tumors. *Densely granulated somatotroph tumors* are characterized by diffuse and intensive PIT1 and GH positivity. The granulation pattern of densely granulated PitNETs is characterized by perinuclear or diffuse LMWCK cytoplasmic positivity **(Figure 1, A-D)**. *Sparsely granulated somatotroph tumors* are defined by diffuse and intensive PIT1 positivity which is surprisingly accompanied by focal, sparse (sometimes even negative) GH staining. The granulation pattern of sparsely granulated somatotroph tumors is defined by the presence of LMWCK positive, sharply demarcated, dot-like perinuclear staining, known as "fibrous bodies" in more than 70% of tumor cells **(Figure 1, E-H)** (4,8). Sparsely granulated somatotroph tumors demand special attention of endocrinologists and surgeons since they frequently show aggressive biological behavior and resistance to somatostatin analogues therapy (9). This can be at least partially explained by the absence of GNAS mutation and the lack of the activation of tumor senescence, both being present in densely granulated somatotroph PitNETs (10,11).

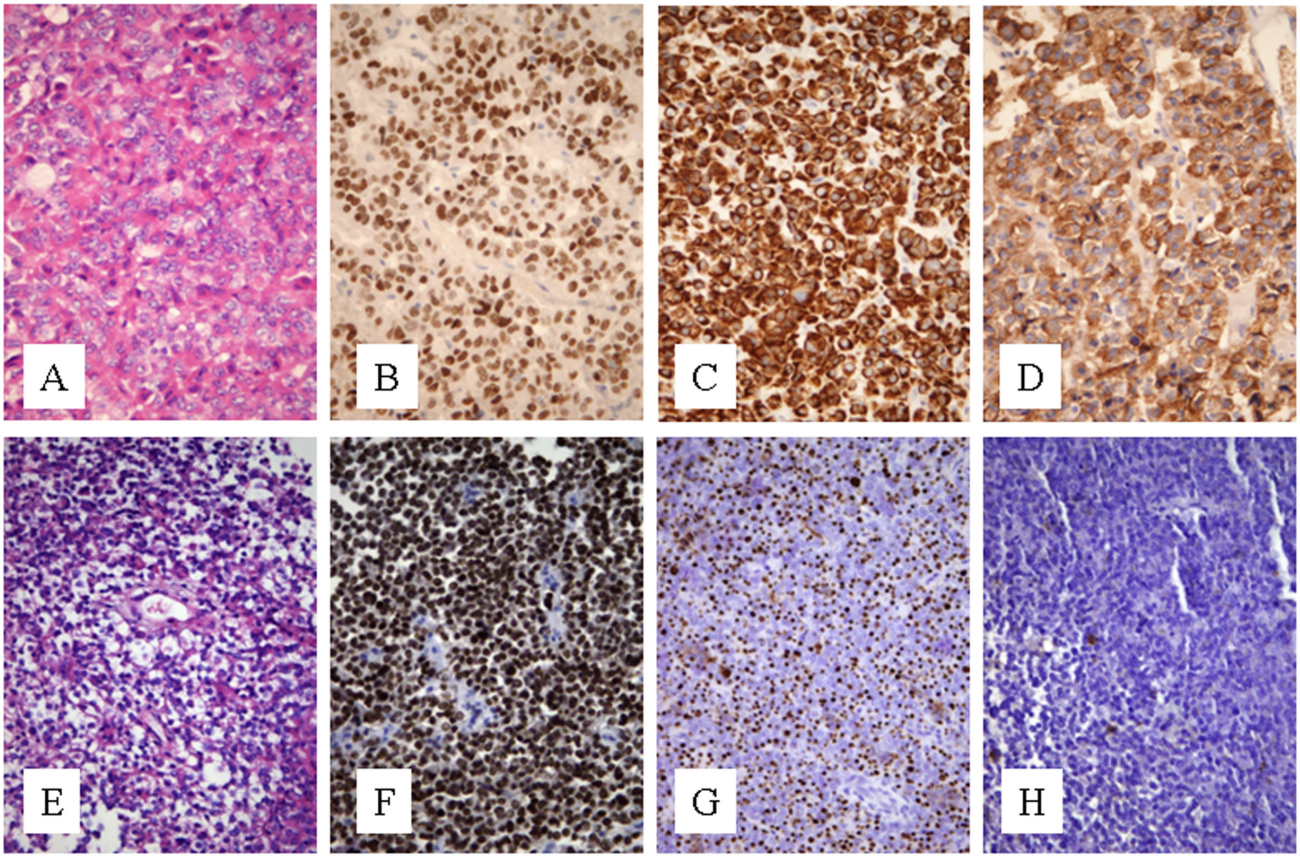

Figure 1. Somatotroph PitNETs. **A.** Densely granulated somatotroph PitNETs are typically acidophilic tumors (HE, x400); **B.** PIT1 nuclear positivity delineates somatotroph differentiation (PIT1, x400); **C.** CK8/18 positivity in densely granulated somatotroph PitNETs is diffuse cytoplasmic (CK8/18, x400); **D.** Densely granulated somatotroph PitNETs typically show diffuse and intensive GH positivity (GH, x400); **E.** Sparsely granulated somatotroph PitNETs are chromophobe tumors (HE, x400), **F.** PIT1 nuclear positivity is typically diffuse and intensive in sparsely granulated somatotroph PitNETs (PIT1, x400); **G.** Numerous spheric, CK8/18 positive perinuclear inclusions, named "fibrous bodies", are diagnostic hallmark of sparsely granulated somatotroph PitNETs (CK8/18, X400) **H.** GH positivity in sparsely granulated somatotroph PitNETs can be sparse, sometimes in scattered single cells (GH, x400).

Lactotroph tumors are the most frequent tumors of the anterior pituitary and they cause hyperprolactinemia. Intriguingly, they are extremely rare in surgical series owing to a very successful treatment with dopamine agonists (12). Currently, surgical treatment of prolactinomas is performed in rare cases resistant to dopamine agonists therapy (13). All lactotroph tumors are positive for PIT1 and estrogen receptor (ER). Their granulation pattern is defined according to the pattern of PRL positivity. *Sparsely granulated lactotroph tumors* are identified by PRL positivity in the Golgi zone, as opposed to rare *densely granulated lactotroph tumors* showing diffuse cytoplasmic PRL positivity (4,8). A great majority of surgically treated lactotroph tumors have previously been treated by dopamine agonists, which cause secondary changes in the tumor tissue including hyalinization, rarefaction of tumor cells, and scant PRL positivity. This is of great importance in cases with a small amount of tumor tissue provided for pathological analysis, since the majority of it can be seen as hyalinized tissue; tumor cells could be scattered and difficult to recognize (4,8). Lactotroph PitNETs in men need exceptional clinical attention due to their propensity for aggressive biological behavior (14).

Mammosomatotroph tumours are relatively rare tumors, most frequently presenting with acromegaly associated with hyperprolactinemia. Besides their PIT1 positivity, they are designated by diffuse GH positivity (similar to densely granulated somatotroph tumors) as well as positivity for PRL and ER (15). They have been presented as a distinct tumor type for the first time in the recent WHO classification (8).

Thyrotroph tumors are traditionally described as very rare tumors consisting of polygonal or spindle cells positive for PIT1 and TSH, causing central hyperthyroidism (16). Owing to a progress in understanding pituitary tumors, we can contemplate a majority of previously diagnosed "TSH-omas" could be currently re-diagnosed as mature and immature PIT1-lineage tumors, due to their frequent clinical presentation including the association of acromegaly and hyperthyroidism (8).

Mature plurihormonal PIT1-lineage tumors and immature PIT1-lineage tumors are recently recognized as new diagnostic categories (8,17), previously being designated as Silent subtype 3 pituitary adenomas (18). They are characterized by a wide spectrum of clinical presentations, ranging from non-functioning tumors to hyperthyroidism, acromegaly or galactorrhea and amenorrhea

(17). Morphologically, mature plurihormonal PIT1-lineage tumors are characterized by acidophilic tumor cells in diffuse arrangement, in contrast to the immature type, characterized by polygonal or spindle-shaped cells resembling thyrotrophs. The only consistent immunohistochemical finding in this exceptional type of PitNET is diffuse and intensive PIT1 positivity. In mature forms, GH, PRL and TSH positivity are frequently observed, in contrast to immature forms, where the percentage of GH, PRL and TSH cells decreases to scattered cells. Although very rare, immature PIT1-lineage tumors demand clinical attention and close follow-up due to the predisposition for aggressive biological behavior (8).

Acidophil stem-cell tumor is an extremely rare type of tumor belonging to the PIT1 lineage. It is an example of tumor composed of precursor cells, like immature plurihormonal PIT1-lineage tumors. They are usually large tumors with hyperprolactinemia which is of a lower level than expected for tumor size. Similarly, with mammosomatotroph tumors, acidophil stem-cell tumor has been presented as a distinct tumor type for the first time in the WHO classification 2022, previously being presented as a subtype of lactotroph tumor. This type of PitNET has unique morphological findings: "giant mitochondria" that can be observed on HE staining as intracytoplasmic vacuole. In immunohistochemistry, acidophil stem-cell tumor is diffusely positive for PIT1, ER and PRL and focally for GH (8).

Mixed somatotroph and lactotroph tumors are composed of two distinct populations of tumor cells; most frequently densely granulated somatotrophs and sparsely granulated lactotrophs (10).

SF1-lineage PitNETs, presented as a single category of gonadotroph tumors, are most frequent in surgical series. Since they are typically non-functioning tumors, their clinical presentation is caused by their compression to cavernous sinuses, optic chiasm, and meninges, causing headaches and visual impairment (19). The introduction of antibodies against anterior pituitary transcription factors caused a significant increase in the number of diagnosed gonadotroph tumors, since a great majority of them have been incorrectly diagnosed as null-cell adenomas, due to the lack of the positive stain for FSH and/or LH (20). The FSH and LH positivity is typically focal when present (4,10).

TPIT-lineage PitNETs include three types of PitNETs, two of them being PitNETs with aggressive biolog-

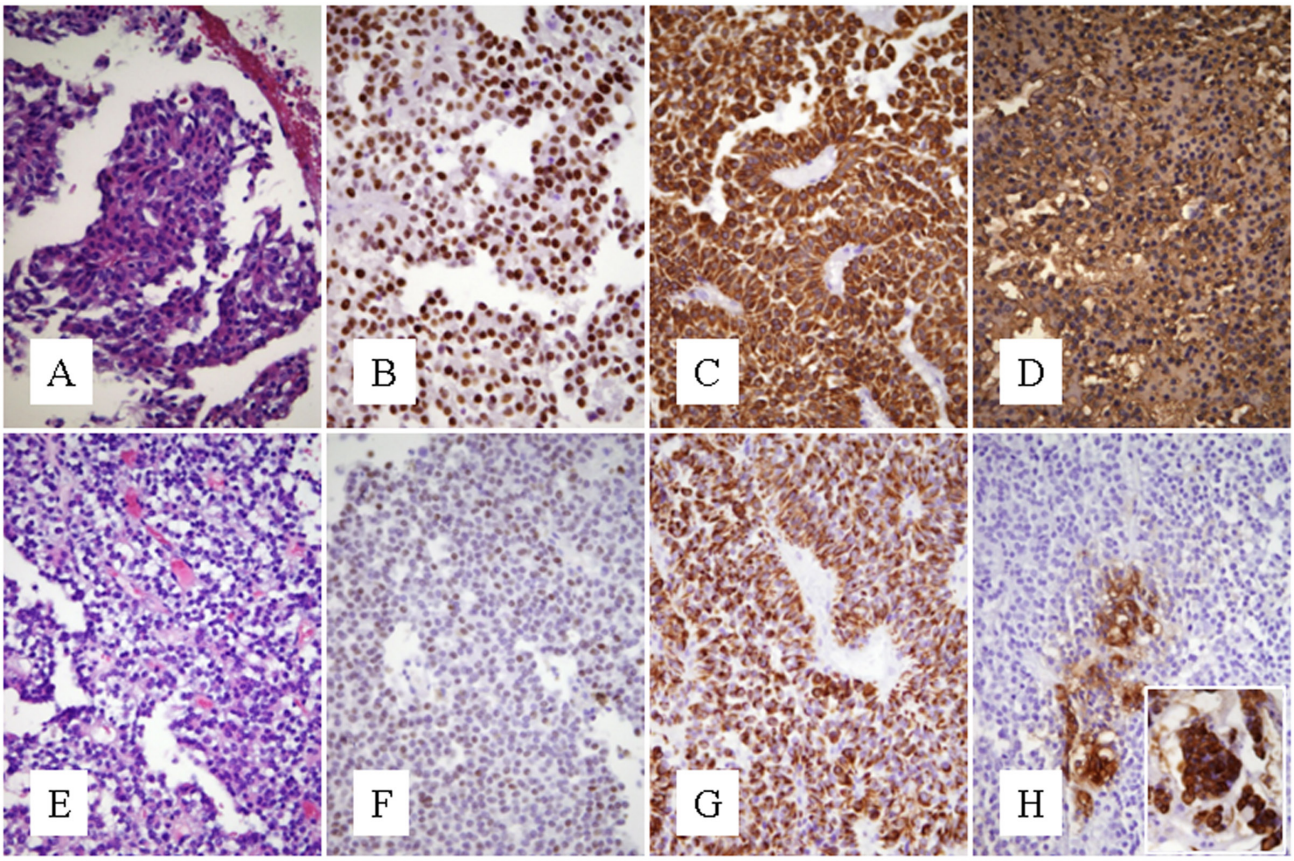

Figure 2. Corticotroph PitNETs **A.** Densely granulated corticotroph PitNETs are basophil tumours on HE stain (HE, x400); **B.** TPIT nuclear positivity is diffuse and intensive in densely granulated corticotroph PitNETs (TPIT, x400); **C.** CK8/18 positivity is typically diffuse and intensive in all corticotroph PitNETs, including densely granulated (CK8/18, x400); **D.** Diffuse and intensive ACTH positivity is the diagnostic hallmark of densely granulated corticotroph PitNETs (ACTH, x400); **E** Tumor cells of silent corticotroph PitNET are typically chromophobe (HE, x40); **F.** TPIT nuclear positivity is focal and of variable intensity in silent corticotroph PitNETs (TPIT, x400); **G.** CK8/18 positivity is typically diffuse and intensive in silent corticotroph PitNETs (CK8/18, x400); **H.** ACTH positivity is typically sparse, sometimes absent, being the diagnostic hallmark of sparsely granulated corticotroph PitNETs (ACTH, x400). Insert Crooke hyaline change in corticotroph cells, as a morphological proof of hypercortisolemia).

ical behavior. *Densely granulated corticotroph tumors* are typically of small size, around few millimeters in diameter, and cause florid Cushing disease (21). On HE slides, they are basophilic tumors, with diffuse and intensive TPIT, LMWCK and ACTH positivity **(Figure 2, A-D)**. Contrary to this, *sparsely granulated corticotroph tumors* are relatively large tumors, frequently invading surrounding structures, causing frequent recurrences (22), with variable Cushing disease (23). On HE slides present as chromophobe tumors, with variable immunohistochemical expression of TPIT and ACTH and diffuse LMWCK positivity. It is worth noting that ACTH positivity could be absent **(Figure 2, E-H)**. The number of diagnosed sparsely granulated corticotroph tumors increased after the introduction of the antibody against TPIT in routine practice. Until then, they were misdiagnosed as null-cell adenomas. Sparsely granulated corticotroph PitNETs frequently show aggressive biological behavior which is frequently linked with ATRX mutation (24). *Crooke cell tumor* is an extremely rare variant of corticotroph tumor composed of cells with Crookes hyaline change, morphological evidence of hypercortisolemia and hormonal feedback inhibition **(Figure 2, H, insert)**. The cytoplasm of Crooke cells is filled with a pale hyaline ring intensely reactive for LMWCK. ACTH-positive granules can be observed only at the periphery of the cell. The accurate diagnosis of this unique tumor is of great importance since they show aggressive biological behavior (8).

PitNETs with no distinct cell lineage are very rare in the era of routine use of antibodies for anterior pituitary transcription factors. The number of diagnosed null-cell tumors, characterized by the lack of expression of any anterior pituitary hormone and transcription factor, significantly decreased after the introduction of the use of antibodies for anterior pituitary transcription factors (25). A great majority of ex-null-cell adenomas became re-diagnosed as gonadotroph tumors (owing to SF1 positivity); a smaller part of ex-null-cell adenomas with aggressive biological behavior was re-diagnosed as silent corticotroph tumors, owing to TPIT positivity (26). Contemporary diagnosis of null-cell tumor, besides negative immunohistochemical stains for all anterior pituitary hormones and transcription factors, requires the exclusion of the possibility of metastases of neuroendocrine tumors from other sites (25), or the diagnostic use of proteomics (27,28). Plurihormonal tumors are also exceedingly rare. By definition, they are composed of tumor cells with multiple cell lineages. The crucial step in the diagnosis of plurihormonal tumor is the exclusion of the possibility of cross-reactions of antibodies for anterior pituitary hormones and transcription factors, as well as reassessment of the quality of the immunohistochemistry (29).

Contemporary molecular diagnostics is not needed in routine diagnostics of PitNETs, since proteomics analyses follow the classification tree established by immunohistochemistry (27,28). The molecular diagnostic approach could be of great importance in rare sellar tumors without detectable lineage of differentiation by immunohistochemistry (25). Namely, some rare forms of PitNETs produce anterior pituitary transcription factors in such low amounts that they could be detected (and subsequently diagnosed and classified) only by proteomics (27,28).

Tumors of the posterior pituitary

Modern understanding of posterior pituitary tumors is that they represent a spectrum of low-grade tumor types with a single common characteristic: thyroid transcription factor 1 (TTF1) positivity (30,31). Spindle-cell oncocytoma, pituicytoma and granular cell tumor of the sellar region originate from pituicytes of the posterior pituitary or infundibulum. Their differentiation relies on their morphological characteristics on HE slides since their immunohistochemical profile is very similar: they show strong positivity for Vimentin and S-100, whilst GFAP, EMA, CD56, CD68 and bcl-2 stains are variable. Pituicytomas are composed of bipolar spindle cells arranged in sheets and short fascicles. Granular cell tumors consist of polygonal cells with granular cytoplasm. Finally, spindle cell oncocytomas are, as the name implies, constituted of spindled or epithelioid tumor cells with brightly eosinophilic granular cytoplasm (5).

TTF1 positivity in a sellar tumor should be regarded with special attention and with numerous additional immunohistochemical staining, bearing in mind that TTF1 is widely present in lung and thyroid tumors, with a chance that the tumour is a metastasis from tumors of these organs.

Meningioma

Sellar and parasellar meningiomas represent 5-10% of intracranial meningiomas (32). Their clinical, endocrinological and radiological presentation resembles the most frequent sellar tumors PitNETs (33). Subsequently, histopathological examination is crucial for the diagnosis, which is sometimes challenging. The only immunohistochemical marker constantly expressed in all types of meningiomas, regardless of the grade, is SSTR2A. Furthermore, this is the only immunohistochemical marker mandatory for the diagnosis of meningioma according to the WHO criteria (5). It should be noted that neuroendocrine tumors also express SSTR2A and that a broader immunohistochemical panel should be used for the correct differential diagnosis. The meningothelial origin of tumor cells can be confirmed by positive immunohistochemical staining for EMA, Vimentin and PR. However, EMA and PR positivity is frequently lost with an increase in meningioma grade. Meningiomas are negative for GFAP, SOX-10, STAT6, Inhibin, Melan A and HMB45. S-100, cytokeratin AE1/AE3 and CD34 positivity are observed in rare cases (34). Bearing in mind the broad spectrum of differential diagnoses of sellar menin-

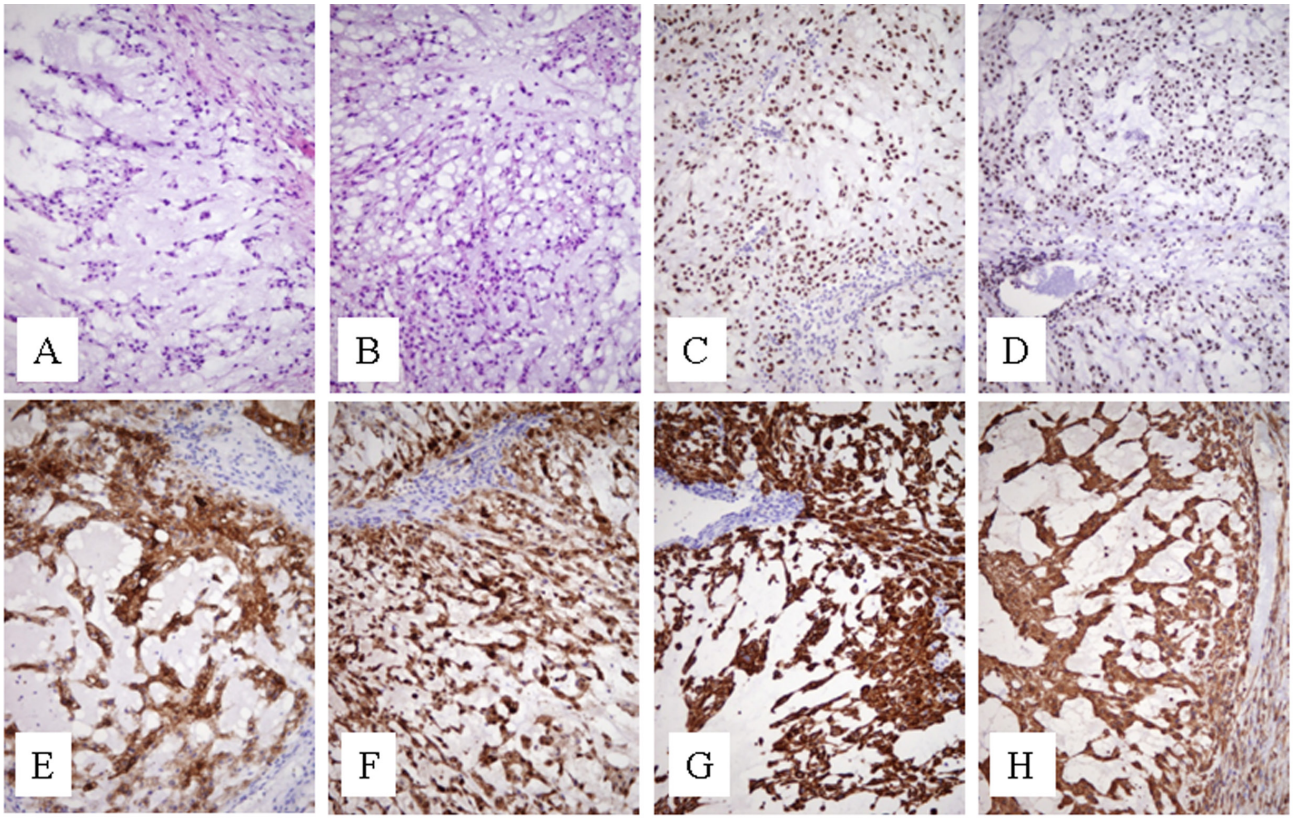

Figure 3. A. Chordomas are characterized by epithelioid cells organized in trabecules, separated by myxoid stroma (HE, x400). **B.** Chordoma cells sometimes have vacuolated cytoplasm, giving the appearance of "physaliphora cells" (HE, x400). **C.** Brachiury positivity is mandatory for diagnosing chordoma (Brachiury, x400); Chordomas are usually positive for EMA (**D.**, EMA, x400), S-100 (**E.**, S-100, x400); panCK (AE1/AE3) (**F.**, panCK (AE1/AE3), x400); INI1 (**G.**, INI1, x400) and Vimentin (**H.**, Vimentin, x400).

giomas, we recommend the use of a wider spectrum of immunohistochemical markers and clinical-pathological correlation for its reliable diagnosis.

Chordoma

Chordomas of the sellar region account for about 0.5% of all sellar masses (3). Nevertheless, they are not surprising lesions on this location since they are midline tumors. Although their histopathological appearance is unique, with lobular architecture, cords of epithelioid cells embedded in the extracellular myxoid matrix and "physaliphorous cells" **(Figure 3, A-B)** (6), immunohistochemistry is mandatory for the diagnosis. Brachiury is a mandatory marker for the reliable diagnosis of chordoma **(Figure 3C)**. It is worth noting that the patterns of expression of Brachiury are variable (35); therefore, additional markers are needed for the diagnosis, such as positivity for Vimentin, S-100, cytokeratins, EMA, INI1 **(Figure 3 D-H)**) and negative stains for vascular (CD34, CD31, ERG, FLI) and neuroendocrine (Synaptophysin, SSTR2A) markers (6).

Clinical and radiological correlation is helpful in cases where the material obtained for the histopathological diagnosis is scant since chordomas frequently affect clivus.

Craniopharyngioma

For decades, craniopharyngiomas have been classified as a tumor type with two subtypes, named adamantinomatous and papillary. The current WHO classification of CNS tumors classifies them as distinct tumor types, corresponding to CNS WHO grade 1 (5). Both types are clinically usually presented with hypopituitarism or signs of increased intracranial pressure (36).

Adamantinomatous craniopharyngiomas constitute all craniopharyngiomas diagnosed in childhood and up to 80% of craniopharyngiomas diagnosed in adults (5). They are composed of epithelium with peripheral palisading, organized in cords, lobules, nodular whorls and trabeculae. Nodules of anucleate ghost-like squamous cells, named "wet keratin", calcifications and areas of microcystic stellate reticulum are characteristic **(Figure 4 A-B)**. Furthermore, xanthogranulomatous reaction, cholesterol clefts, hemosiderin deposits and lymphoplasmacytic infiltrate can be observed as a reaction to the ruptured cystic material, making differential diagnosis with the cyst of Rathke cleft challenging (5). Nuclear p63 positivity is observed in all layers of the epithelium in adamantinomatous craniopharyngiomas **(Figure 4C)**. Nuclear translocation of ß-catenin positivity is seen in scattered cells **(Figure 4D)** (37).

Papillary craniopharyngiomas principally occurs in adults (5). They are composed of non-keratinizing epithelium covering papillary fibrovascular cores **(Figure 4 E-F)**. In contrast to adamantinomatous craniopharyngiomas, wet keratin, stellate reticulum, palisading of the cells and calcifications are absent. Squamous differentiation can be proved by p63 and CK5/6 positivity, whilst

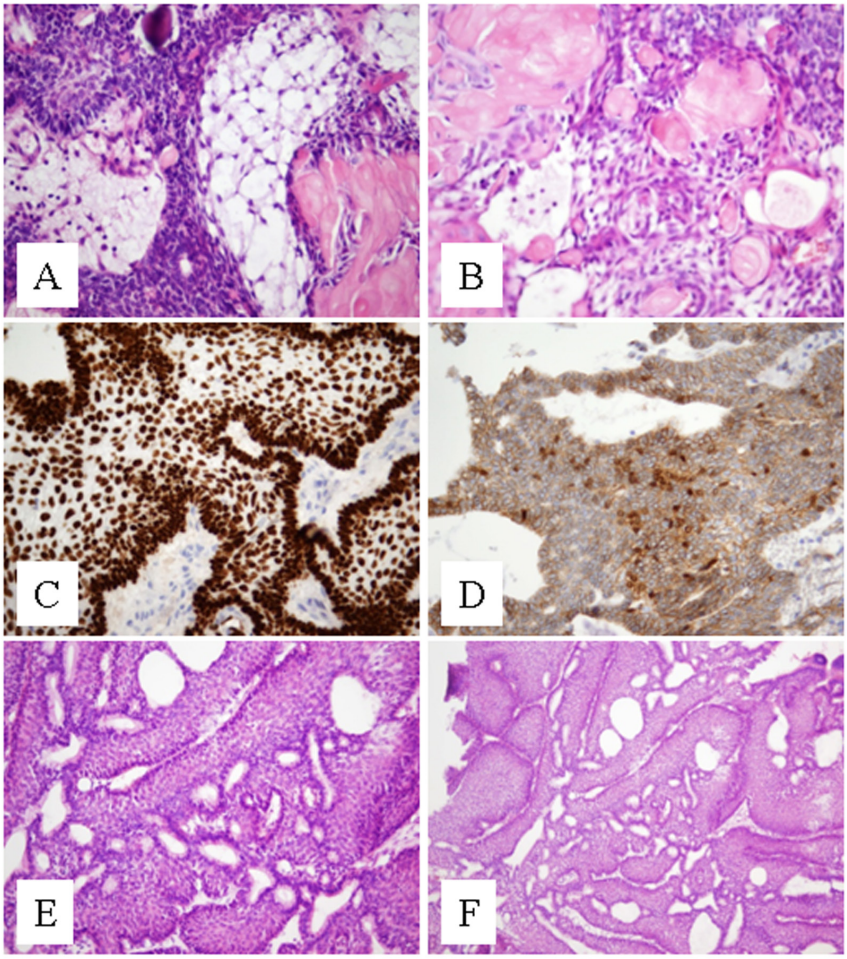

Figure 4. Adamantinomatous craniopharyngioma is composed of cords and trabeculae of cells punctuated by wet keratin (composed of anucleated ghost cells) and stellate reticulum (**A and B**, HE x400). P63 demarcates nuclei in all layers of epithelial cells adamantinomatous craniopharyngioma (**C,** p63, x400). **D.** β-catenin positivity is membranous in the majority of epithelial cells; nuclear translocation of positivity is observed in scattered cells (β-catenin, x400). **E.** Papillary craniopharyngioma is composed of well-differentiated non-keratinizing squamous epithelium (HE, x400); **F.** Papillary architecture can be observed on lower magnification (HE, x200).

ß-catenin positivity is membranous. All papillary craniopharyngiomas harbor BRAF V600E mutation, which can be detected by immunohistochemistry (37). This characteristic is useful in challenging cases of differentiation between papillary craniopharyngiomas and Rathke cleft cyst with squamous metaplasia.

Rathke cleft cyst

Rathke cleft cyst occurs at the junction between the anterior and posterior lobe of the pituitary gland, causing compressive effects on the gland and adjacent structures (38,39). The lining of the cyst varies from cuboidal to cylindric cells, with occasional ciliation on the surface. If present on slides, the content of the cyst is dense, colloidal and amorphous. In immunohistochemistry, the epithelium is positive for cytokeratins (usually CK7, exceedingly CK20) and negative for S-100 and Synaptophysin **(Figure 5)** (40). The differential diagnosis between Rathke cleft cysts and craniopharyngiomas might be challenging and demands a detailed correlation between pathological, endocrinological, neurosurgical and neuroradiological findings (41). Regarding papillary craniopharyngioma, it could be overcome by the use of BRAF V600E antibody, since its immunohistochemical positivity is observed in papillary craniopharyngioma, in contrast to the epithelium of Rathke cleft cyst, where it is negative (42).

Germ cell tumors

After the pineal region, the sellar localization of germinomas is the most frequent (5). They usually affect children, without sex predilection on the sellar region. The diagnosis of teratomas (mature, immature and the variant of somatic-type malignancy), germinomas, embryonal carcinomas, yolk-sac tumors, choriocarcinomas, as well as mixed germ cell tumors should be established with a specific combination of a broad spectrum of antibodies, including OCT4, CD117, PLAP, SALL4, CD30, AFP, ß-HCG and LMWCK for all cases of sellar tumors suspected to germ-cell tumors. Germinomas are characterized by OCT4, CD117, D2-40, PLAP and SALL4 positivity **(Figure 6)** (43). Embryonal carcinomas are positive for OCT4, PLAP, SALL4, CD30 and LMWCK. Yolk-sac tumors show positivity for SALL, AFP and variably for PLAP whilst choriocarcinomas are positive for ß-HCG, LMWCK and variably for PLAP (5). Importantly, all slides should be analyzed with special care, having in mind the possibility that some types of mixed germ cell tumors can be present in extremely small areas (sometimes a square millimeter). The presence of additional components of germ cell tumors prompts changes in oncological treatments of such patients.

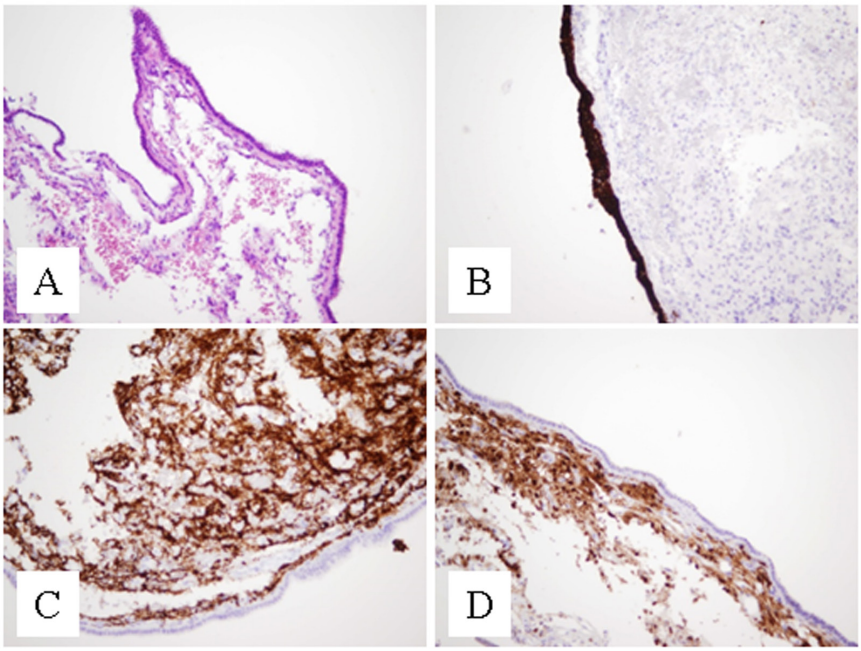

Figure 5. Rathke cleft cyst. **A.** The wall of the cyst is covered by cylindric epithelium (HE, x200). The epithelium is positive for CK7 **(B)** and negative for Synaptophysin (**C,** x200) and S-100 (**D**, x200). Synaptophysin demarcates adjacent anterior pituitary cells **(C)** whilst S-100 demarcates adjacent posterior pituitary cells **(D).**

Metastases

Metastases in the sellar region are rare, encompassing 0.4% of all intracranial metastases and 1.8% of surgically treated sellar lesions (3,44). They most frequently originate from the lungs and breast; metastases originating from the thyroid, cervix, kidney and digestive system were also observed (45). A broad spectrum of immunohistochemical markers (including CK7, CK20, TTF-1, PAX8, CDX2, GATA3) is mandatory for the recognition of the origin of metastasis **(Figure 7)**. This panel should be applied after the negative basic panel for pituitary gland tumors, as well as for all sellar tumors with an expression of neuroendocrine markers, keeping in mind that a metastasis could also come from neuroendocrine tumors of other localization (25). Positivity for TTF-1 and GATA3 should be considered with caution since posterior pituitary tumors express TTF1 and some PitNET types (thyrotroph PitNETs, mature plurihormonal PIT1-lineage tumors and immature PIT1-lineage tumor) express GATA3. These positive stains should be interpreted together with other anterior pituitary transcription factors and hormones.

Hematologic malignancies

Hematologic malignancies are rare in sellar region, mostly being detected in end-stage primary disease (3). Nevertheless, they should be considered as a primary

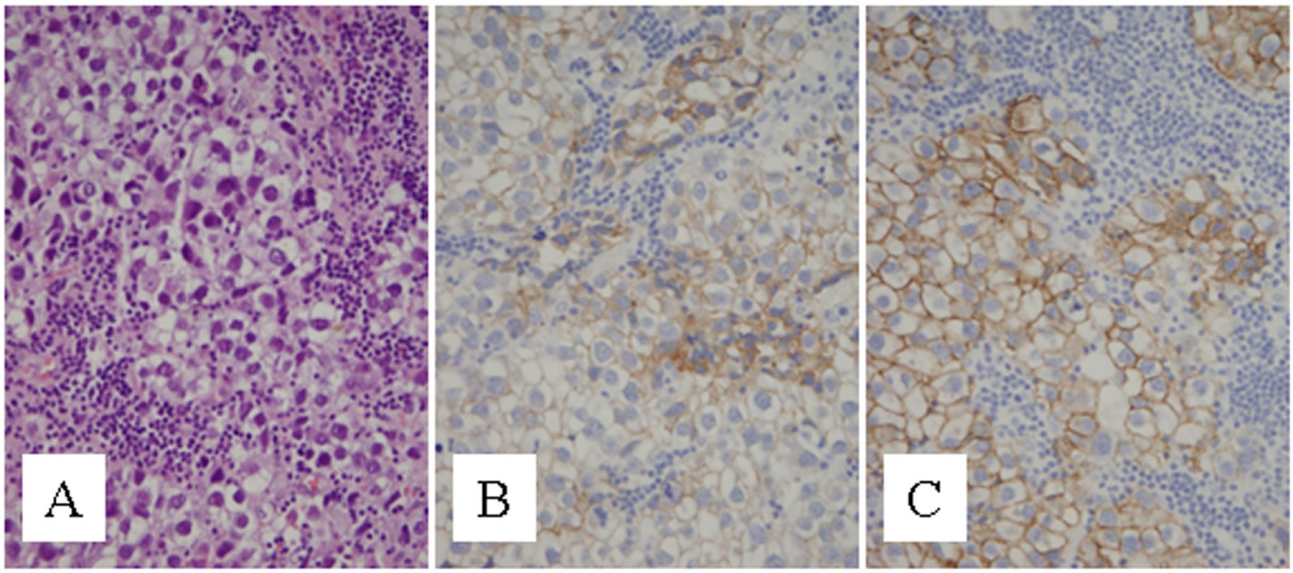

Figure 6. Germinoma. **A.** On HE sections, germinoma is characterized by large polygonal cells with pale cytoplasm, large nuclei with nucleoli, and separated with groups of small lymphocytes (HE, x400). **B.** Germinomas show CD117 positivity in membrane and/or Golgi zone (CD117, x400); **C.** Membranous D2-40 positivity is characteristic of germinoma (D2-40, x400).

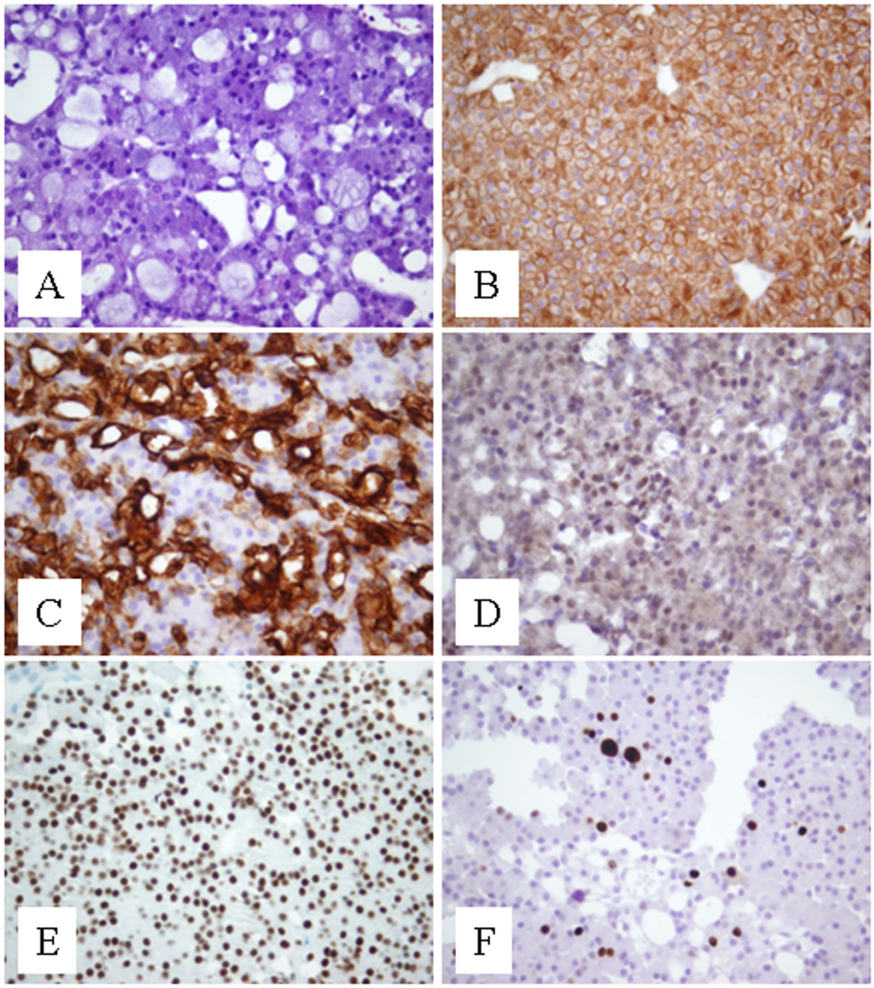

Figure 7. Metastasis of the carcinoma of the kidney in the sellar region is recognized by cribriform histological pattern (**A,** HE, x400), diffuse cytokeratin AE1/AE3 (**B,** x400) and focal cytokeratin 7 (**C,** x400) positivity. Focal nuclear positivity for GATA3 (**D,** x400) and diffuse nuclear PAX8 positivity (**E,** x400) suggest that the metastasis originates from renal carcinoma. Increased proliferative Ki-67 index, compared to other sellar tumors, also suggest the possibility of metastasis (**F,** x400).

manifestation of the disease, although being exceedingly rare. Multiple myeloma, lymphomas (both secondary and primary), as well as acute and chronic leukemia were described in literature (46). Such cases should be carefully distinguished from more frequent hypophysitis and correlated with endocrinological and hematologic findings before establishing the final diagnosis by an experienced hematopathologist (47). A large list of antibodies for hematologic malignancies and molecular analyses are necessary for the proper diagnosis, which overcomes the scope of this review.

Conclusion

We conclude that the heterogeneity of sellar tumors requires extensive experience, principally in the field of neuropathology, in order to cover a wide spectrum of histopathological diversity and avoid pitfalls in their diagnosis. A multidisciplinary approach, including endocrinological, neuroradiological and neurosurgical aspects, is mandatory for establishing an accurate diagnosis, reducing dilemmas and offering the best options for the further treatment of patients with sellar tumors.

Acknowledgements

None

Conflict of interest

None to declare

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PATOLOGIJA TUMORA SELARNE REGIJE: SAVREMENI DIJAGNOSTIČKI PRISTUP

Emilija Manojlović Gačić[1], Martina Bosić[1], Ivan Zaletel[2], Marko Stojanović[3,4], Mirjana Doknić[3,4], Sandra Pekić Đurđević[3,4], Dragana Miljić[3,4], Dejan Rašić[4,5], Sofija Glumac[1]

Sažetak

Selarna regija je kompleksno područje koje se nalazi u sredini baze lobanje, sa hipofizom u centralnom položaju i bliskim anatomskim odnosom sa vitalnim strukturama, što čini osnovu za razvoj brojnih endokrinoloških i neuroloških stanja uzrokovanih ekspanzijom ili sekretornom aktivnošću tumora. U ovom članku ćemo obuhvatiti literaturu koja sagledava histopatološke i imunohistohemijske aspekte selarnih tumora, zajedno sa kliničkim (pretežno endokrinološkim) karakteristikama, koje su ključni elementi za postavljanje dijagnoze. Predstavićemo histopatološki dijagnostički algoritam za najčešće tumore ovog regiona, neuroendokrine tumore hipofize, kao i tumore zadnje hipofize, meningiome, kraniofaringiome, hordome, tumore germinativnih ćelija, hematološke malignitete, metastaze i ciste Ratkeovog špaga. Savremeni histopatološki dijagnostički pristup selarnim tumorima u velikoj meri zavisi od rutinske upotrebe imunohistohemije za širok spektar antitela, kao i od detaljne korelacije sa endokrinološkim, neurološkim, neurohirurškim i neuroradiološkim aspektima, koji redukuju diferencijalno-dijagnostiče dileme, čime se omogućava izbor najboljih opcija za dalje lečenje pacijenata sa selarnim tumorima.

Ključne reči: selarni tumor, neuroendokrini tumor hipofize, imunohistohemija

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REVIEW

The role of radiotherapy in the treatment of ewing sarcoma of bone

Predrag Filipović[1], Marija Popović-Vuković[1], Marina Nikitović[1,2]

[1]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

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Correspondence to:
Predrag Filipović
Institut za onkologiju i radiologiju Srbije
Paterova 14, 11000 Beograd
+381117350683
predrag.filipovic@ncrc.as.rs

Summary

Ewing sarcoma (ES) is the second most common primary bone malignancy after osteosarcoma. The disease most often occurs in adolescence, with peak incidence around the age of fourteen. The most common primary location of the tumor is the bones of the pelvis, followed by the ribs, spine and long bones of the extremities such as the tibia and the fibula. ES shows a tendency towards hematogenous dissemination, primarily in the lungs and bones, much less often lymphogenously. One third of patients have distant metastases present at initial diagnosis, which is why ES is considered a systemic disease.

The treatment of Ewing sarcoma is based on a multimodal approach that includes the use of chemotherapy, surgery and/or radiotherapy. The identification of prognostic parameters enabled the individual treatment of patients based on the assessed risk group. With the application of modern therapeutic protocols, five-year survival for patients with localized disease lies between 60% and 68%, while for patients with metastatic disease, five-year survival is still unsatisfactory and is around 17%.

ES belongs to the group of radiosensitive tumors, and radiotherapy plays a very important role in the local control of the disease, in combination with surgical treatment or independently, and can be applied as radical, preoperative or postoperative radiation therapy. Also, radiation therapy has a role in the palliative approach to the treatment of lung metastases and other metastatic sites.

Considering that the modern multimodal treatment of Ewing sarcoma leads to long-term survival, it is necessary to take into account the expected side effects of the therapy that can reduce the quality of life of treated patients.

Modern radiotherapy techniques such as three-dimensional conformal radiation therapy (3D CRT), intensity modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT) enable precise delivery of the prescribed dose of radiation to target volumes with better sparing of surrounding normal tissues and organs, which leads to a lower incidence of late sequelae of radiation therapy and enables the preservation of the quality of life of treated patients.

Key words: Ewing sarcoma, radiotherapy, radiotherapy techniques

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INTRODUCTION

Epidemiology and etiology of Ewing's sarcoma

After osteosarcoma, Ewing's sarcoma is the most common bone malignancy. The disease can occur in any age group, but it most often occurs in adolescence and young adulthood, and about 86% of tumors occur before the age of forty (1). The incidence of this tumor is estimated at 2.93/1,000,000 per year in the white population and has not changed significantly in the past 30 years (2).

The etiology of this disease has not been fully elucidated, but numerous studies that could clarify the molecular-genetic pathways crucial in the pathogenesis of Ewing's sarcoma are underway.

Pathohistology and genetics

Pathohistologically, Ewing's sarcoma is a tumor of small round blue cells that is often PAS, vimentin and cytokeratin positive (6). Tumors of the Ewing sarcoma family are thought to share a common precursor cell that is of neuroectodermal origin (5). Cytogenetic analyzes showed a certain number of translocations in Ewing's sarcoma cells, which probably have an impact on the tumorigenesis of this disease.

The characteristic translocation for Ewing sarcoma is t(11;22) (q24;q12). This translocation leads to the fusion of the EWS and FL1 genes, which results in the formation of an aberrant protein that plays the role of an oncogenic transcription factor. More recently, new translocations that do not involve the EWS gene, such as BCOR-CCNB3 and CIC-DUX4 fusions (8-9) have been discovered, which do not currently have a clear clinical significance. In addition to gene aberrations, epigenetic changes such as DNA methylation and their possible influence on the pathogenesis of Ewing sarcoma have recently been increasingly studied. Some of the genes that are hypermethylated in Ewing sarcoma cell lines are MGMT, HIC1, CDH1, p16 and p15. (10-11). So far, the only gene whose methylation is associated with a worse prognosis is RASSF1A (12).

Natural History

The disease is most often presented by pain and palpable swelling of the region affected by the tumor. The primary localization of Ewing sarcoma is most often on the bones of the pelvis and then in the region of the ribs, spine and long bones of the extremities such as the tibia and the fibula (4). In about a quarter of patients there is limited mobility of the extremities (13). In the case of localization in the chest area or pelvic localization that accompanies intracavitary tumor growth, it may take a long time for the first symptoms to appear (5). If it is a primary tumor, or if metastatic lesions are localized in the spinal region, in addition to pain, paresthesias may also occur if the tumor compresses the nerve roots (14). As Ewing's sarcoma occurs in adolescence, the pain caused by the tumor is often attributed to "growing pains" which prolongs the time from symptom onset to diagnosis. The time to diagnosis (TtD) in Ewing's sarcoma is 3-6 months and it is the longest TtD in the population of pediatric tumors (15).

Ewing's sarcoma most often metastasizes hematogenously to the lungs and bones, while lymphogenic spread and the affection of regional lymphatics are rare. About 30% of patients have metastatic disease at initial diagnosis (3).

Diagnostics

The first and most common diagnostic method is radiography of the affected region. A radiograph can show tumor osteolysis of bone (aggressive bone osteolysis), lifting of the periosteum by the tumor (Codman's triangle) as well as the extension of calcified spicules into the soft tissue.

The gold standard in diagnostics is magnetic resonance imaging (MR) of the primarily affected region, which must be performed before the biopsy of the tumor and obtaining a pathohistological diagnosis. MR examination enables precise determination of the size of the initial tumor, which is one of the most important prognostic parameters and is necessary in case of later planning of radiotherapy. It is necessary to include the entire bone in the examination due to the possible presence of metastases in the affected bone, which can occur in 14% of cases (16). The tumor shows a signal intensity that is hypointense on T1W sequences, and hyperintense on T2W sequences, in 96% of cases the presence of an extraosseous component of the tumor is characteristic, which shows a significant enhancement of the post-contrast signal enhancement (17).

In order to complete the staging of the disease, it is necessary to perform a computerised tomography (CT) of the thorax and scintigraphy of the skeleton in order to detect possible metastases in the lungs and bones.

Positron emission tomography (PET CT) with Fluorine F 18-fludoxyglucose (18F-FDG PET) is an optional modality for determining disease stage, which has shown high sensitivity and specificity for ES. This method has an increasing role in the detection of skeletal metastases, but due to its poorer resolution, it has a lower sensitivity compared to chest CT in the case of lung metastases (5).

Standard diagnostic methods include bone marrow aspiration and biopsy.

Prognostic parameters and prognostic groups

The most significant prognostic parameter is the presence of metastatic changes at diagnosis. In patients with metastatic disease, the five-year survival rate is less than

30%. An exception are the patients with isolated metastases in the lungs, whose five-year survival is about 50% (18). In the case of localized disease, a favorable prognostic factor is primary localization on the extremity, tumor volume less than 100ml (or in some works below 200ml), and age below 15 years at diagnosis (4). Numerous molecular parameters such as alterations of P53, p16, as well as the presence of vascular endothelial growth factor (VEGF) and CCN3 proteins are associated with an unfavorable prognosis (19-21).

The grouping of the aforementioned prognostic factors is very important for determining the therapy. According to the Euro Ewing protocol, after the sixth cycle of induction chemotherapy, patients are grouped into three therapeutic groups (R1, R2 and R3). In the case when after induction chemotherapy and after surgery, patients have a good histopathological response to chemotherapy and in the case when the tumor volume is below 200 ml, patients are classified in the R1 group. Patients who have a poor histopathological response to chemotherapy, a tumor volume of 200 ml or the presence of lung metastases belong to group R2. Group R3 includes patients who initially had metastatic disease (e.g metastases in the bones and bone marrow). Stratification into the mentioned groups enables adequate prescribing of adjuvant therapy. (23).

TREATMENT OF EWING'S SARCOMA OF BONE

A modern approach to the treatment of Ewing's sarcoma, both localized and metastatic, involves the use of a multimodal approach that includes the use of chemotherapy, surgical treatment and/or the use of radiotherapy. Using modern therapeutic protocols, five-year survival for patients with localized disease is 60% - 68%, while for patients with metastatic disease, five-year survival is still unsatisfactory and is around 17%. (5.3)

Ewing's sarcoma is a systemic disease, and the treatment according to protocols begins with induction chemotherapy, followed by local treatment. The use of induction therapy significantly prolongs the survival time of patients with a localized tumor (22). Also, given that the tumor volume is reduced, optimization of local treatment is possible: the possibility of total resection is improved, and in case of radiotherapy, it leads to a reduction of the radiation volume, which reduces the toxicity of the therapy.

In Europe, in case of a localized tumor, the Euro Ewing protocol is most often applied, which involves the application of induction polychemotherapy with six cycles of vincristine, ifosfamide, doxorubicin and etoposide (VIDE regimen). After four cycles of therapy, the therapeutic response is evaluated and a decision is made about local treatment, which can be in the form of surgical treatment, radiotherapy or a combination of these two modalities. After the local treatment, treatment continues with consolidation chemotherapy (seven cycles of chemotherapy according to the vincristine, dactinomycin, ifofosfamide/cyclophosphamide regimen). (23)

Planning optimal local therapy requires a multidisciplinary approach that involves the participation of experts from these fields. Surgical treatment is carried out with the aim of wide resection of the tumor with confirmation of negative margins of the tumor borders and with acceptable morbidity. If it is not possible to achieve this goal, surgical resection can be marginal or intralesional, or even radical in the form of amputation.

In the case of a metastatic tumor with the presence of lung metastases, the Euro Ewing protocol involves the application of induction therapy according to the VIDE protocol with radiotherapy of the lungs and the primary site of the disease and then consolidation chemotherapy according to the vincristine, dactinomycin ifofosfamide protocol (VAI). An alternative option is high-dose chemotherapy with busulfan and melphalan with autologous blood stem cell transplantation. (23).

RADIOTHERAPY IN THE TREATMENT OF EWING'S SARCOMA

Ewing's sarcoma belongs to the group of radiosensitive tumors, so radiotherapy plays a significant role in its treatment and can be applied in the definitive, preoperative, postoperative and palliative approach.

Definitive radiotherapy

Definitive radiotherapy involves the application of radiotherapy as the only type of local therapy when surgical treatment is not possible. These are most often patients with large tumors that are localized in places unfavorable for adequate surgery, such as tumors on the spinal vertebrae or the pelvis.

When planning radiation therapy, it is important to define the following target volumes: the tumor volume (Gross Tumor Volume - GTV) which includes the tumor visible on CT or MR imaging, the clinical target volume (Clinical Target Volume - CTV) which includes the zone of probable microscopic spread diseases around the GTV, and the planned target volume (Planning Target Volume - PTV) by contouring the margin around the CTV, which, with its width, includes possible inaccuracies in repositioning the patient during the performance of fractionated radiation.

Tumor volume (GTV) is determined based on the initial disease (before induction chemotherapy). In patients who have a tumor that initially protrudes into the chest or abdomen ("pushing" the lung parenchyma and bowel), and is reduced after administration of induction chemotherapy, the GTV is modified to reduce lung or bowel irradiation. CTV includes all possible places of microscopic extension of the disease (scar, biopsy tracks) and is mainly formed by the expansion of GTV by 1.5cm-

2cm radially (which depends on the anatomical localization of the tumor). The CTV must respect anatomical boundaries such as fascial barriers and bone. The PTV is defined by the margin around the CTV. Depending on the localization and immobilization of the patient, it is 0.5 cm to 1 cm. (40).

The dose applied in case of macroscopic disease is from 55.8Gy to 60Gy in daily fractions of 1.8Gy/2Gy (24). If a dose of less than 40Gy is used, local control is worse even for lesions that are below 8cm (25). According to the current EuroEwing protocol, the dose for defintive radiotherapy is 54 Gy with the possibility of a "boost" dose of 5.4 Gy (40).

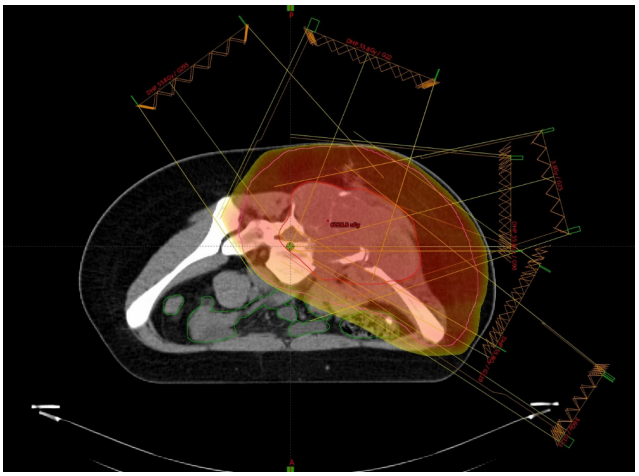

Figure 1. Definitive radiotherapy of a pelvic tumor (Institute for oncology and radiology of Serbia)

Postoperative radiotherapy

Postoperative (adjuvant) radiotherapy involves the application of radiotherapy after surgical treatment in patients with residual microscopic disease, inadequate margins or in patients who have a poor histopathological response to preoperative chemotherapy, i.e. have viable tumor cells >10%. It must be performed in cases of intralesional surgery.

Postoperative radiotherapy improves local control after surgical treatment (26). It is particularly effective in case of marginal or intralesional surgical resection (frequency of local recurrence 5% with and 12% without radiotherapy) (27). In case of wide resection but poor histological response to induction chemotherapy, the frequency of local recurrence is 12% without and 6% with adjuvant radiotherapy (28). The observational analysis of the EURO EWING group study showed (median follow-up was 6 years) that the frequency of local recurrence was 11.9%. It has also been shown that the addition of radiotherapy significantly reduces the frequency of recurrence compared to surgical treatment alone. Of all the groups, the greatest benefit in reducing local recurrence was experienced by patients who had a tumor volume of less than 200 ml and a good response to induction chemotherapy (29).

When planning postoperative radiation therapy, by definition, there is no GTV, but it is useful to delineate the preoperative GTV based on preoperative/prechemotherapy diagnostics in order to facilitate the formation of the CTV. Primarily, on the basis of the preoperative GTV, CTV1 is formed, which should include all places of potential microscopic spread, including prostheses, drainage lines, and the surgical scar. Depending on the anatomical localization, CTV1 is formed as GTV + 1.5cm to 2cm radially. Anatomical barriers to tumor spread must be taken into account. In case when the inclusion of the entire surgical scar implies the formation of a large radiation volume that will cause significant morbidity, only a part of the scar can be included. The second part of radiation therapy includes CTV2, which is formed by the expansion of the GTV by 1-2 cm (depending on the anatomical localization). The formation of PTV is equal to the formation in the planning of preoperative and definitive radiotherapy.

Adequate immobilization, which enables adequate reproducibility of the therapeutic position, is extremely important for extremity tumors. The CTV includes 2 cm from the GTV of the tumor in the bone and another 2 cm craniocaudal to the pre-chemotherapy extraosseous tumor mass. It is preferable to spare the joints and epiphyseal plates. It is also necessary to spare part of the circumference of the entire extremity in order to preserve adequate lymphatic drainage and reduce the incidence of lymphedema. It is considered necessary to preserve at least 10% of the limb circumference on each axial section of the therapeutic CT.

In tumors of the cervical spine and skull, GTV - CTV margins can be below 1.5 cm and can be corrected based on anatomical sections and to spare critical structures such as the spinal cord or optic chiasm.

Pelvic tumors often initially have a large volume that protrudes into the pelvic and abdominal cavity. Often, after induction chemotherapy, the tumor shrinks and normal tissues such as the intestines return to their normal position. The CTV needs to be adjusted so that the intrapelvic and intraabdominal organs are not unnecessarily in the air field.

In chest wall tumors, the formation of the CTV is similar to the formation of the CTV in case of pelvic tumors (post-chemotherapy tumor reduction, correction of the CTV to avoid unnecessary irradiation of the heart and lungs.). In the event that the tumor involves the pleura with the presence of pleural effusion, it is necessary to irradiate the entire hemithorax with a "boost" to the primary site of the disease.

In case of spinal and paraspinal tumors, it is necessary that the GTV includes the entire tumor as well as the extraosseous extension. CTV should include the vertebra above and the vertebra below the involved vertebra as well as the surgical scar and surgical endoprostheses (in case of previous surgical intervention). (23).

According to the EuroEwing 2012 protocol, the dose for postoperative radiotherapy is 54 Gy, where 45 Gy is

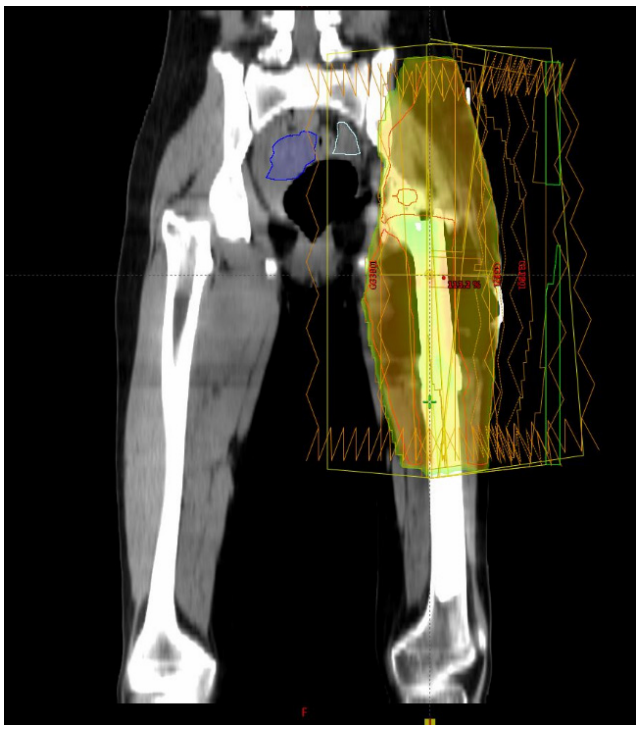

Figure 2. Postoperative radiotherapy of Ewing's sarcoma of the extremity (Institute for oncology and radiology of Serbia)

prescribed for a larger radiation volume with an additional "boost" dose of 9 Gy for a reduced radiation volume (40).

Preoperative radiotherapy

Preoperative radiotherapy is indicated in cases where tumor progression occurs during induction therapy, as well as when adequate reduction of the tumor do not occur after induction therapy and positive margins are expected during surgical treatment.

Preoperative (non-adjuvant) radiotherapy was first incorporated into the EISECC 92 protocol with the idea of reducing iatrogenic tumor dissemination during surgery and in cases where positive or "narrow" (<1mm) resection margins are expected after chemotherapy (30). The use of non-adjuvant radiotherapy has no effect on the event-free survival, while local control is satisfactory and amounts to 6% after five years. (30).

The delineation of target volumes when planning neoadjuvant therapy is identical to the delineation of volumes for definitive radiotherapy.

According to the current EURO EWING protocol, the dose for preoperative radiotherapy is 50.4 Gy in 28 radiation fractions. In case of an extremely large radiation volume that endangers the surrounding normal organs and tissues, the dose can be reduced to 45 Gy in 25 fractions (40.)

Palliative radiation therapy

Palliative radiation therapy in Ewing's sarcoma plays a significant role in the treatment of lung, bone and endocranial metastases, as well as in rapidly progressing primary tumors.

In case of lung metastases, whole lung irradiation (WLI) is used. For patients with isolated lung metastases, the analysis of the EISECC study showed the benefit of whole lung radiotherapy (four-year EFS was 40%) (31). The clinical target volume includes the pleural cavity of the surfaces of both lungs. The PTV margin is 1 cm and it is preferable to use respiratory gating. (36). According to the current EuroEwing protocol, the dose used during WLI is 15Gy in 10 fractions for patients below 14 years of age, while the dose for patients aged 14 years and older is 18Gy in 12 fractions (36).

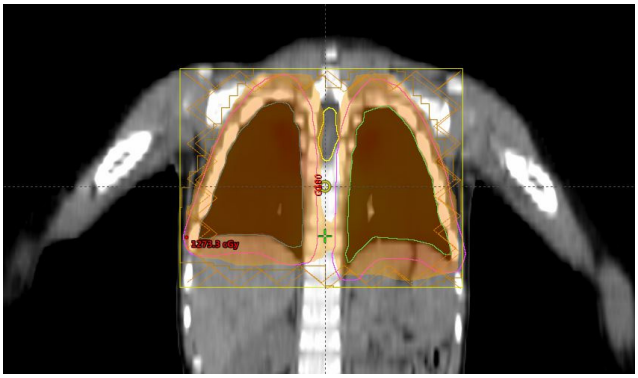

Figure 3. Whole lung radiotherapy (Institute for oncology and radiology of Serbia)

In the presence of extrapulmonary metastases, it is important to undergo a local treatment of both the primary tumor and metastases (the three-year disease-free period in case of local treatment of metastases and primary tumor is 39%, while in case of local treatment of only the primary tumor or only metastases is 17%)(32). When it comes to bone metastases, definitive radiation therapy (radical dose) can be applied to all bone metastases and the primary tumor simultaneously. When delineating bone metastases, it is important to create a smaller radiation volume so that less than 50% of the bone marrow is included in the total radiation volume. Irradiation of more than 50% of the bone marrow can lead to a significant myelosuppressive effect that may preclude the use of systemic therapy. In case of numerous bone metastases that cannot be irradiated at the same time as the primary lesion, the radiotherapy of the metastases is carried out after the radiotherapy of the primary tumor. Nowadays, stereotactic radiotherapy is increasingly used in the treatment of bone metastases. With this technique, a dose of 35-40 Gy in 5 fractions can be administered to bone metastases smaller than 5 cm (33).

In the case of the presence of endocranial metastases, stereotaxic radiotherapy is recommended if there are adequate indications. In other cases, irradiation of the entire endocranium (Whole Brain Radiotherapy - WBRT) is carried out with a dose of 30 Gy in 15 fractions (23).

If there is a rapidly progressive tumor, it is possible to perform palliative radiation therapy with TD 36Gy in 12 sessions (23).

RADIOTHERAPY TECHNIQUES IN THE TREATMENT OF EWING SARCOMA

Technological advances in radiotherapy (modern linear accelerators with multi-lamellar collimators), improved immobilization systems, as well as new knowledge in the fields of radiobiology and physics have enabled the application of conformal techniques, which involve the modulation of beams so that the planned target volume and dose distribution correspond to the irregular shape of the tumor. The high degree of conformality also allows reduced high-dose irradiation of the surrounding healthy tissues. (32). Conformal techniques include: three-dimensional conformal radiotherapy (3D CRT), intensity modulated radiotherapy (IMRT) and volumetric arc therapy (VMAT) (34).

IMRT is a form of modern 3D conformal radiotherapy that enables high conformation of the radiation volume (especially concave tumor volumes that are near the organs at risk). Radiation volumes of a complex shape can be achieved by dividing the beam into smaller beams that can have different intensities and treat small volumes within the target volume, which enables the application of a higher radiation dose to the target volume. In addition to the above, the presence of multi-lamellar collimators allows shaping the beam to the shape of the tumor (35).

A study by Mounessi et al. comparing 3D CRT and IMRT technique in the treatment of pelvic tumor localization showed that IMRT has significant advantages over 3D CRT. Primarily, the IMRT technique makes it possible to achieve a better conformation of the radiation volume. Also, by using the IMRT technique, better protection of the small intestine is achieved (the volume of the small intestine that receives doses from 2Gy to 60Gy is lower when using the IMRT technique). In case of the bladder and rectum, there are no differences in the mean dose that these organs receive, but the volumes that receive 30Gy, 40Gy, 45Gy and 50Gy are smaller in case of the IMRT technique (36).

The VMAT technique also represents a modern form of conformal radiotherapy, which owes its high conformality to the continuous delivery of the dose, which is achieved by arcing the head of the apparatus, as well as to the continuous corrections of the beam using a multi-lamellar collimator located in the head of the apparatus. Using the VMAT technique, it is possible to achieve even a higher degree of conformality than with the IMRT technique, but at the cost of irradiating a large volume of surrounding healthy tissue with low doses.

Radiotherapy of Ewing's sarcoma is challenging because the pediatric population has a higher sensitivity and lower tolerance to radiation therapy compared to adults. The application of proton radiotherapy in the treatment of Ewing's sarcoma is being increasingly examined. In case of Ewing's sarcoma of the spinal column, proton therapy has an advantage over IMRT in terms of an improved index of conformality and homogeneity, significantly lower doses to the lungs, heart and liver, as well as reduced integral doses to the whole body (37-38).

ADVERSE EFFECTS OF RADIOTHERAPY

Unwanted effects of radiotherapy can be divided into early and late.

Early side effects of radiotherapy occur during radiation therapy and can last up to several weeks upon the end of radiotherapy treatment. These side effects are usually reversible. They are most often manifested by the presence of skin changes in the form of radiodermatitis (erythema of the skin, dry or wet desquamation), as well as by a myelosuppressive effect, which is most often manifested by leukopenia and neutropenia. The degree of myelosuppressive effect depends on the volume of active bone marrow that is included in the radiation volume.

Late complications occur in a period of time from several months to several years after the treatment and are irreversible in their nature. The severity and type of late complications depend on the localization of the irradiated region as well as the radiation dose.

About 41% of patients treated for Ewing's sarcoma have no late complications from combined therapy after 5 years (41).

The most common late complications of radiation therapy are abnormalities in bone growth, fracture of the irradiated bone, chronic lymphedema, chronic pain, fibrosis of the treated extremity, and limited range of motion.

A retrospective study investigating long-term treatment complications in a population of 101 patients showed that the most common chronic complications are related to the musculoskeletal system. Most of the mentioned patients were treated with multimodal therapy. 12 patients had asymmetry of bone growth, 12 patients had shortening of the length of the treated limb, 2 patients had chronic lymphedema, 3 patients reported the presence of chronic pain. Less frequent complications were problems with dentition and speech in patients irradiated to the head and neck region (42). Considering the fact that irradiation of epiphyseal plates stops bone growth, the most common late skeletal complications of radiotherapy represent abnormalities in the growth of the treated bone. The degree of bone growth abnormalities depends on the localization of the irradiated epiphyseal plate, the patient's age and the total radiotherapy dose (5).

Bone fractures can be a late complication of Ewing's sarcoma. Based on a retrospective study of 93 patients, it was shown that the most common fracture site is the proximal femur. From the mentioned cohort, 9 patients had a pathological fracture after radiotherapy. Of those 9 patients, in 3 patients the cause of the fracture was active recurrent disease or secondary malignancy. All patients

who had a bone fracture after radiotherapy that was not related to recurrent disease or secondary malignancy were irradiated with doses higher than 40Gy (43).

A significant late complication of radiotherapy is the appearance of tumors induced by radiotherapy. These tumors appear within the limits of the radiation volume after a latent period of more than four years. Also, the histopathological type of the tumor differs from the type of the primary tumor (44).

It is considered that the pediatric population has a tenfold risk for the development of induced malignancy than the adult population. Considering the long-term survival of patients treated for Ewing's sarcoma, frequent check-ups are necessary and should be carried out over a longer period of time (45).

CONCLUSION

Radiotherapy has an important role in the treatment of Ewing's sarcoma of the bone. The biggest benefit of radiotherapy is the improvement of local disease control both in the postoperative and in the definitive approach. Even in metastatic disease, radiation therapy plays a significant role, especially in the treatment of lung metastases. The application of modern conformal radiotherapy techniques enables a better conformation of the beam volume to the shape of the tumor and a better sparing of healthy tissues, which results in a lower incidence of late complications of radiotherapy. Modern techniques also allow escalation of the dose to the primary tumor, which theoretically can lead to an even better degree of local control.

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ULOGA RADIOTERAPIJE U LEČENJU EWING SARKOMA KOSTIJU

Predrag Filipović[1], Marija Popović-Vuković[1], Marina Nikitović[1,2]

Sažetak

Ewingov sarkom (ES) predstavlja drugi najčešći primarni malignitet kostiju nakon osteosarkoma. Bolest se najčešće javlja u adolescentnom dobu, sa pikom incidencije oko četrnaeste godine. Najćešću primarnu lokalizaciju tumora predstavljaju kosti karlice a potom rebra, kičma i duge kosti ekstremiteta kao što su tibija i fibula. ES pokazuje tendenciju ka hematogenoj diseminaciji, pre svega u pluća i kosti, znatno ređe limfogeno. Jedna trećina pacijenata ima prisutne udaljene metastaze prilikom incijalne dijagnoze, zbog čega se ES smatra sistemskom bolešću.

Lečenje Ewing sarkoma se zasniva na multimodalnom pristupu koji obuhvata primenu hemioterapije, hirurgije i/ili radioterapije. Definisanje prognostičkih parametara omogućava svrstavanje pacijenata u rizične grupe prema kojima se određuje plan lečenja. Primenom savremenih terapijskih protokola petogodišnje preživljavanje za pacijente sa lokalizovanom bolešću iznosi između 60% - 68% dok je kod pacijenata sa metastatskom bolešću petogodišnje preživljavanje i dalje nezadovoljavajuće i iznosi oko 17%.

ES spada u grupu radiosenzitivnih tumora, te radioterapija ima veoma značajnu ulogu u lokalnoj kontroli bolesti, u kombinaciji sa hirurškim lečenjem ili samostalno i može se primeniti kao radikalna, preoperativna ili postoperativna zračna terapija. Takođe, zračna terapija ima ulogu u palijativnom pristupu u tretmanu plućnih metastaza i drugih metastatskih mesta.

S obzirom na to da lečenje Ewing sarkoma dovodi do dugogodišnjeg preživljavanja, neophodno je voditi računa o očekivanim neželjenim efektima terapije koje mogu umanjiti kvalitet života lečenih pacijenata.

Savremene radioterapijske tehnike kao što su trodimenzionalna konformalna zračna terapija (3D-CRT), intezitetom modulisana zračna terapija (IMRT) i volumetrijski modulisana lučna terapija (VMAT) omogućavaju preciznu isporuku propisane doze zračenja na ciljne volumene uz bolju poštedu okolnih normalnih tkiva i organa, što dovodi do niže učestalosti pojave kasnih sekvela zračne terapije i omogućava očuvanje kvaliteta života lečenih pacijenata.

Ključne reči: Ewing sarkom, radioterapija, radioterapijske tehnike

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Izdavač i vlasnik:
MEDICINSKI FAKULTET UNIVERZITETA U BEOGRADU

Uredništvo i administracija:
11105 Beograd, Dr Subotića br. 8, soba 311

Tehnički urednik:
Radević Vladimir

Lektor za engleski jezik:
doc. dr Danka Sinadinović

Tehnički sekretar:
Tijana Tošić

Grafički dizajn:
Prof. dr Slobodan Štetić
Fakultet pedagoških nauka u Jagodini Univerziteta u Kragujevcu