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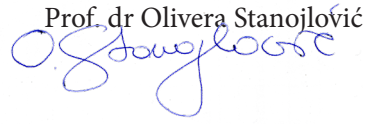

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ORIGINAL ARTICLE

LONG-TERM SURVIVAL AFTER HEART TRANSPLANTATION: THE EXPERIENCE OF THE REPUBLIC OF SERBIA HEART TRANSPLANTATION PROGRAM

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Summary

Introduction/Aim: Heart transplantation (HTX) is the gold standard for the treatment of patients in symptomatic terminal stadium of heart failure. This study aimed to investigate the long-term results of the HTX and to determine the independent survival predictors after HTX within The Serbian Heart Transplantation program.

Methods: A retrospective observational study was performed. The study included 44 patients that were subjected to heart transplantation from 2013 to 2021 within the The Serbian Heart Transplantation program. All the patients were included in the National heart transplantation waiting list according to the contemporary ISHLT criteria. The study included all the patients subjected to HTX, as well as the ones who received long-term circulatory support preoperatively. The data were obtained from the medical history records and follow-up visits.

Results: The average survival of all patients was 1611.8 days (1306.9 - 1916.7). The median length of patient follow-up was 637.4 days (1-2028 days). Six-year patient survival was 70.5%. Cox's regression analysis determined that independent predictors of worse outcome were as follows: age of the recipient, body mass index of the recipient, previous CVI, preoperative chronic renal insufficiency, pulmonary hypertension, infection in the postoperative course, as well as the need for mechanical circulatory support in the immediate postoperative course.

Conclusion: The Serbian Heart Transplantation program demonstrated the survival rate comparable to the HTX centers worldwide and in ISHLT registry. The independent risk factors should be carefully analyzed for our study population and taken into consideration when planning the procedure.

Keywords: heart transplantation, survival, independent predictors

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INTRODUCTION

Terminal stage of heart failure is defined by repetitive hospitalizations due to decompensation, progressive worsening of symptoms, alteration of myocardial function and augmentation of heart cavities despite maximal medical and interventional therapy.(1, 2) This group of patients is classified in NYHA III and IV class, as well as stage D according to the classification of the American Heart Association. (3)

Heart transplantation (HTX) is the gold standard for the treatment of patients in symptomatic terminal stadium of heart failure. Despite the use of optimal medical therapy, resynchronization therapy, alternative surgical or interventional methods, patients with terminal heart failure should be considered as candidates for HTX. (4) Survival of these patients without HTX is 50% in 1-2 years. Primary indications for HTX in adults are non-ischemic cardiomyopathy (53%) and ischemic cardiomyopathy (38%), acquired valvular heart disease (3%) and retransplantation (3%). (5) Candidates are included in the National list of transplantation candidates after performing a wide range of diagnostic procedures. The most important diagnostic procedures for listing heart transplant candidates are cardiopulmonary exercise test and right heart catheterization. (6)

The first heart transplant in the Republic of Serbia was performed in 1989 at the Institute for cardiovascular diseases "Sremska Kamenica", followed by four heart transplantations at the Institute for cardiovascular diseases "Dedinje" in 1990s. After the period of silence the program was reinstituted in the year 2013. Since then until December 2022, 44 heart transplantations were performed and in more than 120 patients short and long-term mechanical circulatory support were implanted within the National transplantation program at the University Clinical Center of Serbia.

This study aimed to investigate the long-term results of HTX and to determine the independent survival predictors after HTX within the Serbian heart transplantation program.

MATHERIALS AND METHODS

Study design

A retrospective observational study was conducted. The study included 44 patients who were subjected to heart transplantation form 2013 to 2021 in the Department for cardiac surgery, University Clinical Center of Serbia.

Indication for the HTX was given by heart failure. The team consisted of: cardiac surgeons, transplantation cardiologists, anesthesiologists, interventional cardiologists, echocardiographers and the other specialists if needed. All patients were included in the National heart transplantation waiting list according to the contemporary ISHLT criteria. (4) The study included all the patients subjected to HTX, as well as the ones who received long-term circulatory support preoperatively.

Surgical technique

All surgeries were done by different transplantation and explantation teams. The donor allocation process was led by the coordinator from the University Clinical Center of Serbia alongside the coordinators from local donor hospitals. Organs were explanted either within the University Clinical Center of Serbia or at local donor hospitals (Medical Military Academy, University Clinical Center of Nis, University Clinical Center of Vojvodina and University Clinical Center of Kragujevac).

After the potential donor was declared brain dead, he/she was subjected to the necessary diagnostic procedures according to the national transplantation protocol in order to be declared as suitable for organ procurement by a network of coordinators. After the donor was declared suitable and the approval was given, the explantation team was directed to the donor hospital. For the donors who had a high level of alosenzitisation (PRA>20%) the result of cross match was obtained before the organ procurement. After the arrival in the donor hospital, the final assessment of the organ was done by the head of the organ harvesting team and the head of the transplantation team was informed and made the final decision regarding the beginning of the procedure. The donor heart was arrested and rinsed by the Custodiol HTK solution and transported in three sterile bags submerged in the same solution stored in the medical refrigerator. All implantation procedures were done with the use of extracorporeal circulation, using bicaval or biatrial technique according to the surgeon preference. After surgery, graft rejection was monitored with regular endomyocardial biopsies, alongside coronary angiography and intravascular ultrasound according to the local protocol.

Data procurement

Data were obtained from the medical history records and follow-up visits. Demographic characteristics, comorbidities, hemodynamic measurements, operative characteristics, postoperative complications and intrahospital mortality. Mortality and organ rejection in the follow-up period were registered from the electronic database and from documentation of follow-up visits. Variables were defined according to the variable definitions from ISHLT guidelines, Euroscore and STS-score definitions.

Statistical analyses

Descriptive statistics was calculated for the baseline demographic and clinical features and treatment outcomes.

Graphical and mathematical methods tested the normality of distribution. As appropriate, continuous variables were presented as means with standard deviations or medians with 25th-75th percentiles. Categorical variables were presented as numbers and percentages. Differences between groups were analysed using Student's t-test for continuous variables (or the Mann-Whitney test) and the Pearson chi-squared test for categorical variables. Survival analysis was performed using the Kaplan-Meier method, and the groups were compared using the log-rank test. In addition, Kaplan-Meier survival curves were truncated at a timepoint in follow-up, when at least 10% of patients were still at risk, to avoid visual misinterpretation.(7) The significance level was set at 0.05, and all testing was two-sided. Statistical analysis was performed using the IBM SPSS Statistics for Windows, version 21.0. (Armonk, NY, USA) package.

RESULTS

Patient characteristics

Forty four patients who underwent heart transplantation were included in the study. The average age of transplanted patients was 46 years (16-64 years). Out of the total number of patients, 39 (88.6%) were male, while 5 (12.4%) transplanted patients were female. Most patients had dilated cardiomyopathy (63.6%) and ischemic cardiomyopathy (29.5%). The majority of patients had A blood group 43.2%, 22.7% had O blood group, 15.9% had B blood group whereas 18.3% of patients had AB blood group. The preoperative characteristics of patients are shown in **Table 1**. The average body mass index was 25.6 (15.3 - 35.8). About 22.7% of patients had a previous cardiac surgery, while 13.6% of them had a Left ventricular assist device (LVAD) mechanical circulatory support implanted as a bridge to heart transplantation. Nineteen (43.2%) patients had pulmonary hypertension preoperatively, while 11 (25%) were on inotropic circulatory support before transplantation.

Table 1. Preoperative characteristics of patients

BMI	±sd	25.6 ± 4.7
HTA	n (%)	14 (31.8)
HLP	n (%)	14 (31.8)
DM	n (%)	10 (22.7)
CKD	n (%)	12 (27.3)
COPD	n (%)	8 (18.2)
PVD	n (%)	3 (6.8)
Stroke	n (%)	4 (9.1)
Smoking	n (%)	21 (47.7)
Previous cardiac surgery	n (%)	10 (22.7)
LVAD	n (%)	6 (13.6)
ICD	n (%)	17 (38.6)
Inotropic support	n (%)	11 (25)
Pulmonary hypertension	n (%)	19 (43.2)

Values are presented as n (%). CKD - chronic kidney disease, COPD - chronic obstructive pulmonary disease, DM - diabetes mellitus, HLP – hyperlipidaemia, HTA - arterial hypertension, PVD – peripheral vascular disease, BMI – body mass index, LVAD – left ventricular assist device, ICD- intracardial defibrillator.

Donor and operative characteristics

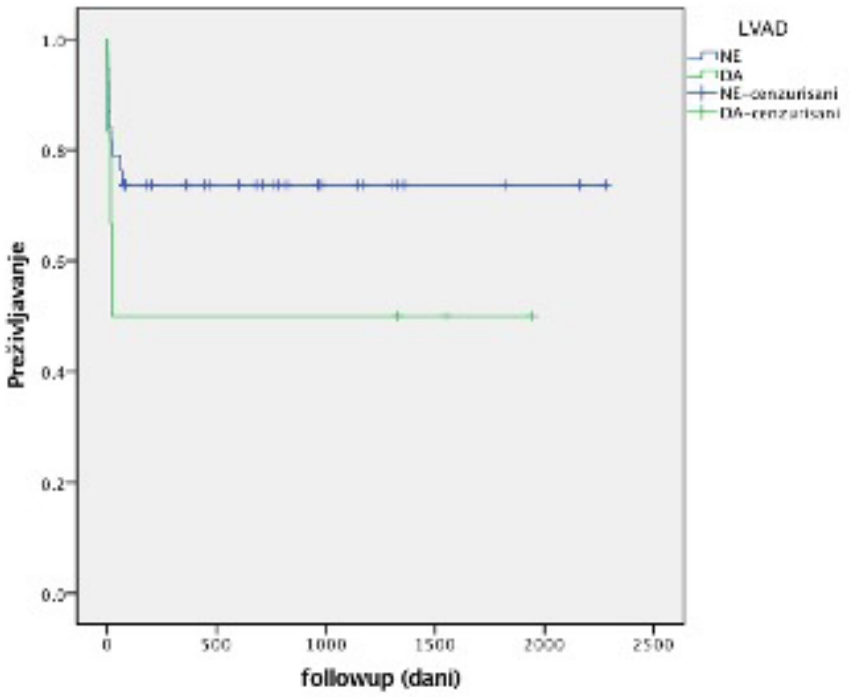
The average donor age was 41.6 years, while the youngest donor was 15 years old, and the oldest was 66 years old. Male donors made up for 65.9%, 43.2% of them had A blood group while 40.9% had O blood group. The average ischemic time was 116.3 ± 39.6 minutes, while the average duration of extracorporeal circulation was 161.6 ± 51.8 minutes. Biatrial transplantation technique was performed in 33 (78.6%) patients, while bicaval technique was performed in 9 (21.4%) patients.

Survival and organ rejection

The average survival of all patients was 1611.8 days (1306.9-1916.7). The median length of patient follow-up was 637.4 days (1-2028 days). Six-year patient survival was 70.5%. By comparing survival in patients, no statistically significant difference was found between male and female patients (log-rank p=0.734). Also, no statistically significant difference was found in the survival of patients who underwent a previous cardiac surgery (log-rank p=0.085). **Graph 1** shows the survival analysis of patients who had mechanical circulatory support previously implanted as a bridge to transplantation. No statistically significant difference in survival was found in this group of patients (log-rank p=0.228). Patients with preoperatively diagnosed pulmonary hypertension had statistically significantly worse survival than other patients (log-rank p=0.023, **Graph 2**).

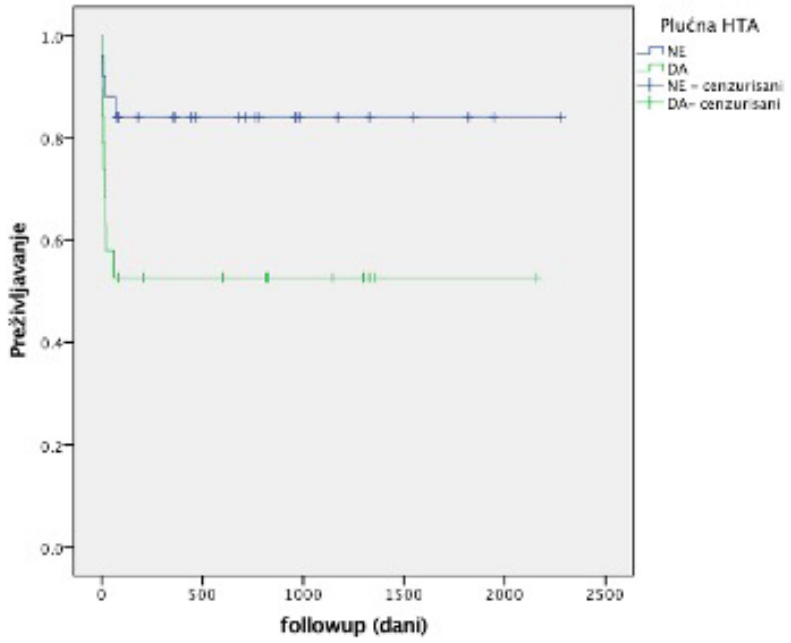
The analysis of survival in relation to the donor's age found no statistically significant difference between the donors older than 45 years and those younger than 45 years (log-rank p=0.641). Besides, no statistically significant difference in survival was found between the patients operated with biatrial technique and those operated with bicaval technique (log-rank p=0.239). Cox's regression analysis determined that the following were independent predictors of worse outcome: recipient's age, recipient's body mass index, previous CVI, preoperative chronic renal insufficiency, pulmonary hypertension, infection in the postoperative course, as well as the need for mechanical circulatory support in the immediate postoperative course (ECMO, IABP) **(Table 2)**.

In the postoperative follow-up, 13 (29.5%) patients developed some degree of organ rejection. Among the patients who developed rejection, 30.7% developed grade



Graph 1. Survival of patients after heart transplantation with preoperative long-term circulatory support

I, 76.9% developed grade II, and only one patient developed grade III. There was no statistically significant difference in the survival of patients who developed some degree of graft rejection and those who did not (log-rank p=0.453). Cox's regression analysis determined that the following were independent predictors of rejection: recipient's age, recipient's body mass index, preoperative allosensitization PRA>20%, as well as ischemic time **(Table 3)**. Graft vasculopathy (CAV) developed in 4 (9.1%) patients. Patients who developed CAV did not have statistically significantly worse survival than other patients (log-rank p=0.213). Preoperative diagnosis of diabetes mellitus was determined as an independent predictor for the development of CAV, with a hazard ratio of 1.006 (CI 95% : 1.001- 1.012; P=0.018). In the postoperative follow-up period, the occurrence of infections requiring hospitalization was recorded in 3 (6.8%) patients. During the follow-up period, 9 (20.5%) patients developed renal insufficiency, while one patient developed malignancy.



Graph 2. Survival of patients after heart transplantation with preoperative pulmonary hypertension

Table 2. Independent predictors for survival after HTX

variable	P value	HR	95% CI
Age	0.019	1.077	1.012-1.147
Body mass index	0.015	1.172	1.031-1.333
Previous stroke	0.011	5.496	1.490-20.273
CKD	0.014	0.951	0.914-0.990
Pulmonary hypetrtension	0.018	1.006	1.001-1.012
Postoperative infection	0.044	3.381	1.035-11.038
ECMO,IABP postoperatively	0.016	6.625	1.419-30.928

CKD - chronic kidney disease, ECMO – extracorporeal life support, IABP – intraaortic ballon pump, HR – hazard ratio, CI – confidence interval.

Table 3. Characteristics of the recipients

variable	*P value*	HR	95% CI
age	0.012	0.947	0.907-0.988
Body mass indeks	0.017	0.840	0.729-0.970
PRA>20%	0.018	0.149	0.031-0.724
Ischemic time	0.049	1.014	1.000-1.028

HR – hazard ratio, CI – confidence interval.

DISCUSSION

As no therapeutic option has been found yet to reduce the mortality of patients in the terminal stage of heart failure, heart transplantation remains the gold standard in their treatment. However, 5.000-6.000 heart transplants are performed annually worldwide., although the need is much greater, due to the lack of donors this remains an option for a limited number of patients.(5)

Worldwide, over 50% of patients who are candidates for heart transplantation have been diagnosed with nonischemic cardiomyopathy, 37% have been diagnosed with ischemic cardiomyopathy, while only 1% of them have been diagnosed with restrictive cardiomyopathy. In our study population, 63.6% had nonischemic cardiomyopathy, 29.5% had ischemic cardiomyopathy, while only 1.5% had restrictive cardiomyopathy.(2) Survival analysis did not establish a difference between these groups of patients after a long-term follow-up period. The average age of transplant patients in the ISHLT report was 54 years. There was also a trend of an increase in the number of transplanted patients over 60 years of age, and a certain small number of centers transplanted hearts to patients who were 70 years of age. (8) In our center, the average recipient age was 46, where the youngest recipient was 16 years old, and the oldest was 66 years old. The lower average age of patients can be explained by the fact that according to the protocol of our transplantation program, the upper limit for transplant candidates has been set at 65 years.

The entry into increasingly widespread clinical use of devices for long-term mechanical circulatory support as a bridge to heart transplantation has led to the fact that, according to the ISHLT registry, 36% of patients had this type of mechanical support implanted preoperatively. (9) In our population, mechanical circulatory support was preoperatively implanted in 13.6% of patients. Survival analysis showed that patients with a preoperatively implanted LVAD had worse survival, while it was not identified as an independent risk factor.

Advances in immunosuppressive therapy led to the introduction of heart transplantation into clinical practice in the 1980s, and they are the main reason for the development of this method. The greatest risk of death is in the first year upon transplantation, when immunosuppressive therapy is most intensive. The greatest progress in the field of heart transplantation was made precisely in the reduction of mortality in the first postoperative year. In the largest number of world centers, mortality in the first year is around 10%. The most common causes of death in the first year are acute graft rejection, infection, CAV, malignancy, and renal failure. In our study population, infection was registered as an independent predictor of mortality after transplantation. After the first year, the median survival is 13 years.(10) After 5 years, survival is 70-75%, which is confirmed by the results of our study, where six-year survival rate of 70.5% was recorded.

Elderly patients showed that recipient age was an independent predictor of both early and late post-transplant mortality. Patients in their 40s and mid-50s have the lowest risk. Patients younger than 40 years of age have a higher risk of worse outcome probably due to a stronger immune response and a higher risk of developing graft rejection. On the other hand, patients older than 60 years of age also have a higher risk of worse outcome due to a greater chance of developing malignancy and infection because of weakened immune system, as well as a higher frequency of comorbidities before transplantation.(11) Also, in our study the age of the recipient was an independent predictor of mortality with a ratio of 1.077 (95% (36): 1.012-1.147; P=0.019).

Pulmonary hypertension proved to be one of the most important independent predictors of worse outcome, mostly due to the inability of the donor heart to cope with the increased afterload to which it had not been previously conditioned. Therefore, fixed pulmonary hypertension with pulmonary artery systolic pressure > 60 mmHg, TPG > 15 mmHg and PVR > 6 WU is an absolute contraindication for heart transplantation. (4, 12) In our population, preoperative pulmonary hypertension was identified as an independent predictor of mortality with a hazard ratio of 1.006 (95% (36): 1.001-1.012; P=0.018).

The incidence of graft rejection is highest in the first few months upon transplantation, while hyperacute rejection by preformed antibodies is certainly the most fatal complication in the immediate postoperative course. (9) In our study, there was no hyperacute graft rejection,

which can be explained by the fact that in preoperatively verified allosensitive patients did not initiate procedures prior to obtaining crossmatch analysis. Previous allosensitization proved to be an independent predictor of rejection and increased postoperative mortality. A PRA test result > 20% was shown to be a non-significant predictor of organ rejection with worse outcome after heart transplantation. In our study, allosensitization with PRA values > 20% proved to be an independent predictor of postoperative rejection with a hazard ratio of 0.149 (95% (36): 0.031-0.724; P=0.018). The incidence of some degree of organ rejection varies between 15-36%, depending on the centre, and rejection was shown to be the cause of death in 9% of cases after the first post transplantation year.(13)

In our study, 13 (29.5%) patients developed some degree of organ rejection. Among the patients who developed rejection, 30.7% developed grade I, 76.9% developed grade 2, and only one patient developed grade 3. There was no statistically significant difference in the survival of patients who developed some degree of graft rejection and those who did not. As independent predictors of organ rejection, ischemic time, recipient's BMI, and recipient's age were singled out.

Graft vasculopathy is a form of coronary graft disease characterized by diffuse intimal proliferation. It is characterized by angiographic diffuse narrowing of the epicardial coronary arteries, with a decrease in the lumen of the distal segments and occlusion of small branches. Five years after heart transplantation, about 25% of patients develop some degree of CAV, while after 10 years, about 50% of patients develop some form of this entity. In our study, 9.1% of patients had CAV after a five-year follow-up period, and statistical analysis did not prove to be an independent predictor of mortality. In this population, diabetes mellitus proved to be an independent predictor of the development of CAV, which supports the fact that hypertension as a consequence of this disease can be the cause of CAV. The lower percentage of CAV in our population can be explained by the very low representation of marginal donors in our transplant program.(14)

On average, heart transplant patients can be expected to live 10 years, although many of them live much longer. Return to work, physical activity, pregnancy and good quality of life are today a reality for these patients. Graft dysfunction, CAV and the occurrence of malignancy are the main problems that limit long-term survival. New methods of immunosuppression, as well as monitoring the state of the immune system and better understanding of the pathophysiology of CAV will enable even greater long-term survival after heart transplantation.

CONCLUSION

The analysis of mortality within the group of patients who underwent a heart transplant as a part of the transplant program in the Republic of Serbia determined that six-year survival rate was 70.5%. Independent predictors of outcome after a long-term follow-up period were as follows: recipient's age, recipient's body mass index, previous CVI, preoperative chronic renal insufficiency, pulmonary hypertension, infection in the postoperative course, as well as the need for mechanical circulatory support in the immediate postoperative course (ECMO, IABP). Independent predictors of organ rejection after heart transplantation in our study population were as follows: recipient's age, recipient's body mass index, preoperative allosensitization PRA>20%, as well as ischemic time.

Conflict of Interest Disclosure: None declared.

Author Contributions:

- **Milos Matkovic** - Conceptualization; Investigation; Methodology; Writing-original draft
- **Emilija Nestorovic** - Methodology; Writing—review & editing
- **Nemanja Aleksic** - Methodology; Writing—review & editing
- **Ilija Bilbija** - Conceptualization; Statistical analysis; Methodology; Writing—review & editing
- **Dejan Markovic** - Methodology; Writing—review & editing
- **Svetozar Putnik** - Investigation; Methodology; Supervision; Writing—review & editing.

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UDALJENI REZULTATI NAKON TRANSPLANTACIJE SRCA: ISKUSTVO TRANSPLANTACIONOG PROGRAMA REPUBLIKE SRBIJE

Milos Matković[1,2], Emilija Nestorović[1,2] Ilija Bilbija[1,2], Nemanja Aleksić[1,2], Dejan Marković[2,3], Svetozar Putnik[1,2]

Sažetak

Uvod/ciljevi: Transplantacija srca predstavlja zlatni standard za lečenje pacijenata u simptomatskom terminalnom stadijumu srčane insuficijencije. Cilj studije bio je da ispita dugoročne rezultate posle transplantacije srca i da ispita nezavisne prediktore preživljavanja nakon operacije u okviru transplantacionog programa Republike Srbije.

Metode: Urađena je retrospektivna opservaciona studija koja je uključila 44 pacijenta koji su bili podvrgnuti transplantaciji srca od 2013. do2021. godine u okviru Programa transplantacije srca Republike Srbije. Svi pacijenti su stavljeni na Nacionalnu listu čekanja za transplantaciju srca prema savremenim ISHLT kriterijumima. Studija je obuhvatila sve pacijente koji su bili podvrgnuti transplantaciji, kao i one koji su preoperativno dobijali dugotrajnu cirkulatornu podršku. Podaci su dobijeni iz istorija bolesti i kontrolnih pregleda.

Rezultati: Prosečno preživljavanje svih pacijenata bilo je 1611,8 dana (1306,9 –1916,7). Srednja dužina praćenja pacijenata bila je 637,4 dana (1–2028 dana). Šestogodišnje preživljavanje pacijenata bilo je 70,5%. Statističkom analizom utvrđeno je da su nezavisni prediktori lošijeg ishoda: starost primaoca, indeks telesne mase primaoca, prethodni CVI, preoperativna hronična bubrežna insuficijencija, plućna hipertenzija, infekcija u postoperativnom toku, kao i potreba za mehaničkom cirkulatornom potporom postoperativno.

Zaključak: Program transplantacije srca u Republici Srbiji pokazao je stopu preživljavanja koja je komparabilna sa centrima širom sveta u ISHLT registru. Nezavisne faktore rizika treba pažljivo analizirati u okviru naše populacije i uzeti ih u obzir prilikom planiranja procedure.

Ključne reči: transplantacija srca, preživljavanje, faktori rizika

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ORIGINAL ARTICLE

HYBRIDE IMAGING IN ADVANCED MELANOMA

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Summary

Aim: To evaluate the usefulness of 18F-fluorodeoxyglucose positron emission tomography with computed tomography (18F-FDG PET/CT) in patients with advanced melanoma.

Method: This study included 264 consecutive patients with melanoma who were sent for the 18F-FDG PET/CT. The inclusion criteria were as follows: histopathologically verified melanoma stage III or IV, the absence of other malignancy/infection; glycemia ≤ 11 mmol/l. The final study population consisted of 220 patients. After the first 18F-FDG PET/CT, the follow-up examination was performed after 11.81±7.99 months, for therapy response evaluation.

Results: Pathological 18F-FDG PET/CT was present in 154 patients. Sensitivity of 18F-FDG PET/CT was estimated as 99%, specificity as 47%. There was no statistically significant difference between 18F-FDG PET/CT findings and gender (p> 0.05), and MDCT examination (p = 0.678). However, 18F-FDG PET/CT upstaged 45% patients, especially these with widespread disease. SUV max and inguinal disease localization (in patients who had lower extremities as primary localization of disease) were associated with progression free survival (PFS) (p < 0.05). SUV max (HR 1.03, CI 1.00-1.12, p=0.05) and locally advanced disease (HR 12.02, CI 1.13-148.00, p=0.04) were independent predictors of PFS. A follow up 18F-FDG PET/CT revealed active disease in 22/26 patients. Therapy type (immunotherapy or target therapy) did not correlate significantly with the 18F-FDG PET/CT follow up result (p=0.760, *ρ=-0.354*).

Conclusion: 18F-FDG PET/CT has good sensitivity in the evaluation of advanced melanoma. Small lesions and brain localization reduce specificity of the examination, then MDCT, MR are advised. Predictive factors SUV max and locally advanced disease, are more important than the timing of follow-up 18F-FDG PET/CT, since they were predictors of PFS. Follow up 18F-FDG PET/CT should be done at least in 6 months, only if there is suspicion of the presence of active disease.

Keywords: advanced melanoma, 18F-FDG PET/CT

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INTRODUCTION

Melanoma is a solid tumor formed by malignant transformation of melanocytes. It is most often localized on the skin although it can also be found on the mucous membranes of the head and neck, the urogenital tract, the gastrointestinal tract, eyes (1-3).

Surgical treatment of primary melanoma, as well as local and regional metastases, is the gold standard in the treatment of melanoma patients. Chemotherapy has a limited role in patients with melanoma, and it is mainly used for the purpose of palliative care (1). Radiotherapy in patients with melanoma also becomes important in special indications (bone metastases or metastases in the central nervous system). Therapeutic strategies, such as immunotherapy and immunotherapy with checkpoint inhibitors, have significantly improved the treatment of metastatic disease.

Early diagnosis and adequate assessment of disease is crucial for the timely treatment of these patients (4,5). For these very reasons, it is necessary to define adequate criteria for monitoring and diagnosis of these patients.

Guidelines usually suggest the use of multi detector computed tomography (MDCT) or magnetic resonance (MR) imaging in patients with advanced melanoma. Nowadays, many international studies and guidelines indicate the usefulness of positron emission tomography with computed tomography with fluorodeoxyglucose (18F-FDG PET/CT) in these patients (1,6,7).

Thus, international studies indicate the usefulness of 18F-FDG PET/CT in the evaluation of patients with melanoma, given the high 18F-FDG avidity of melanoma (8-15). However, there is no universal international consensus regarding the use and frequency of 18F-FDG PET/CT in the evaluation of patients with advanced melanoma (16, 17). For that reason, the evaluation of this topic is important and ongoing.

The aim of this study was to determine the usefulness of 18F-FDG PET/CT and its timing in the evaluation of patients with advanced melanoma.

MATERIALS AND METHODS

Study population

This study included 264 consecutive patients with melanoma who were sent for the 18F-FDG PET/CT examination to the National PET Center, University Clinical Center of Serbia in the period from January 2010 to May 2020 to obtain the assessment of the prevalence of the disease. The study was designed as ambidirectional (retrospective-prospective study); in 2018 it became prospective, having obtained the Ethics committee's approval.

The criteria for inclusion in this study were as follows: (a) histolopathologically verified melanoma; (b) Stage III or IV disease; (c) the existence of clinical and /or radiological indicators of disease activity; (d) the absence of other malignancy, as well as the absence of infection; and (e) glycemia ≤ 11 mmol / l.

Out of the initial population of 264 patients, 44 patients were excluded from the study (lung cancer – 8, head cancer – 8, thyroid cancer – 7, breast cancer – 4, lymphoma –5, gastrointestinal cancer – 9, presence of infection – 3). This way, the final study population of 220 patients was obtained (mean age 57 ± 15 years, 128 men and 92 women).

This work was done in accordance with the ethical principles of the Helsinki Declaration and prospective part of the study was approved by the Ethics committee of the Faculty of Medicine, Center of Nuclear Medicine University of Belgrade, written consent was obtained from all participants (IRB 668/6; 19/4/2018).

Procedures

For all included patients, the following data were collected prior to performing the 18F-FDG PET/CT examination: (a) demographic characteristics (collected by the epidemiological questionnaire); (b) clinical data, descriptions of previous diagnostic procedures -multidetector computed tomography (MDCT) or magnetic resonance imaging (MR) (obtained from medical histories).

Twenty-six patients were invited for follow-up 18F-FDG PET/CT examination. These patients had pathological findings in the first 18F-FDG PET/CT examination and they had their therapy changed. The examination was performed 11.81±7.99 months after the first 18F-FDG PET/CT in order to evaluate the therapeutic response, collect the therapy data, disease symptoms, and diagnostic procedures.

Data Acquisition, Reconstruction, and Image Analysis

The 18F-FDG PET/CT scan was performed on a 64-slice hybrid PET/CT scanner (Siemens Biograph, Siemens Medical Solutions USA Inc., Hoffman Estates, Illinois, USA). The patients fasted for 8 hours before receiving an intravenous injection of 18F-FDG at a dose of 5.5 MBq/kg. PET/CT acquisition (imaging) began 60 minutes after the intravenous injection. A three-dimensional whole body PET scan (14-15 bed, 3 min/bed) and a low-dose CT was performed. Multidetector CT had the following characteristics: voltage 120 kV, with automatic "real-time" voltage height modulation (CareDose4D with a basal level of 45 mA); slice thickness 5 mm; pitch 1.5; rotation time 0.5 s. CT, PET (attenuation corrected) and PET/CT fusion images were processed on a SYNGO SIEMENS workstation (Syngo MMWP, Siemens AG, Berlin and Munich, 2008, Germany).

The findings of 18F-FDG PET/CT were categorized as positive or negative, based on visual and quantitative assessment. The accumulation of 18F-FDG was quantitatively analyzed as the maximum standardized value of radiopharmaceutical uptake (SUV max). SUV max was calculated as the concentration of activity at the end of the recording that was corrected for individual body weight and dose of intravenously injected 18F-FDG: SUV max = tissue activity (count / pixel / second) × calibration factor/dose of intravenously injected 18F-FDG (MBq/kilogram of body weight) (18).

SUV max equal or higher than 2.5 was considered pathological. Findings were considered positive if there was an increased accumulation of FDG compared to normal accumulation in organs (visceral organs, lungs, skin, brain parenchyma or bone ...). The follow-up 18F-FDG PET/CT examination was performed under the same conditions as the first one, in terms of administering the same amount of radiopharmaceuticals, the same time period and the method of acquisition, reconstruction and image analysis. 18F-FDG PET/CT images were interpreted by two independent physicians (nuclear medicine specialists). If there was a discrepancy between these findings, the final judgment was obtained through their consensus.

Statistical Analysis

The X2 test was performed to assess the difference between pT stage and gender. Independent sample T-test was used to assess difference between age and pT stage. The difference between 18F-FDG PET/CT result and gender was obtained with X2 test, as well as the difference between disease localization and disseminated disease. The evaluation of difference between primary melanoma site and the presence of distant metastases was also obtained with X2 test.

Independent sample T-test was used to asses differences between SUV max levels in distant metastases and positive sentinel lymph nodes, in different primary sights of disease. The difference between the MDCT and 18F-FDG PET/CT findings was assessed by the X2 test. A Cox proportional hazard model was done to determine whether age, gender, MDCT, primary localization of disease and SUV max affected the disease outcome. SUV max was used as dichotomous variable (pathological findings were above SUV max 2.5). In patients with multiple lesions, SUV max was calculated as mean of all lesions. Univariate and multivariate predictive models with confounding factor control were applied. Also, Kaplan-Meier analysis was used to determine how the localization of the disease, therapy and the pathological finding of 18F-FDG PET/CT (SUV max >2.5) could affect survival in patients with advanced melanoma. The X2 test was performed to assess the difference between genders regarding the frequency of pathological findings on 18F-FDG PET/CT examination. Sensitivity (Sn) and specificity (Sp) of 18F-FDG PET/CT in detection active disease in melanoma patients was assessed by ROC analysis. Positive predictive value, negative predictive value and diagnostic accuracy of 18F-FDG PET/CT scans were also determined. Determination of the difference between the value of SUV max during the first and follow-up examination was performed using the Paired Sample T test. The results of continuous numerical variables are presented as values of arithmetic mean ± standard deviation (SD), while P value below 0.05 was considered statistically significant.

RESULTS

Characteristics of the study population

The study population consisted of 220 patients with melanoma aged 57 ± 15 years (128 men and 92 women). All patients had advanced melanoma (stages III and IV), with high pT stage III (30.1%), IV (30.1%), II (24.1%), I (15.7%). High pT stage was frequently present in older patients (X2 test, $p<0.001$), regardless of gender (X2 test, $p=0.755$).

The majority of the study population had a previous surgical intervention. Metastasectomy was performed in the presence of solitary lesions, usually liver metastases in patients with eye melanoma. Immunotherapy with pembrolizumab/nivolumab was applied in 19 patients (pembrolizumab 14/19; nivolumab 5/19), while 4 patients received target therapy (enkorafenib+bimetinib). After initial 18F-FDG PET/CT examination, pathological findings were present in 154 patients (70%), 91 men and 63 women.

There was no statistically significant difference in the finding of 18F-FDG PET/CT in relation to gender (X2 test, $p> 0.05$). More disseminated disease was present in patients who had primary melanoma of the head region and the back, but this was not statistically significant (X2 test, $p=0.835$). Primary site of disease did not significantly affect the occurrence of distant metastases (X2 test, $p=0.893$) **(Figure 1)**.

Patients with primary melanoma localized on lower extremities had significantly higher SUV max in distant metastases than in regional lymph nodes (11.70±7.4 vs. 1.50±4.05), ($p<0.05$, CI -20.71- 1.68), which was not the case in other localizations. The most common disease localizations are shown in **Table 1**.

18F-FDG PET/CT finding and MDCT finding

18F-FDG PET / CT examination revealed an active malignancy in 154 patients (70%), with mean SUV max value 8.07 ± 8.92. MDCT examination detected active malignancy in 66% of patients. The 18F-FDG PET/CT

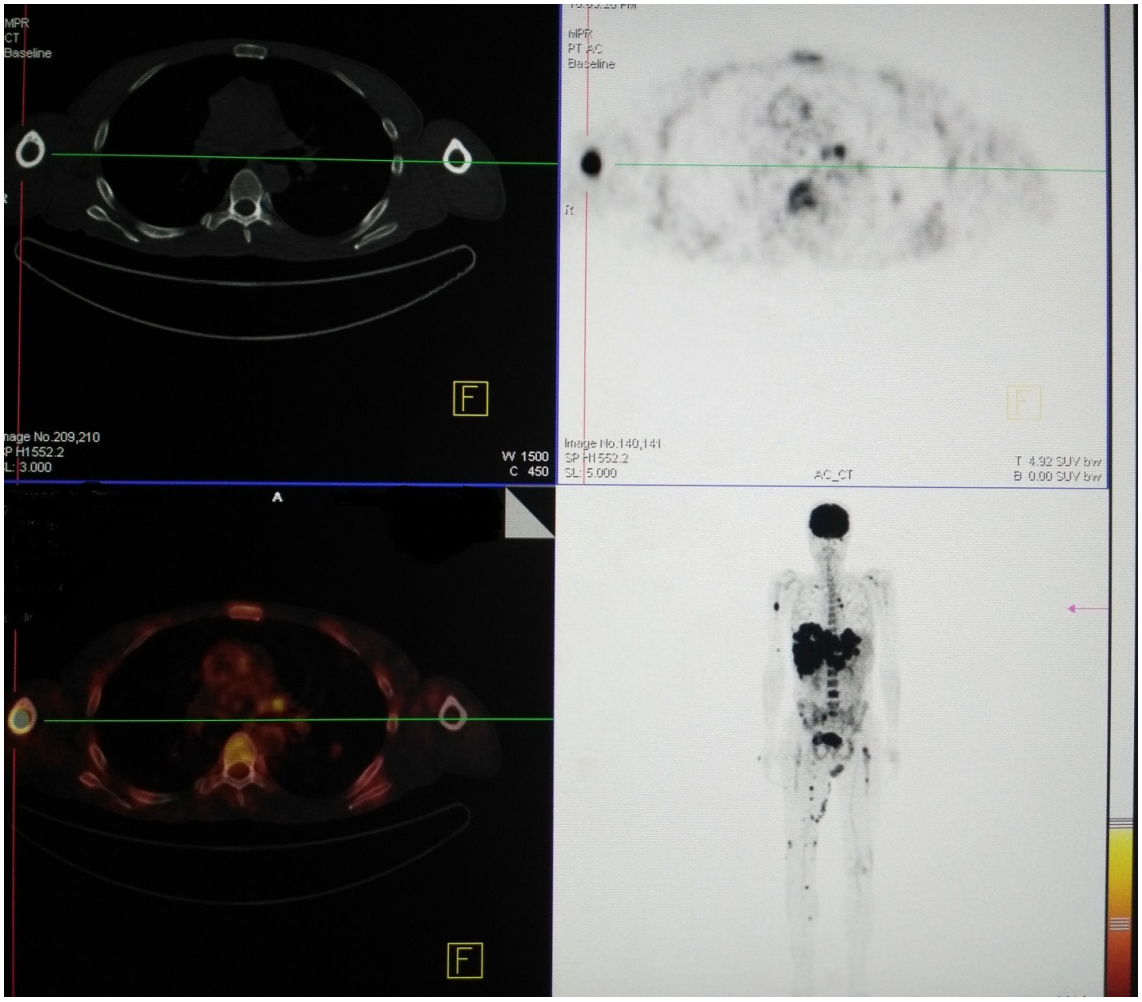

Fig 1. 18F-FDG PET / CT revealed disseminated disease and upstaged disease in this 64 years old patient. The disseminated disease on MIP (maximal intensity projection image), the active disease is present in the right humerus, mediastinal lymph nodes, liver and other bones (vertebrae, iliac bone, femur). High metastatic burden resulted in early death, 4 months after the 18F-FDG PET/CT examination.

examination found the same disease localizations as MDCT in 4% of patients. In almost half of the patients, the 18F-FDG PET/CT examination revealed a more widespread disease than it was seen on MDCT. In the majority of patients 18F-FDG PET/CT detected new sites of disease since they were out of focus in single region MDCT (e.g., only thorax/ abdomen MDCT was done, and new sites of disease were present in other regions on 18F-FDG PET/CT).

Table 1. The most common disease localizations found on 18F-FDG PET / CT examination

	Primary site of disease											
Metastasis on 18F-FDG PET/ CT	Head and face	Neck	Back	Chest	Abdomen	Gluteal region	Upper extremities	Lower extremities	Unknown primary localization	Multiple primary localization	Eye	Genital region
Sentinel lymph node metastasis	1	0	3	1	0	0	1	0	0	0	0	0
Distant metastasis												
Distant lymph nodes	30	7	50	11	4	4	15	44	16	4	5	5
Lungs	3	2	10	0	2	1	3	6	4	0	0	0
Brain	3	0	1	1	1	0	0	1	0	0	0	2
Bones	7	4	9	1	2	1	1	11	3	1	0	1
Liver	5	1	9	1	2	1	2	7	2	1	1	2
Subcutaneous tissue	6	1	11	2	1	2	3	16	3	1	0	0

* Majority of patients had multiple metastases on 18F-FDG PET/CT examination

In 7% of patients, the finding was pathological on MDCT and normal on 18F-FDG PET/CT. This was present in small lung lesions (2 %) or in lesions with FDG high background uptake (brain 5 %).

The opposite situation was present in 11% of patients where MDCT was normal and 18F-FDG PET/CT pathological. These patients had small diameter lymph nodes (smaller than 10 mm), which were classified on MDCT as normal, but they had FDG uptake higher than background and reclassified as pathological. In 33% of patients, both examinations were pathological but showed completely different localizations of the disease. 18F-FDG PET/CT usually detected disease in distant extremities, lymph nodes, even liver metastases, while MDCT was better at detecting active disease in brain, intestines and urinary tract. Based on the X2 test, there was no statistically significant difference in the finding of MDCT examination and 18F-FDG PET/CT examination (p=0.678). However, upstaging of disease was done by PET/CT in 45% of patients.

18F-FDG PET/CT finding and its sensitivity

Sensitivity of 18F-FDG PET/CT in disease detection was estimated as 99%, specificity as 47%, while negative predictive value was 87% and positive predictive value 43%. Diagnostic accuracy of the test was estimated at 69%, ROC cut off was 7.6 and ROC "area under the curve" was 0.730. **(Figure 2)**

Follow-up 18F-FDG PET/CT examination

Twenty-six patients (8 men and 18 women, mean age 54.04 ± 17.09 years) came for a follow-up 18F-FDG PET/CT examination, which was scheduled 11.81±7.99 months after the first one.

In the follow up, ten patients had a progression of disease. Partial remission was present in 2 patients, stable disease in 10 patients. There was no statistically significant decrease in the SUV max value compared to the previous examination (8.66 ± 8.84, 8.80 ± 14.94) (p>0.05). The therapy type (immunotherapy or target therapy) did not significantly correlate with 18F-FDG PET/CT follow up result (p=0.760, ρ=-0.354). However, four patients, who received immunotherapy (pembrolizumab), had complete metabolic response. **(Figure 3)**

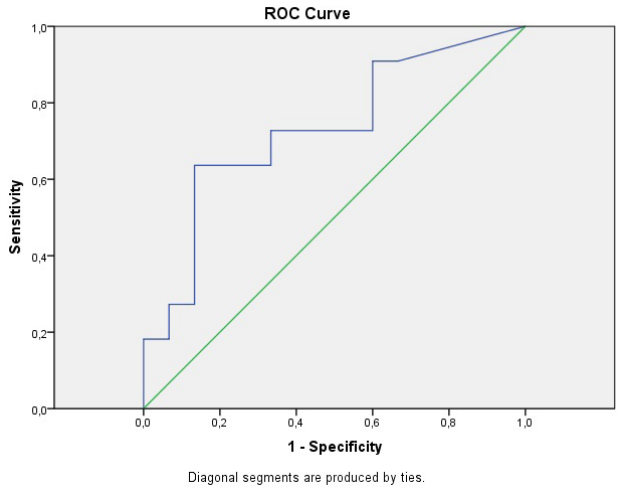

Fig 2. Statistical ROC analysis (area under the curve-0.73)

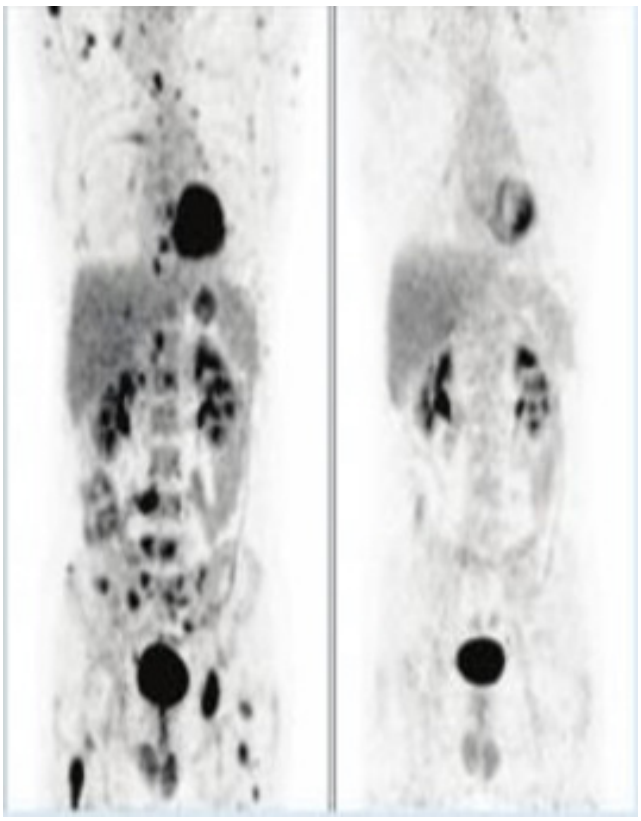

Fig 3. Complete metabolic regression of disease on control the 18F-FDG PET / CT examination. Left image-baseline 18F-FDG PET / CT revealed widely spread disease (axillary, mediastinal, retroperitoneal, para iliac and inguinal lymph nodes, right humerus, ribs, left and right femur). The right image-follow up 18F-FDG PET / CT indicates metabolic regression after immunotherapy

Influence of 18F-FDG PET/CT on disease prognosis

A univariate analysis of Cox regression analysis showed that SUV max was a predictor of a worse progression free survival (PFS), (HR 1.030 95% CI 1.00-1.06; P < 0.05). In a multivariate Cox regression analysis SUV max and locally advanced disease were independent predictors of a PFS **(Table 2)**. Other variables such as: age, gender, MDCT result, primary localization of disease had no effect on the disease outcome.

Based on the Kaplan-Meier survival analysis (Long rank test) it was observed that presence of the disease in the inguinal localization, in patients whose lower extremities were primary localization of disease, was associated with PFS (p = 0.03). **(Figure 4)** It was similar with the patients who had an increased SUV max level on 18F-FDG PET/CT examination, regardless of the primary localization of the disease (p = 0.04). **(Figure 5)**

Table 2. Univariate and multivariate Cox regression models predicting progression free survival in 220 patients with advanced melanoma

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Age	1.02	0.98-1.06	0.38	1.00	0.90-1.02	0.59
Sex (male vs. female)	0.99	0.33-2.97	0.63	1.02	0.26-6.10	0.83
SUV max	1.03	1.00-1.06	**0.04**	1.03	1.00-1.12	**0.04**
MDCT result (pathological vs. normal)	0.01	1.00-2.60	0.58	1.10	1.12-5.71	0.96
Primary localization of disease	1.10	0.94-1.29	0.23	6.68	0.05-834.99	0.44
Locally advanced disease	0.60	0.07-5.38	0.65	12.02	1.13-148.00	**0.04**

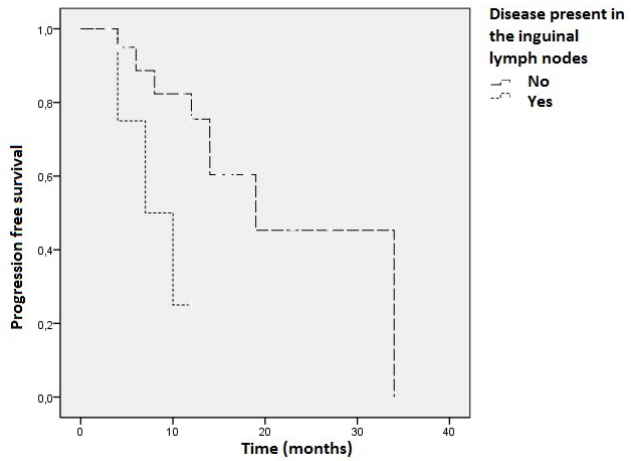

Fig 4. Kaplan-Meier curves for PFS stratified by presence of pathological inguinal lymph nodes on 18F-FDG PET/CT (Long rank test, p = 0.03

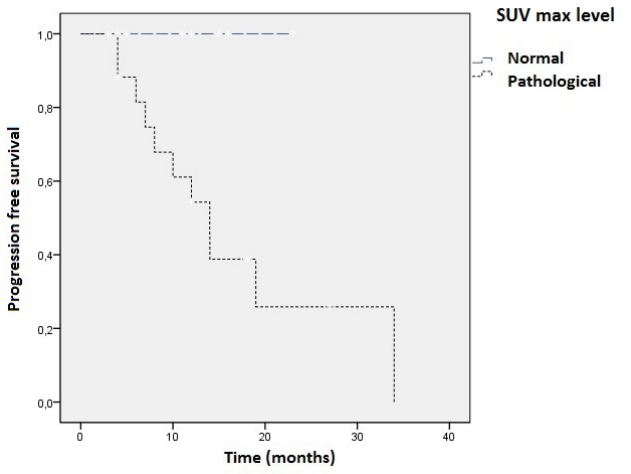

Fig 5. Kaplan-Meier curves for PFS stratified by SUV max level (cut off 2.5) on 18F-FDG PET/CT (Long rank test, p = 0.04

Therapy did not had statistically significant effect on PFS (Long rank test, p=0.34).

DISCUSSION

The frequency of newly diagnosed melanomas is gradually increasing, about 3-8% annually. More than a half of the patients with melanoma will have a relapse of the disease to local lymph nodes (20%), or to regional lymph nodes (50%), and 30% of patients will have distant metastases (1). This fact suggests the importance of adequate monitoring of the patients with melanoma, which would enable early detection of relapse, and thus, the initial treatment of the disease. Conventional monitoring of patients includes clinical examination, dermatoscopy, laboratory parameters of disease progression, ultrasound of regional lymph basins, as well as other diagnostic procedures (MDCT, MR) depending on the stage of melanoma at the time of diagnosis. The frequency and type of follow-up examinations vary depending on the national guidelines.

In addition to ultrasound analyses and MDCT/MR, the guidelines have recently suggested the use of 18F-FDG PET/CT diagnostics. The Danish National Melanoma Guide recommends 18F-FDG PET/CT examinations at 6, 12, and 24 months in patients with melanoma who have stage II B higher (19). On the other hand, American Melanoma Guides suggest 18F-FDG PET/CT or MDCT or MR heads every 3-12 months in patients with stage IIB-IV (6). Our guide to melanoma suggests the use of 18F-FDG PET/CT diagnostics only in disease stages greater than III (1). Obviously, there is no clear international consensus regarding the frequency and type of control 18F-FDG PET/CT scans (contrast CT or non-contrast CT). This can be explained by the fact that it is an expensive and insufficiently available diagnostic procedure, especially for the conditions of countries with low national income.

Therefore, the aim of this study was to discover advantages and disadvantages of 18 F-FDG PET/CT and its timing in population of advanced melanoma in our environment.

This study included 220 patients with melanoma, who met the criteria of the Serbian National Melanoma Guide when referred to 18F-FDG PET/CT examination. Hence, it was a population of patients with stage IIIa-IV disease. High pT in our population was frequently present in older patients regardless of gender.

In our study population, 18F-FDG PET/CT was positive in 70% of patients (91 men and 63 women). The disease was most often disseminated, with distant metastases, equally present in both genders. A more spread

disease was usually found in patients with primary melanoma of the head and back region.

Since most patients also had MDCT, a comparison of these two procedures was done. There was no statistically significant difference between the findings of 18F-FDG PET/CT and MDCT (p = 0.678). However, in the majority of patients 18F-FDG PET/CT found new localizations of the disease and they were upstaged.

There were 7% of patients in whom MDCT showed a more widespread disease than 18F-FDG PET/CT (usually localized in the central nervous system and in small lesions). Surely, small dimensions below 3 mm cannot be adequately assessed by 18F-FDG PET/CT diagnostics, since they are below the spatial resolution of the device. Due to a "partial volume effect", small volumes will be underestimated in terms of calculating SUV max. Thus, SUV max will be unrealistic, and, in that way, it could incorrectly suggest a benign etiology of the disease (18). On the other hand, certain localizations such as the brain parenchyma are not adequate for assessing the presence of primary and secondary tumors on the 18F-FDG PET/CT, since this organ intensively physiologically binds radioactively labeled glucose. This way, discrimination of pathological and healthy tissue will be more difficult, so the use of MR is recommended (8, 18).

The advantages of 18F-FDG PET/CT diagnostics are reflected in the fact that it involves imaging a large body area (half-body / whole-body study), and thus can detect previously unrecognized localizations of the disease. SUV max is one of the most frequently used parameters for objective quantifying the accumulation of radiopharmaceutical. It is good for evaluating the metabolic activity of the disease and for monitoring the therapeutic effect. In order to reduce the possibility of false negative results, 18F-FDG PET/CT should always be done 3-4 weeks after the completion of chemotherapy, and 3 months after radiotherapy.

Using the low dose protocol reduces the patient's irradiation by some 30%, while preserving the quality of the study (20). Thus, 18F-FDG PET/CT has increasing usefulness in clinical practice.

Based on the results of our study, the sensitivity of the 18F-FDG PET/CT was estimated at 99%, and its specificity at 47%, while the positive predictive value was calculated at 43% and the negative predictive value at 87%. The diagnostic accuracy of the test was 69%. Most studies have concordant results and also report high sensitivity values of 18F-FDG PET/CT, probably due to the inclusion of high-risk patients (Stage III and IV) (21, 22). According to some authors, the sensitivity of the procedure went up to 100%, since the inclusion criteria of the study included a positive 18F-FDG PET/CT result (23).

Although the sensitivity in our study was high, the specificity was low (47%). This can be explained by the fact that FDG uptake is not sensitive to a certain pathohistological type of tumor and it can be elevated in some benign conditions, that can be falsely classified as suspicion of metastases. Pathohistological verification is advised, since it remains the gold standard for evaluation of disease activity.

On the other hand, Vensby at al. also report in their study high negative predictive values of 18F-FDG PET/CT, which once again indicates good abilities of 18F-FDG PET/CT to rule out the presence of relapse. Low positive predictive values were mainly present in patients with a low probability of recurrence (21).

Since, 18F-FDG PET/CT upstaged half of the study population, therapy was changed in all of them. After 11.81 ± 7.99 months, the patients were invited for a follow-up 18F-FDG PET/CT examination. However, only 26 patients came for the follow-up 18F-FDG PET/CT and in 22 of them the test was positive. During the follow-up examination, the disease was most often present in distant metastases (90 %), rarely as locally advanced (10 %). There was no statistically significant correlation between the type of therapy (immunotherapy/target therapy) and 18F-FDG PET/CT result. These results must be taken with a limitation considering the small number of subjects on immuno/target therapy as well as the time of its inclusion.

Also, the comparison of both types of therapy could not be done adequately, given that almost all patients received immunotherapy since target therapy can only be received within the framework of clinical trials in our country because otherwise it has not been assigned a license for use. Additionally, this therapy can be given only to patients with verified BRAF mutation. However, our results show that in the follow up, almost two years after the first examination, all patients were alive. Stable disease was present in 10 patients, 2 had partial remission, 10 experienced a progression of disease and complete remission was present only in 4 patients. Perhaps the higher number of relapses was caused by postponed responses to immunotherapy and prompt responses to target therapy. Perhaps it would be better to perform a follow up examination after a shorter period of time, for example after 6 months. Although 18F-FDG PET/CT is an expensive procedure, especially for developing countries, its importance lies in the fact that it can assess the spread of the disease with high sensitivity, much better than other diagnostic procedures.

Some authors state that more frequent monitoring of 18F-FDG PET/CT examination in patients with melanoma could be useful for early detection and treatment of relapse. Thus, patients would have better survival (21). However, Rueth et al. showed that this approach lead to minimal improvement in survival of these patients (15). Koskivuo et al. and Danielsen et al. quote that false-positive findings were usually present in asymptomatic patients who had no clinical suspicion of disease relapse (16,17). This example demonstrates the importance of performing an 18F-FDG PET/CT examination only in

clear indications, as otherwise it may result in patient anxiety, unnecessary diagnostics, and treatment costs.

Our results suggest that 18F-FDG PET/CT, as a highly sensitive procedure, has the ability to stage disease more accurately than MDCT. Prognostic factors can be more useful than the exact timing of the follow-up 18F-FDG PET/CT examination. SUV max and locally advanced disease were independent predictors of progression free survival. Additionally, increased SUV max and the presence of the disease in the inguinal localization (in patients whose lower extremities were the primary localization of disease) were associated with worse progression free survival.

The majority of our patients in the first examination and in the follow up examination had a primary localization of the disease in the lower extremities, and the finding should be interpreted keeping this fact in mind. The presence of active disease in the inguinal lymph nodes should be considered as a locally advanced disease, which precedes further distant spread.

Other diagnostic procedures have different indications for the lymph node evaluation. Ultrasound or lymphoscintigraphy have the role in preoperative staging of regional lymph nodes and postoperative follow up, while dynamic lymphoscintigraphy is used for detection of sentinel lymph node when melanoma is localized on hull (1).

This study has certain limitations. One of them is a low response rate to the follow-up 18F-FDG PET/CT examination. Another limitation of the study is the inhomogeneity of the study population (some of them had different kind of surgery, some received immuno/target therapy before the first 18F-FDG PET/CT). The other limitation was a small number of patients who underwent immunotherapy. Immunotherapy (pembrolizumab and nivolumab) was introduced into clinical use only after 2016. Until today, other types of therapy have not received legal permission for use in our country. That's why the number of patients with target therapy is minimal, because it can be only given under cover of clinical trials. Nevertheless, this study included a significant number of patients (stage III and IV of disease) which represents one of the most numerous populations in international journals evaluating this topic, compared to other mainly retrospective studies with smaller study groups (24-27).

CONCLUSION

18F-FDG PET/CT shows high sensitivity in the detection of active disease in advanced melanoma. In patients with small diameter lesions and lesions in the central nervous system, the use of other diagnostic procedures is recommended (MR/MDCT). It seems that SUV max value and locally advanced disease are independent predictors of a worse PFS in these patients. 18F-FDG PET/CT has advantages in terms of informativeness, the existence of predictive parameters and quantitative parameters that allow early detection of disease activity and thus an early treatment. These characteristics justify the high cost of examination, which should be performed in precisely defined indications.

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HIBRIDNE DIJAGNOSTIČKE PROCEDURE U EVALUACIJI MELANOMA

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Sažetak

Cilj ovog rada je bio da utvrdi korisnost 18F-FDG PET/CT u evaluaciji pacijenata sa uznapredovalim melanomom.

Metodologija: U ovu studiju su uključena 264 uzastopna pacijenta sa uznapredovalim melanomom, koja su upućena na 18F-FDG PET/CT. Kriterijumi za uključivanje su bili: patohistološki verifikovani melanom III/IV stadijuma, odsustvo drugih maligniteta/infekcija, glikemija ≤ 11 mmol/l. Konačnu populaciju činilo je 220 pacijenata. Nakon prvog 18F-FDG PET/CT, obavljen je kontrolni pregled nakon 11.81±7.99 meseci, u cilju procene efikasnosti terapije.

Rezultati: Patološki 18F-FDG PET/CT je bio prisutan kod 154 pacijenta. Senzitivnost procedure je procenjena na 99% a specifičnost 47%. Nije bilo statistički značajne razlike između 18F-FDG PET/CT nalaza, pola i MDCT pregleda (p>0,05). 18F-FDG PET/CT je pogoršao stejdžing kod 45% pacijenata, posebno onih sa raširenom bolešću. SUV max i ingvinalna lokalizacija bolesti (kod pacijenata sa primarnim tumorom lokalizovanim na donjim ekstremitetima) su uticali na preživljavanje bez progresije bolesti (PFS), (p < 0,05). SUV max (HR 1,03, p<0,05) i lokalno uznapredovala bolest (HR 12,02, p<0,04) bili su nezavisni prediktori PFS. Kontrolni 18F-FDG PET/CT otkrio je aktivnu bolest kod 22/26 pacijenata. Tip terapije (imunoterapija ili target terapija) nije značajno korelirao sa 18F-FDG PET/CT rezultatom praćenja (p=0,760, ρ=-0,354).

Zaključak: 18F-FDG PET/CT ima dobru senzitivnost u evaluaciji uznapredovalog melanoma. Mali dijametar lezija i prisustvo bolesti u moždanom parenhimu smanjuju specifičnost pregleda. SUV max i lokalno uznapredovala bolest su prediktori PFS. Kontrolni 18F-FDG PET/CT treba raditi na 6 meseci jedino ako postoji sumnja na prisustvo aktivne bolesti.

Ključne reči: uznapredovali melanom, 18F-FDG PET/CT

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ORIGINAL ARTICLE

IMPLICATIONS OF COVID-19 PANDEMIC ON LAPAROSCOPIC AND ABDOMINAL SURGERY FOR BENIGN ADNEXAL CONDITIONS – SINGLE CENTER EXPERIENCE

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Summary

Introduction/Aim: COVID-19 pandemic posed a challenge in patient treatment and caused problems in the organization of health systems in many countries. The study aimed to analyze and quantify the influence of COVID-19 pandemic on performing surgeries for benign adnexal conditions with classic (open abdominal) and minimally invasive (laparoscopic) approach at the Clinic for Gynecology and Obstetrics University Clinical Center of Serbia.

Material and Methods: The study retrospectively analyzed all patients who were operated due to benign adnexal masses at our Clinic during the past five years. We compared numbers and types of operations before and during the pandemic.

Results: The study included 2166 patients who significantly more often had laparoscopic (61.9%) than open surgeries (38.1%). Surgeries of benign adnexal masses were elective in 53.2% cases, whereas in 46.8% cases those were emergency surgeries. Before the pandemic laparoscopic surgeries (mostly cystectomies) were on the rise. A reduction in laparoscopic and open surgeries was seen in the year 2020 (p=0.001). Moreover, the majority of surgeries were emergency surgeries (76.2%; p=0.001). Nevertheless, this decrease was significant only for laparoscopic cystectomies (p=0.001), but not for adnexectomies (p=0.224) and salpingectomies (p=0.762). Likewise, the 2020 reduction in open cystectomies (p=0.073), adnexectomies (p=0.836) and salpingectomies (p=0.241) was not significant either. During 2021, the number of surgical procedures for benign adnexal masses started to rise again (p=0.023). No intra-hospital COVID-19 infections were registered.

Conclusion: The total number of operations of benign adnexal masses in our Clinic decreased and was limited to emergencies, which was mostly reflected in minimally invasive surgery.

Keywords: COVID-19, gynecological surgery, adnexal pathology, laparoscopy, laparotomy

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INTRODUCTION

Coronavirus (SARS-COV-2) affected almost the entire world in just a few months and it was affecting it for more than two years. It posed not only challenge in patient treatment but also caused problems in the organization of health systems in many countries (1, 2). COVID-19 patients overcrowded hospitals which produced numerous organizational challenges. Intensive care units and other perioperative facilities became clinical care units for COVID-19 patients. These additional units also required staff. Therefore, many practitioners such as general and internal medicine specialists, surgeons, anesthesiologists along with nurses and support personnel were transferred to provide support in these new COVID-19 units (3).

To allow utilization of necessary technical resources and personnel for the treatment of COVID-19 patients, the majority of eminent medical societies recommended postponing nonessential medical visits and prioritizing only emergency surgeries while delaying elective surgeries of benign diseases and attempting alternative medical management wherever possible (4, 5). Nevertheless, the issue of health impairment due to postponed elective procedures, forced health care systems to adapt to the fluctuation of COVID-19 incidence and find a balance in the treatment of COVID-19 patients and non-COVID-19 patients. Consequently, in the past year more and more elective surgeries are again performed including gynecological surgeries (6).

There is another discussion regarding the optimal surgical approach to both emergency and elective surgeries. Some early recommendations indicated that laparotomy should be performed instead of laparoscopy in both emergency and elective conditions to avoid potential risk of virus dissemination in the operating theater during surgery due to the use of gas insufflation, possibility of gas leakage and creation of aerosols from electro-surgery (7). However, studies showed that there was no strong evidence to support claims that laparoscopy increased and open surgery prevented spreading of COVID-19 infection. Therefore, leading medical societies currently recommend the use of minimally invasive procedures whenever indicated and whenever possible (8).

The situation with organizing healthcare system during pandemic was similar in Serbia as we followed all current recommendations. This exceptional emergency situation affected the organization of gynecological services as well, especially surgical treatment of non-COVID patients with benign conditions requiring vaginal and laparoscopic operations. However, there are still no empirical data of the effect of pandemic on gynecological surgery in Serbia.

The aim of this study was to analyze and quantify the influence of COVID-19 pandemic on performance of surgeries for benign adnexal conditions with classic (open abdominal) and minimally invasive (laparoscopic) approach at a regional tertiary referral center – Clinic for Gynecology and Obstetrics University Clinical Center of Serbia.

MATERIAL AND METHODS

This retrospective study was performed at the Clinic for Gynecology and Obstetrics University Clinical Center of Serbia over the period of the past five years (2017 to 2021 year). The study conforms to the legal standards and was approved by the Ethics' Committee of the clinic. The study included all patients who were operated due to histopathologically confirmed benign adnexal masses. They were divided into groups according to the year when the operation was performed (before and after COVID-19 pandemic onset: 2017-2019 and 2020-2021) as well as into subgroups according to the surgical approach (open or laparoscopic). Patients' general data (age, Body Mass Index – BMI), indication and type of procedures, number of days in hospital, as well as postoperative complications including intra-hospital infection with COVID-19 were taken from medical records (medical histories and operative protocols). Patients' data were kept confidential and only researchers had access to them. The obtained data were analyzed by methods of descriptive (frequency, percent, mean, standard deviation) and analytical statistics (χ^2 test, t-test) and using the SPSS 20 software.

RESULTS

Study included 2166 patients who on average had 35.3 +/-9.7 years of age. The examined patients were obese (BMI≥25kg/m2) in just 6.4% (p=0.001) of cases. Overall significantly more patients had laparoscopic surgery (1341; 61.9%) than open approach surgery (825; 38.1%) **(Table 1)**.

Table 1. Frequency of performed surgeries per year

Surgery indication and approach		2017	2018	2019	2020	2021	Total
Salpingectomy	laparoscopy	62	79	71	68	77	357
	open	47	24	25	18	34	148
Cystectomy	laparoscopy	186	169	220	113	124	812
	open	92	83	80	61	87	403
Ovariectomy and/or adnexectomy	laparoscopy	31	49	38	29	25	172
	open	60	59	54	52	49	274
Total		478	463	488	341	396	2166

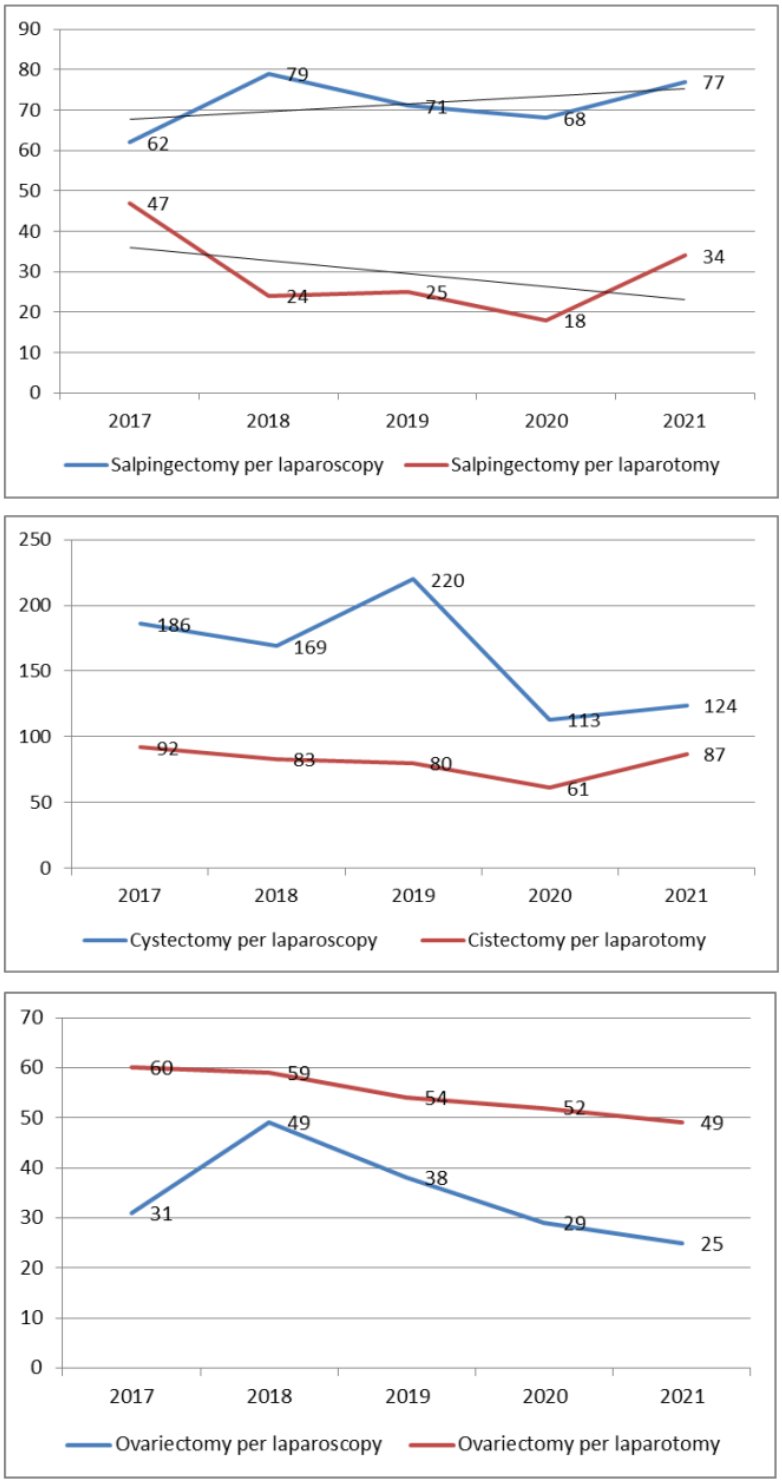

Figure 1. Total number of surgeries for benign adnexal masses per year

Furthermore, throughout the analyzed period all types of surgery were significantly more often performed with laparoscopic surgery than with open approach (cystectomies p=0.011, salpingectomies p=0.004 and adnexectomies p=0.004). Surgery of adnexal masses in our sample was elective in 53.2% of cases and due to emergency conditions in 46.8% (p=0.284) of cases. The emergency of surgery did not affect the choice of surgical approach (p=0.145). Still, women who were obese (p=0.023) were more often operated using the open approach.

Before the pandemic outbreak the number of patients operated laparoscopically slightly but gradually increased, mostly due to the rise in laparoscopic cystectomies (p=0.050). Contrary, the frequency of open surgeries for benign adnexal masses was declining especially when it comes to open salpingectomies (p=0.003) **(Table 1)**.

A substantial drop in the overall number of laparoscopic and open surgeries was clearly seen in the first year of pandemic (p=0.001) **(Figure 1)**. Moreover, the majority of surgeries were performed due to emergency condi-

tions (76.2%; p=0.001). Nevertheless, this decrease was significant only for performed laparoscopic cystectomies (p=0.001), but not for the rate of laparoscopic adnexectomies (p=0.224) and salpingectomies (p=0.762) **(Figure 2)**. Moreover, the 2020 reduction in the frequency of open cystectomies (p=0.073), adnexectomies (p=0.836) and salpingectomies (p=0.241) was also not significant. During the second year of the pandemic, the overall number of surgical procedures for benign adnexal masses started to rise again (p=0.023) **(Table 1)**.

Only two emergency surgeries of benign adnexal masses were performed in COVID-19 positive women, both executed using the open approach. No other patients developed symptoms or had a positive COVID-19 test during their stay in hospital, regardless of surgical approach or the type of surgery. Postoperative period was uneventful in all examined patients. Patients were discharged from the clinic on average on the 1.3 +/- 0.8 postoperative day after a laparoscopic surgery and on the 5.7 +/- 0.4 day after an open surgery for benign adnexal masses (p=0.001).

Figure 2. Types of laparoscopic surgeries and open surgeries per year

DISCUSSION

Surgery is a treatment of choice for numerous gynecological disorders, including benign and malignant uterine and adnexal pathologies. Since the beginning of the COVID-19 pandemic changes in the clinical routine and policies have posed certain issues concerning gynecological surgical treatment. Management of COVID-19 patients led to the redeployment of staff and resources causing a significant reduction in the total number of surgeries in many hospitals around the world, which was mostly reflected in elective non-emergency procedures, vaginal and minimally invasive gynecological surgery (9). In this unusual situation of crisis gynecologists were compelled to make a balance between patient treatment and prevention of COVID-19 infection, both for the patients and the staff. They strictly had to decide if a patient's situation needed urgent intervention and if so what would be the best treatment option and a surgical approach (laparoscopy or laparotomy) (10).

Surgical treatment of gynecologic conditions in COVID-19 pandemic was influenced by published recommendations for modifications of daily clinical practice issued by professional societies (11). At the beginning of pandemic different professional associations, including gynecological ones, released the Joint Statement saying that elective surgeries for benign conditions should be postponed, and if possible, alternate medical treatments should be considered, while in cases of malignancy or urgent conditions surgical treatment must not be delayed, but should be undertaken with precaution and using all preventive measures against COVID-19. A surgical treatment of COVID-19 positive patients who do not require emergency surgery due to life-threatening conditions should be postponed until full recovery while implementing all possible alternative treatments along with treatment against COVID-19. The decision of the surgical approach (laparoscopy, mini laparotomy or laparotomy) should be individualized and based on available conditions for a safe procedure as well as classic medical indications (4, 5).

When the same time periods before and after pandemic onset were compared in different centers a decrease in the volume of elective and emergency surgical procedures ranged from 26% up to 87% (12, 13). This decrease was most obvious in case of elective surgeries, while the frequency of emergency procedures was even increased in some institutions (14). Moreover, authors assessing surgical treatment of abdominal pathologies observed significantly higher patient morbidity and mortality during COVID-19 pandemic causing an increase in admissions to the intensive care unit for the patients of similar age (12, 14). When gynecological and obstetrical services during pandemic were assessed a sharp negative trend was observed mostly during spring 2020, while surgical rates recovered to pre-pandemic levels by the end of 2020 (12, 13).

Laparoscopy generally has numerous benefits for patients such as shorter recovery time and hospital stay, a lower risk of post-surgical complications, a reduced risk of pain and consequently the use of pain medication, less bleeding and risk of hemorrhage during the operation (15). Therefore, minimally invasive surgical procedures are generally indicated when it comes to surgery of benign adnexal pathologies except in case of suspicious ovarian malignancy, the presence of large tumors in the pelvis or findings on adnexal regions larger than 10 cm in diameter (16). However, at the beginning of the pandemic concerns were made regarding the safety of laparoscopy. COVID-19 virus (SARS-CoV-2) is a respiratory agent transmitted by respiratory droplets. Laparoscopic surgery is based on pneumoperitoneum formation by inflating the abdominal compartment with CO2. That means that laparoscopic surgeries are aerosol-generating procedures during which it might be possible to aerosolize viral particles and contaminate the operating room (17). Therefore, laparoscopic surgery might pose a greater risk of virus exposure which can occur during intubation and extubation, during CO2 inflating and expulsion as well as through surgical smoke of electrosurgical and ultrasonic devices which could all lead to aerosol induced infection. This concern was made because previous studies demonstrated increased transmission of other respiratory pathogens during laparoscopic surgeries. However, to date, no concrete evidence was found to prove that respiratory viruses are transmitted through electrosurgical smoke or aerosolized gas (18, 19). For that reason,

the finding of different microbial infections so far was not considered to be an absolute contraindication for the use of the laparoscopic approach, although specific protective measures are always recommended to avoid possible exposure to viral particles. Consequently, it was supposed that if similar protective measures are used in case of COVID-19 infection laparoscopic surgery could be safely performed (15, 20). Studies performed during the past two years have confirmed that with adequate preventive and protective measures laparoscopic surgery is possible without a significant increase in health risks for either patients or healthcare workers. Finally, different associations of endoscopic surgeons including gynecological ones proclaimed that laparoscopic approach should be undertaken whenever possible in preference to laparotomy in accordance with the current measures for safety of surgeons and patients (4, 5). One more reason for such recommendation is another benefit of laparoscopy presented in better use of hospital resources which is especially practical during the time of pandemic. Still, it is suggested to minimize the use of electrosurgical procedures, especially laser tissue ablation, ultrasonic scalpels, monopolar and advanced bipolar devices (21, 22). High-efficiency particulate air (HEPA) filters, ultra-low particulate air (ULPA) filters and closed smoke evacuation filtering devices are recommended if available to protect the medical staff against all potential risks of COVID-19 transmission. Moreover, some authors believe that if the patient is so urgent that there is not enough time for COVID-19 testing, laparotomy should be performed to prevent all risks (15, 23).

In recent years in our institution as well as worldwide, operations on the fallopian tubes are mostly performed laparoscopically. The number of laparoscopic fallopian tube operations remained similar during the analyzed period. On the other hand, the number of laparotomy operations was on the decline, but not significantly. Analyzing the number of operations of simple benign ovarian cysts in our clinic, it was obvious that in the period before the pandemic outbreak, the largest number of them was performed laparoscopically, while during the past two years there has been a significant decrease in the number of laparoscopic cystectomies. Unlike tubal surgery and ovarian cystectomy, removal of the ovaries with or without the fallopian tubes in our clinic was generally more often performed by laparotomy. The pandemic did not significantly impact either the overall number of performed adnexectomies or the approach used for these procedures.

Currently most institutions recommend that at admission all patients requiring urgent surgery should be questioned for having contacts with COVID-19 positive people over the past 14 days, screened for signs and symptoms of infection and also tested (quick antigen test) for COVID-19 infection (18, 24).

Our clinic has incorporated all current recommendations for COVID-19 prevention. According to the protocol of our Clinic all patients had to be pre-operatively tested for COVID-19 using a PCR test. Patients admitted for elective surgery (laparoscopic and open) are obligated to take a test 24 to 72 hours prior to the scheduled surgery and present a negative finding at admission to the clinic. In case of a positive finding, surgery is postponed until full recovery. On the other hand, emergency patients are all tested during the admission process. In case of positive test all precautions are taken (isolation from other patients, use of personal protective equipment of personnel, etc.) to prevent spreading the virus. If during their stay in hospital a patient shows signs and symptoms of COVID-19 she is immediately isolated, PCR tested and treated according to the current guidelines.

The strength and novelty of our study was the fact that it for the first time quantified the influence of COVID-19 pandemic on the performance of surgeries for benign adnexal conditions. Moreover, by examining a prolonged period of time we confirmed the safety of laparoscopic surgery even during the time of viral pandemic. However, the study limitation might be concentrating only on surgery of adnexal benign adnexal masses out of all different gynecological pathologies that are treated surgically. Still, according to clinical experience we hypothesized that the impact of reduced number of surgeries especially laparoscopic ones would have the most impact on adnexal pathologies and therefore they were chosen for investigation.

CONCLUSION

Following the recommendations of relevant surgical societies and due to the need for moving and redeployment of doctors and nurses, COVID-19 pandemic was proven to have a significant negative impact on the number of laparoscopic cystectomies in our hospital, while the number of laparoscopic salpingectomies and adnexectomies slightly decreased. Open surgeries were reduced in the first pandemic year, but not significantly, as most of such procedures were performed due to emergency conditions. During the second year of the pandemic, the overall number of surgeries recovered. Finally, our findings of no registered intra-hospital COVID-19 infections throughout the two-year period showed that with adequate preventive and protective measures, minimally invasive approach for surgery was possible and did not significantly compromise health of either patients or medical professionals.

Author Contributions: MD was in charge of the concept or design of the work. BM, JD, TD and LA performed the acquisition, analysis, and interpretation of data. MD, BM and JD wrote the draft of the manuscript. All authors revised the final version of manuscript.

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IMPLIKACIJE PANDEMIJE KOVIDA 19 NA LAPAROSKOPSKU I ABDOMINALNU HIRURGIJU BENIGNIH ADNEKSALNIH STANjA – ISKUSTVO JEDNOG CENTRA

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Sažetak

Uvod/Cilj: Pandemija Kovida19 predstavljala je izazov u lečenju pacijenata i izazvala je probleme u organizaciji zdravstvenih sistema u mnogim zemljama. Studija je imala za cilj da analizira i kvantifikuje uticaj pandemije Kovida19 na izvođenje operacija benignih adneksalnih stanja klasičnim (otvorenim abdominalnim) i minimalno invazivnim (laparoskopskim) pristupom na Klinici za ginekologiju i akušerstvo Univerzitetskog kliničkog centra Srbije.

Materijal i metode: U studiji su retrospektivno analizirane sve pacijentkinje koje su operisane zbog benignih adneksalnih masa na našoj Klinici u poslednjih pet godina. Uporedili smo brojeve i vrste operacija pre i tokom pandemije.

Rezultati: Studija je obuhvatila 2166 pacijentkinja koje su značajno češće imale laparoskopsku (61,9%) nego otvorenu operaciju (38,1%). Operacija benignih adneksalnih masa bila je elektivna u 53,2% slučajeva, a hitna u 46,8% slučajeva. Pre pandemije, laparoskopske operacije (uglavnom cistektomije) bile su u porastu. U 2020. godini zabeleženo je smanjenje broja laparoskopskih i otvorenih operacija (p=0,001). Štaviše, većina operacija je bila hitna (76,2%; p=0,001). Ipak, ovo smanjenje je bilo značajno samo za laparoskopske cistektomije (p =0,001), ali ne i za adneksektomije (p =0,224) i salpingektomije (p =0,762). Isto tako, smanjenje broja otvorenih cistektomija (p =0,073), adneksektomija (p =0,836) i salpingektomija (p =0,241) u 2020. godini takođe nije bilo značajno. Tokom 2021. godine ponovo je počeo da raste broj hirurških zahvata u slučaju benignih adneksalnih masa (p =0,023). Nisu registrovane intrahospitalne infekcije Kovidom 19.

Zaključak: Ukupan broj operacija benignih adneksalnih masa u našoj Klinici se smanjio i prilagodio samo hitnim stanjima, što se najviše odrazilo na minimalno invazivnu hirurgiju.

Ključne reči: Kovid 19, ginekološka hirurgija, patologija adneksa, laparoskopija, laparotomija

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ORIGINAL ARTICLE

TINNITUS RISK FACTORS AND TREATMENT IN ADOLESCENTS

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Summary

Introduction: Tinnitus is conscious perception of sound without an external sound stimulus. The origin of the name has its root in the Latin word *tinnire* (to ring). The sound can be buzzing, ringing, hissing, and is rarely heard as voice, music, or several different sounds simultaneously. Tinnitus occurs in one-third of people at least once in their lifetime and is chronic in 10-15% of the adult population. In children and adolescents aged 5 to 19 years, the range of tinnitus prevalence is quite wide (from 5% to over 40%), depending on how tinnitus is defined in the study. This article aims to provide an up-to-date overview of tinnitus risk factors and treatment in adolescents.

Methods: The authors searched PubMed, Embase, and Cochrane Review databases using the following keywords: *tinnitus, adolescents, risk behavior, risk factors,* and *treatment*. The inclusion criterion has an article published in Serbian or English without time restriction.

Results: Common risk factors for tinnitus among adolescents are female gender, noise exposure, hearing loss, marijuana and tobacco smoking, exposure to second-hand smoke, and sleep deprivation. Recently, some nutritional risk factors have been added: reduced water intake, niacin and protein deficit, and consumption of fizzy drinks, fast food, and white bread. The results of the current tinnitus treatments, including pharmaceutical, surgical, and behavioral ones, are unsatisfactory, causing frustration both in patients and physicians. Currently, there is no registered medicine for tinnitus.

Conclusion: Tinnitus is one of the greatest enigmas of modern medicine. As tinnitus is still considered incurable, we point out major risk factors among adolescents that should be targeted in primary prevention.

Key Words: tinnitus, adolescents, risk behavior, risk factors, treatment

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INTRODUCTION

Tinnitus is a conscious perception of sound in the absence of actual sound stimuli (1). The name originates from the Latin *tinnire* – to ring (2). It is mainly perceived as ringing, jingling, wheezing, buzzing, and sometimes as voice, music, or even multiple sounds simultaneously. Two main types of tinnitus are subjective tinnitus, where sound is perceived only by the patient, and objective tinnitus, where it can also be heard by the doctor (3). The pathophysiological mechanism of subjective tinnitus is still unknown. On the other hand, objective tinnitus can be caused by carotid or vertebral artery stenosis, myoclonic contraction of the tensor tympani muscle, abnormal contraction of nasopharyngeal muscles, etc. (3) Sound-causing objective tinnitus originates from the body itself where it is transferred to the ear where the impulse is generated and then conducted via the acoustic nerve to the auditory cortex. Since this sound has its source, by eliminating it, the perception will be eliminated (3). On the other hand, with subjective tinnitus, this can't be done. In half of all cases, tinnitus presents bilaterally. When presented unilaterally, it is more frequently perceived in the left ear (4). Rarely, the sound is perceived in the middle part of the head (4).

Every third person reported to have had tinnitus at least once in a lifetime. The chronic form of tinnitus is reported in 10-15% of adults (5,6). Data on the prevalence of tinnitus in children and adolescents are very inconsistent, ranging from 4.7% to 46% (7). This wide range is caused by differences in the methodology of different studies as well as characteristics of the pediatric population since data collected from preschool and elementary school children can be unreliable. Low prevalence is explained by the fact that children rarely report this symptom unless directly asked (8-10). On the other hand, lack of objectivity in this age group, as well as the tendency to overestimate the frequency of this symptom is considered to be the reason for certain studies to have reported a high frequency of tinnitus in this population.

As opposed to this younger pediatric population, data on tinnitus prevalence in adolescents are considered to be somewhat more reliable. In the most recent study conducted among Belgrade adolescents, Tomanic et al. (11) determined that around 13% of high school students presented with constant tinnitus, which is significantly more compared to Swedish adolescents (6%) (12), but much less compared to their Turkish peers (28.3%) (13).

Earlier, tinnitus was almost always associated with hearing loss in the elderly. However, due to the contemporary lifestyle, individuals are exposed to a more significant number of potential risk factors which increase the risk of tinnitus development, especially in healthy young adults. Nowadays, harmful effects of socioeconomic, ecological, behavioral, hereditary, and dietary factors are recognized.

Despite its high prevalence among children, tinnitus unaccompanied by hearing loss is not recognized enough in this population. Considering the fact that psychophysical well-being is essential for proper growth and development, the issue of tinnitus in this sensitive period can be considered a public health problem of particular interest.

This review aims to present known risk factors as well as tinnitus treatment in adolescents.

METHODS

Search Strategy

Two independent authors researched *Medline, Embase,* and *Cochrane* databases without time restriction. Articles in Serbian and English were considered. Book chapters found in relevant journal articles were also reviewed. The following keywords were used: *tinnitus, adolescents, risk behavior, risk factors,* and *treatment*. Due to method heterogeneity in the selected studies, only a narrative synthesis of the results was performed.

RESULTS OF THE RELEVANT LITERATURE REVIEW

Risk Factors

Earlier studies have shown a correlation between tinnitus and ear damage, especially damage related to hearing loss (14-16). However, in recent decades, studies have proven that tinnitus occurs in other auditory and non-auditory diseases as well (17). Also, a more significant number of risk factors for tinnitus development have been recognized, to which younger population is especially exposed **(Table 1.)**

Genetic factors

Studies on the prevalence of the hereditary form of tinnitus are very rare. A large study conducted on 198 families in Europe (18) showed that tinnitus was 1.7 times more likely to occur in individuals whose brother or sister had also reported having tinnitus. In a study from 2017 conducted by Maas et al. on twins in Sweden (Swedish Twin Registry) born between 1900 and 1985, tinnitus cases were classified into subtypes according to laterality (unilateral versus bilateral); they found that chances of inheriting bilateral tinnitus were 0.56, and for unilateral tinnitus 0.27 (19). In a recent study in Serbia, heredity had a significant effect on the probability of tinnitus development (20). Tinnitus was more common in adolescents whose close relatives had some form of tinnitus (acute, constant, intermittent or occasional tinnitus) (p=0.036) (20).

Table 1. Tinnitus risk factors in adolescence

Auditory factors	Non-auditory factors
Hearing impaired • Hearing loss • Presbycusis	Age and gender: • Older teen ages • Female gender
	Genetic factors (a more frequent occurrence in siblings)
	Behavioral factors: • Noise exposure (being in noisy places, headphone use) • Active tobacco smoking • Passive tobacco smoking • Drug use (primarily marijuana) • Gambling
	Dietary factors: • Positive correlation: fizzy drinks, fast food, coffee • Negative correlation: fruit, vegetables, wholegrain bread
	Psycho-emotional factors • Anxiety or depression • Insomnia
Specific diseases and pathological conditions associated with tinnitus: • High blood pressure • Thyroid disease • Head and neck injuries • Different types of surgeries • Multiple sclerosis • Viral infections • Auditory nerve tumors • Ototoxic medicines (quinine and aspirin) use • Anemia • Hyper-/hypothyroidism • Hyperinsulinemia and others	

Age and gender

When considering age and gender, recent meta-analyses and systematic reviews have shown that females of the pediatric population have a higher risk ratio (1.37) for tinnitus development compared to males (21). Multiple publications that studied tinnitus frequency also considered gender distribution and found that tinnitus prevalence in boys was between 20% and 35.6% and in girls between 17% and 42.4% (22-26). In the first Serbian study of tinnitus in adolescents, conducted in 2013, it has been noted that girls suffered from tinnitus more frequently than boys (15% vs. 9%) (27).

Park et al. found that children aged 12 to 18 years had a higher tinnitus prevalence than other age groups (28). In their research of the pediatric population with normal hearing, Aksoy et al. found that children aged 12 to 14 most often reported tinnitus, while 16-year-olds complained least often (29). In a study by Widen and Erlandsson on over 1000 adolescents from Sweden, aged 13 to 19 years, the prevalence of tinnitus was found to be about 8%, and this symptom was more frequent in older adolescents compared to younger adolescents (30).

Hearing impaired

Impaired hearing is still considered one of the most important risk factors associated with tinnitus. According to Baguley, hearing loss followed by subsequent neurological compensation along the auditory pathway is the most probable model of tinnitus development (31). A number of studies indicate that tinnitus is more common among children with hearing loss compared to children with normal hearing (32, 33). In his study from 2018, Lee found that the prevalence of tinnitus in adolescents with and without hearing loss differed, with a risk ratio of 2.39 (34). Other studies also support a higher prevalence of tinnitus in children with impaired hearing (between 23.5% and 62.2%) compared to peers with normal hearing (7.5% and 46.0%) (35-37). The most recent meta-analysis by Lee and Kim emphasizes that the most significant tinnitus risk factors in adolescents were noise exposure (OR= 11.3), hearing loss (OR= 2.4), female gender (OR= 1.4), and older ages (34).

Behavioral factors

Even though numerous studies have shown that noise exposure is a clear risk factor for tinnitus development, as well as a symptom that impairs quality of life to a large degree, adolescents do not seem to acknowledge its importance. During YANS questionnaire validation in Serbia, Tomanic et al. noted that an extremely high number of responses to the question about not needing to use earplugs in clubs and other noisy places indicates that awareness of the harmful effects of noise is very low among young people (38). The high prevalence of smoking, alcohol and substance abuse,

in adolescents with tinnitus, was noted in numerous studies (12,27,34). Several extensive cohort studies have confirmed that apart from active smoking, passive smoking, i.e. staying in rooms filled with tobacco smoke, is a very significant risk factor for tinnitus in the pediatric population (28,39-40). In their study, Lee and Kim also confirmed a significant correlation between active smoking and tinnitus in adolescents (41). Due to the specific developmental period, adolescents are more susceptible to the harmful effects of tobacco smoke and damage to the electromechanical transduction of external auditory cells may be more severe than in older smokers (42).

In the study by Marmut et al., the most dominant tinnitus risk factor in boys was drug use, primarily marijuana (OR = 13.1), whereas in girls it was passive smoking (OR 1,3/ per two hours of exposure) (27). Another study conducted six years later in Serbia found a positive correlation between tinnitus and drug use, being in noisy places, high blood pressure, headphone use, head injury, anxiety or depression, thyroid disease, and smoking (20). Tinnitus was also negatively affected by sedative use, anemia, sinusitis, noise exposure at home, duration of night sleep and noise exposure at school, but these correlations were not statistically significant (20).

Dietary factors

The correlation between nutritional factors and tinnitus in children and adolescents has not been investigated so far. In spite of growing interest and numerous studies in tinnitus, only a few studies researched the correlation between dietary factors and tinnitus and mostly in adult population. The study conducted by Tomanic et al. in 2020 was the first publication that examined the connection between dietary factors and tinnitus in an adolescent population (11). These authors found that fresh fruit, vegetables, and wholegrain bread intake may be negatively correlated to tinnitus, while fizzy drinks and fast food may raise the odds of tinnitus in adolescents (11).

Psycho-emotional factors

In one-third of adolescents, tinnitus is associated with significant psycho-emotional factors (anxiety and depression), which must be recognized and treated (43). Indeed, Levi et al. confirmed that psychiatric disorders in adolescence acted as risk factors for tinnitus (43). According to Nagel, tinnitus may be a cause of anxiety, depression, and insomnia and can impair life quality in younger people (44). At the same time, Stallman recognizes tinnitus as a severe symptom that often leads to a decrease in overall functionality and aggravates achieving academic results in the student population (45). Populational study in Korea indicated that an additional tinnitus risk factor was the average length of sleep ≤ 6 hours (AOR = 1.7) compared to ≥ 9 hours (21). The problem of not achieving an adequate sleep length is recognized in adolescents worldwide, as well as in Serbia, where it is found that 32.6% of boys and 27.6% of girls sleep less than recommended for this age group (46). Tomanic et al. also found a statistically positive correlation between gambling and tinnitus development in Belgrade high schoolers (20).

Specific diseases and pathological conditions associated with tinnitus

Tinnitus in all age categories can be an accompanying symptom of various diseases, resulting from taking numerous medications after head and neck injuries, different types of surgeries, excessive noise exposure, and numerous other causes (2). As mentioned before, tinnitus is a very significant symptom of hearing loss (47,48). It can also occur due to specific physiological processes in the inner ear, such as presbycusis or ototoxicity from various drugs (49). In rare cases, it can be caused by neurological diseases such as multiple sclerosis, viral infections, auditory nerve tumors, medicines such as quinine and aspirin that have ototoxic potential, and other health problems such as anemia, hyper-/hypothyroidism, hyperinsulinemia, and others (2). Research confirms that mental disorders on the anxiety-depressive spectrum may also contribute to the occurrence of tinnitus (50). Vice versa, people with tinnitus may subsequently develop depressive symptoms and severe social limitations (50). However, the direction of this relationship remains questionable, as it is not clear whether anxiety and depression cause tinnitus or occur consequently. In addition, common causes of tinnitus in young people may be various stress-inducing factors (50).

Treatment

Despite its widespread presence in the population, the effects of tinnitus treatment are very unsatisfactory, causing great frustration among patients and general practitioners, neurologists, and otolaryngologists alike (51). In most cases, tinnitus is diagnosed based on anamnestic data. In sporadic cases, pulsating tinnitus can be detected by auscultation.

There is currently no registered medication for tinnitus. To date, tinnitus treatment attempts with antidepressants, benzodiazepines, anticonvulsants, glutamate antagonists, counter-vertigo drugs, vitamins, magnesium, and zinc all influenced the comorbidity of tinnitus but had no significant effect on tinnitus itself (52). As it is not possible to eliminate etiological factors for subjective tinnitus, other treatments remain. Sound therapy is performed with either hearing aids (53) or tinnitus-masking sound generators (53). Relaxation therapy for distress or cognitive behavioral therapy are also used (54). Tinnitus retraining therapy is a combination of tinnitus sound therapy and cognitive behavioral therapy that, through

neural modulation, achieves optimal tinnitus habituation, reduces stress, and improves the quality of life with tinnitus (55). Low-frequency magnetic trans-cranial stimulation of the brain has also been applied with the aim of producing weak electrical currents in the brain to reduce neural excitability (56). Attempts have also been made with laser therapy, in most cases without therapeutic effect on tinnitus (57).

When considering surgical interventions, cochlear implantation produces good results in sensorineural hearing impairment, which is often accompanied by tinnitus. Still, in 9% of the treated patients, there is worsening of tinnitus, and in 4% of patients tinnitus occurs even though it did not exist before surgery (58). When compression of the vestibule-cochlear nerve by blood vessels is present, decompression surgical techniques can reduce tinnitus (59). Generally speaking, the effects of these tinnitus therapies are limited, indicating that preventive action on tinnitus risk factors is of primary and paramount public health importance.

CONCLUSIONS

The prevalence of chronic tinnitus in adolescents is relatively high, but our knowledge of this medical disorder is still insufficient. There are no approved medications for tinnitus, and the results of treatments are disappointing. Therefore, preventing tinnitus in young people should be a public health focus. This review of tinnitus risk factors in young people may be helpful for decision-makers to take urgent countermeasures.

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FAKTORI RIZIKA I LEČENJE TINITUSA KOD ADOLESCENATA

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Sažetak

Uvod: Tinitus je svesna percepcija zvuka bez spoljašnjeg zvučnog stimulusa. Naziv ovog simptoma potiče od latinske reči tinnire (zvoniti). Zvuk se najčešće percipira kao zujanje, zvonjenje, šištanje, a u manjem broju slučajeva kao glas, muzika ili nekoliko različitih zvukova istovremeno. Tinitus će se kod svakog trećeg čoveka javiti bar jednom u životu, a hronično je prisutan kod 10-15% odraslih. Kod dece i adolescenata, uzrasta od 5 do 19 godina, raspon prevalencije tinitusa je prilično širok (od 5% do preko 40%), u zavisnosti od toga kako je tinitus definisan u studiji. Ovaj rad ima za cilj da pruži najnoviji pregled faktora rizika za nastanak tinitusa i uvid u dosadašnje terapijske pristupe njegovom lečenju kod adolescenata.

Metode: Autori su pretraživali baze podataka PubMed, Embase, and Cochrane Review koristeći ključne reči: tinitus, adolescenti, rizično ponašanje, faktori rizika i lečenje. Kriterijum za uključivanje je bio da je članak objavljen na srpskom ili engleskom jeziku bez zadatih vremenskih okvira.

Rezultati: Uobičajeni faktori rizika za tinitus među adolescentima su ženski pol, izloženost buci, gubitak sluha, pušenje marihuane, duvana i izloženost pasivnom pušenju, kao i nedostatak sna. Nedavno su uočeni i dodatni faktori rizika povezani sa navikama u ishrani: nedostatak unosa vode, deficit niacina i proteina i konzumacija zaslađenih gaziranih pića, brze hrane i belog umesto integralnog hleba. Rezultati trenutnog lečenja tinitusa, bilo farmaceutski, hirurški ili bihejvioralni su nezadovoljavajući, što izaziva frustraciju kod pacijenata, ali i lekara. Trenutno ne postoji registrovani lek za tinitus.

Zaključak: Tinitus je jedna od najvećih enigmi moderne medicine. S obzirom da se tinitus još uvek smatra neizlečivim, ukazujemo na glavne faktore rizika među adolescentima na koje je značajno usmeriti primarnu prevenciju.

Ključne reči: tinitus, adolescenti, rizična ponašanja, faktori rizika i lečenje

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ORIGINAL ARTICLE

RISK FACTORS AND SURVIVAL RATE FOR PRIMARY THYROID LYMPHOMA: A CASE-CONTROL STUDY

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Summary

Aim. The aim of the study was to evaluate demographic and clinical characteristics of patients with primary thyroid lymphoma (PTL), to identify risk factors associated with PTL and determine overall survival.

Methods. We performed a retrospective case-control study of patients operated for PTL from 1995 to 2017. There were 41 patients with PTL who formed the cases group. The control group consisted of 82 patients with Hashimoto thyroiditis without concurrent thyroid disease. In statistical analysis we used standard descriptive statistics, logistic regression analysis, Kaplan-Meier survival curves and log rank test.

Results. In the cases group there were 35 patients with non-Hodgkin lymphoma and six patients with Hodgkin lymphoma. The cases group and the control group had a predominantly female population (>90%). In the control group nearly 70% of patients were younger than 55 years, while in the cases group over 60% of patients were older than 55 years. Risk factors for the development of PTL in patients with Hashimoto thyroiditis are older age, long standing Hashimoto thyroiditis, elevated level of TSH and a suspicious FNAB finding. Independent risk factors for PTL are older age (>55 years) and long standing Hashimoto thyroiditis (>10 years). The mean overall survival for patients with PTL is 92.8 months. Patients with longstanding Hashimoto thyroiditis have a shorter survival (84 month).

Conclusion. Patients older than 55 years with longstanding Hashimoto thyroiditis have a higher risk of developing PTL. Additionally, patients with longstanding Hashimoto thyroiditis have worse prognosis compared to other patients with PTL.

Key words: primary thyroid lymphoma, Hashimoto thyroiditis, risk factors, survival

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INTRODUCTION

Lymphoma is the seventh most common malignancy for both sexes together, with extra-nodal involvement in approximately 25–40% of cases.[1] Primary thyroid lymphoma (PTL) represents 2.5% to 7% of all extra-nodal lymphomas and comprises up to 5% of all thyroid malignancies.[2] PTL, although a rare type of thyroid tumour, is the most common nonepithelial thyroid neoplasm together with neoplasms arising from mesenchymal elements.[3] Most PTL are of B-cell origin, predominantly non-Hodgkin lymphoma which accounts for nearly 98% of all cases. Hodgkin lymphoma is much less common and accounts for less than 2% of PTL.[4] Non-Hodgkin lymphomas represent a histologically heterogeneous group of tumours with the most common type being diffuse large B-cell (DLBC) lymphoma followed by mucosal-associated lymphoid tissue (MALT), follicular lymphoma and small lymphocytic lymphoma.[5]

Thyroid DLBC lymphoma, apart from being the most common type, is unfortunately the most aggressive type as well. Disseminated disease is present (stage IVE) in nearly 60% of these patients at the time of diagnosis and the overall five year survival is below 50%.[6] Fortunately, if patients are diagnosed in stage IE of the disease, with a modern multimodal approach, a five-year survival is as high as 90%.[7] The gold standard for treatment of PTL consists of a combined modality therapy that includes chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and external beam radiation.[6] In rare cases, such as patients with intrathyroid MALT lymphoma (stage IE), surgery alone can be curative. More often, the most important role of surgery is confirmation of diagnosis through surgical excision of tissue and definitive pathohistological examination. Furthermore, surgery is useful in the palliation of obstructive symptoms for large thyroid lymphomas.

The best known risk factor for PTL is long standing Hashimoto thyroiditis. It is estimated that it takes 20-30 years for Hashimoto thyroiditis to progress to PTL.[8] The risk of PTL in patients with Hashimoto's thyroiditis is 67-80 times higher than in those without thyroiditis.[6, 8, 9] Other known risk factors are age and gender. PTL most typically occurs in middle-aged to old female individuals.[10, 11]

The aim of the study was to evaluate demographic and clinical characteristics of patients with PTL and identify risk factors associated with it. Establishing risk factors for PTL is important since early recognition of such patients is essential as it is curable if treated in earlier stages of the disease.

MATERIAL AND METHODS

We performed a retrospective case-control study of patients operated for PTL, at a tertiary referral academic hospital, in the period from January 1995 to January 2017. In this period there were 41 patients with PTL that underwent thyroid surgery at our hospital, and these patients formed the cases group. The inclusion criteria for the control group were patients operated for Hashimoto thyroiditis that do not have other concurrent thyroid disease (i.e. thyroid cancer). For each patient with PTL we randomly chose two controls using the following system: one control was the first patient that met the inclusion criteria and underwent surgery before the patient from the cases group; while the other control was the first patient that met the inclusion criteria and had surgery after the patient from the cases group. This way we formed a randomized control group that consisted of 82 patients with Hashimoto thyroiditis without concurrent thyroid disease.

Data of all patients included in the study were retrieved from the electronic database of the surgical department. We collected and analysed the following variables: sex (female/male), age (≤55/>55 years), duration of disease - Hashimoto thyroiditis (≤10/>10 years), preoperative level of TSH (≤10.0/>10.0 mlU/L), preoperative level of T4 (≤104.96/>104.96 nmol/L), cytology findings, type of operation and definitive pathohistological findings. For TSH, instead of using the median value of TSH, that was not statistically significant when analysed, we used a cut-off point of 10mlU/L to identify patients with long-term insufficient levels of hormones. We used this value because it was common practice among endocrinologists at one time not to introduce levothyroxine substitution before TSH levels exceeded 10mlU/L. For T4 we used the mean value (Kolmogorov-Smirnov and Shapiro-Wilk test not significant, $p>0.05$). Cytology findings were first graded using the Bethesda system for reporting thyroid cytopathology.[12] Afterwards we grouped Bethesda I-II as benign and III-VI as potentially malignant to obtain a dichotomous variable. The logic being that Bethesda III-VI will usually be referred for surgery, whereas I-II could have other outcomes other than surgery (repeat FNAB, follow-up). Type of operation included the following procedures: tumour biopsy, tumour reduction, thyroid lobectomy, total thyroidectomy (includes near-total thyroidectomy). Definitive pathohistological findings for the cases group were reported as Hodgkin lymphoma or Non-Hodgkin lymphoma. The reason for such a division was because Non-Hodgkin lymphoma was frequently reported as such, without further information whether it was DLBC lymphoma, MALT lymphoma or lymphoma of another subtype. This was the initial pathohistological finding that was received by the surgical department and which was available from our electronic database. These patients were further referred to the haematology department where the pathohistological findings were further analysed before commencing specific therapy. Unfortunately these data were not available for us and retrieving this information would be timely and dubious. Data regarding patient survival (whether the patient is still alive

Table 1. Descriptive statistics of cases and controls

Variable	Cases		Controls				Chi-square test		
	n[1]	%[2]	n	%	n	%	n	%	p[3]
Sex (female vs. male)	37	90.2	4	9.8	78	95.1	4	4.9	0.439
Age (years) (≤55 vs. >55)	16	39.0	25	61.0	56	69.1	25	30.9	**0.002**
Duration of disease[4] (years) (≤10 vs. >10)	26	66.7	13	33.3	71	93.4	5	6.6	**0.001**
TSH[5] (mlU/L) (≤2.43 vs. >2.43)	17	51.5	16	48.5	33	49.3	34	50.7	1.000
TSH (mlU/L) (≤10.0 vs. >10.0)	27	81.8	6	18.2	65	97.0	2	3.0	**0.015**
T4[6] (nmol/L) (≤104.96 vs. >104.96)	10	41.7	14	58.3	36	57.1	27	42.9	0.146
FNAB[7] (Bethesda) (I-II vs. III-VI)	20	60.6	13	39.4	43	93.5	3	6.5	**0.000**
Extent of operation[8] (less thanTT vs. TT)	24	58.5	17	41.5	5	6.1	77	93.9	**0.000**
FNAB	n		%		n		%		
Bethesda I	2		6.1		0		0		N/A[9]
Bethesda II	18		54.5		43		93.5		
Bethesda III	1		3.0		0		0		
Bethesda IV	0		0		2		4.3		
Bethesda V	8		24.2		1		2.2		
Bethesda VI	4		12.1		0		0		
Type of operation	n		%		n		%		
Tumour biopsy	8		19.5		0		0		N/A
Tumour reduction	13		31.7		0		0		
Lobectomy	3		7.3		5		6.1		
Total thyroidectomy	17		41.5		77		93.9		

1 Number of patients; 2 Percentage of patients; 3 Statistical significance; 4 Duration of Hashimoto disease; 5 Thyroid-stimulating hormone; 6 Thyroxine; 7 Fine needle aspiration biopsy; 8 Less than total thyroidectomy vs. total thyroidectomy (including near-total thyroidectomy) 9 Not applicable

or not and the date when they passed away) were obtained through direct contact with the patients or members of their families using contact details from our database.

Standard descriptive statistics was used to describe the variables included in the study. To determine independent risk factors for PTL we used univariate (ULRA) and multivariate logistic regression analysis (MLRA). All variables that were statistically related to PTL in ULRA at the level of significance of $p<0.05$ were further included in the MLRA model. Variables that had less than 80% of data available were not included in the MLRA. The level of statistical significance of $p<0.05$ was considered statistically significant, while $p<0.001$ was considered highly statistically significant. Kaplan-Meier survival curves were used to determine overall survival, while the log rank test was used to determine specific probability of survival for each of the observed significant variables. IBM SPSS Statistics, version 20.0.0 (SPSS Inc., Chicago, Illinois, USA) was used to perform the statistical analysis.

RESULTS

Descriptive statistics of variables analysed in the study are presented in **Table 1**. In our study of 123 patients (41 cases and 82 controls) there were 93.5% females and 6.5% males with sex ratio 14:1. There were more patients (58.5%) in our younger age group (≤55 years) than in the older group. The youngest patient was 20 years old, while the oldest patient was 82 years old. The mean age was 52.92 years (SD ±13.76 years), with a normal distribution (Kolmogorov-Smirnov test not significant, $p=0.200$). In the cases group there were 90.2% females and 9.8% males, with sex ratio 9:1. The youngest patient was 22 years old, while the oldest patient was 82 years old. The median age was 60 years (IQR25–75 44.5–71.0), with a skewed distribution (Shapiro-Wilk test significant, $n<50$, $p<0.001$). In the control group there were 95.1% females and 4.9% males, with sex ratio of nearly 20:1. The youngest patient was 20 years old, while the oldest patient was 74 years old. The mean age was 51.04 years (SD ±11.43 years), with a normal distribution (Kolmogorov-Smirnov test not significant, $p=0.200$). There was no statistical significance between the cases and control group in relation to sex or age (as a continuous variable) according to Pearson Chi-square (respectively $p=0.439$ and $p=0.142$).

Although there was no statistical difference in relation to age as a continuous variable, a statistically significant difference between cases and controls was noted (OR 3.56, 95% CI 1.60-7.80, $p=0.001$) when age was an-

Table 2. Univariate and multivariate logistic regression analysis

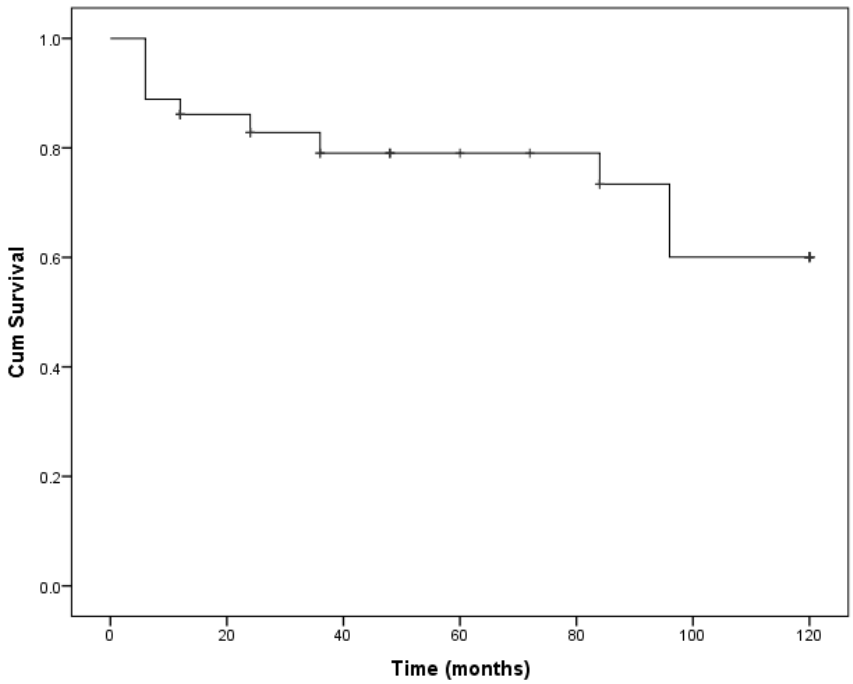
Variable	N[1] (%)	OR[2]	95% CI[3]	p[4]
Univariate logistic regression analysis				
Sex (female vs. male)	100	2.11	0.50-8.90	0.310
Age (years) (≤55 vs. >55)	100	3.50	1.60-7.67	**0.002**
Duration of disease[5] (years) (≤10 vs. >10)	92.7	7.10	2.30-21.87	**0.001**
TSH[6] (mlU/L) (≤10.0 vs. >10.0)	81.3	7.22	1.37-38.06	**0.020**
T4[7] (nmol/L) (≤104.96 vs. >104.96)	70.7	1.87	0.72-4.84	0.199
FNAB[8] (Bethesda) (I-II vs. III-VI)	64.2	9.32	2.38-36.40	**0.001**
Multivariate logistic regression analysis (N>80%, p<0.05)				
Age (years) (≤55 vs. >55)	100	4.44	1.61-12.22	**0.004**
Duration of disease (years) (≤10 vs. >10)	92.7	7.01	1.73-28.35	**0.006**
TSH (mlU/L) (≤10.0 vs. >10.0)	81.3	3.73	0.68-20.52	0.130

1 Percentage of data available for variable 2 Odds ratio; 3 Confidence interval; 4 Statistical significance; 5 Duration of Hashimoto disease; 6 Thyroid-stimulating hormone; 7 Thyroxine; 8 Fine needle aspiration biopsy

alysed as age groups of patients (≤55 years vs. >55 years); meaning there were significantly more patients with PTL in the older age group. A similar pattern was noted for patients with the duration of Hashimoto thyroiditis of more than 10 years (OR 7.38, 95% CI 2.39-22.80, p=0.001) and for patients with TSH level greater than 10 mlU/L (OR 7.22, 95% CI 1.37-38.06, p=0.015). In the control group, the most common finding of FNAB (93.5%) was Bethesda II (Hashimoto thyroiditis), while in the cases group there was a surprisingly high percentage of Bethesda II (54.5%), followed by Bethesda V and VI (24.2% and 12.1% respectively). When FNAB was examined as a dichotomous variable, there were highly significantly more patients in the cases group with a potentially malignant cytological finding (Bethesda III-VI), (OR 9.32, 95% CI 2.38-36.40, p=0.000). In the cases group the most common operation type was total thyroidectomy (41.5%), followed by tumour reduction (31.7%), while in the control group the vast majority of patients had a total thyroidectomy (93.9%). Naturally, examining the extent of operation there was a highly statistically significant difference between the cases and controls group (OR 0.46, 95% CI 0.15-0.138, p=0.000), which was expected.

The results of ULRA and MLRA are presented in Table 2. According to ULRA, risk factors for the development of PTL in patients with Hashimoto thyroiditis are older age (>55 years), OR 3.56, 95% CI 1.63-7.80, p=0.001; long standing Hashimoto thyroiditis (>10 years), OR 7.10, 95% CI 2.30-21.87, p=0.001; elevated level of TSH (>10 mlU/L), OR 7.22, 95% CI 1.37-38.06, p=0.020; and a suspicious FNAB finding (Bethesda III-VI), OR 9.32, 95% CI 2.38-36.40, p=0.001.

Figure 1. Overall survival of patients with primary thyroid lymphoma



According to the results of MLRA, independent risk factors for the development of PTL in patients with Hashimoto thyroiditis are an older age (>55 years), OR 4.54, 95% CI 1.65-12.49, p=0.003 and long standing Hashimoto thyroiditis (>10 years), OR 7.01, 95% CI 1.73-28.35, p=0.006. We did not include the variable FNAB in MLRA, even though it proved to be significant in ULRA, because the data was available for only 64.2% of patients.

Kaplan-Meier survival curve of overall survival for patients with PTL is shown on **Figure 1**. The mean survival time was 92.8±7.5 (95% CI 78.1-107.4) months. Kaplan-Meier survival curves of overall survival for patients with PTL in relation to age group and duration of Hashimoto thyroiditis are shown in **Figure 2** (graph A and graph B). The median survival time for patients with PTL was 96.0±7.6 months (95% CI 81.2-110.8, log rank p=0.029) in relation to the age group (graph A), and 84.0±59.0 months (95% CI 0.0-199.6, log rank p=0.036) in relation to the duration of Hashimoto disease (graph B). The median survival time for patients with PTL in relation to the extent of operation did not prove to be significant (log-rank p>0.05)

DISCUSSION

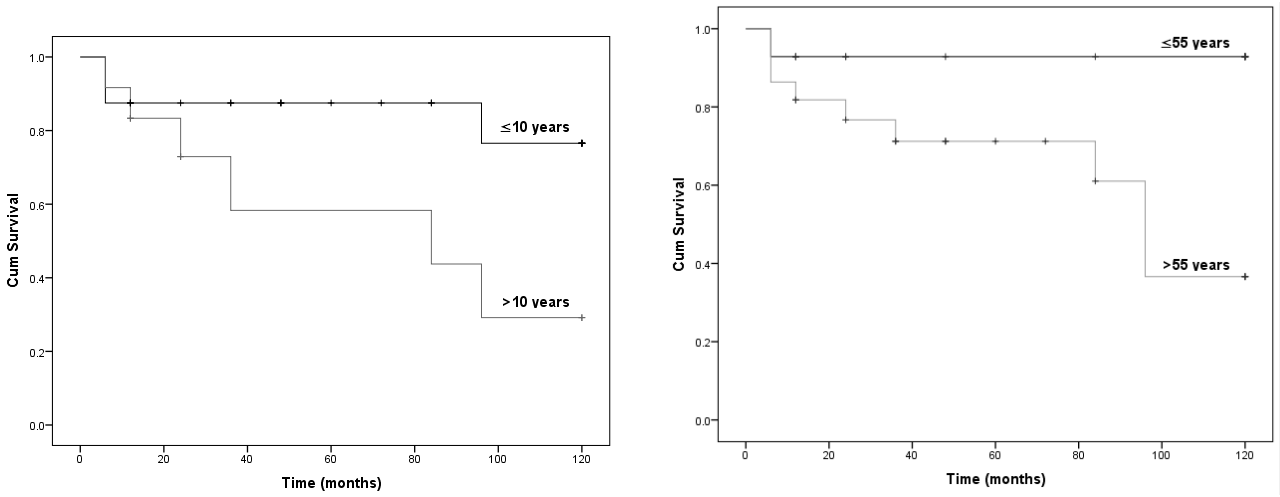
In our study, over 90% of patients with PTL were females. In comparison to other studies, our study had a rather high percentage of females, compared to the rates reported in literature ranging from 49% to 80%.[4, 5, 11, 13-15] Naturally, a high rate of female patients is expected since Hashimoto thyroiditis affects mostly females and is considered an aetiological factor for PTL.[15] Such a high rate of females in our study could further be attributed to the fact that there is a well-established gender-related detection bias in the setting of developing countries, such as Serbia, where women are screened earlier and more thoroughly for diseases than men who are often reluctant to attend medical consultations.[16] In our control group of patients with Hashimoto thyroiditis there was an even higher rate of females (over 95%). Although female gender is a well-known risk factor for PTL it was not statistically proven to be a risk factor in our study, probably because there was such a high ratio of females in both the cases group and the control group.

The youngest patient with PTL in our study was 22 years old. In literature, the youngest reported patient with PTL was 21.[17] The oldest patient in our study was 82 years old. In literature the oldest reported patient was 90.[11] We did not find many studies where age group was evaluated as a risk factor and no studies where a cut-off of 55 years was used as a risk factor. Although there were more patients (58.5%) altogether in our younger age group (≤55 years), there were significantly more patients with PTL in the older age group (61%). The study of Kuribayashi-Hamada et al. used 65 years as a cut-off point and, like us, found more patients with PTL in the older age group (80%).[18] Also, a study by Jin S. et al. showed that the age group of patients was an independent risk factor for PTL, and that the risk was the highest in the oldest age group (>80 years).[19] In our study, we found a much lower cut-off point (age group >55 years) to be an independent risk factor for PTL.

A previous history of Hashimoto thyroiditis is a well-known factor for PTL.[5, 9, 13] Patients with Hashimoto's thyroiditis have 67 to 80 times higher risk of developing PTL.[20, 21] It is estimated that it takes two to three decades for this transformation to happen.[8] In our study this time was much shorter; and a history of Hashimoto thyroiditis of more than ten years was adequate for it to be an independent risk factor for developing PTL.

In our study, to estimate the long-term effect of hypothyroidism we used the cut-off point for TSH (10.0mlU-/L) for reasons previously explained. This proved to be a risk factor for PTL. It is interesting to note that these patients were mainly patients that did not have a concom-

Figure 2. Overall survival of patients with primary thyroid lymphoma in relation to age group (A) and in relation to duration of disease (B)



itant long-term history of Hashimoto thyroiditis. This implies that it is possible that elevated TSH levels have a different mechanism, different from Hashimoto thyroiditis, which can lead to PTL. The evidence that can sustain this theory can be found in patients with PTL and with a recognised resistance to thyroid hormone. Patients with resistance to thyroid hormone can have extremely elevated TSH levels without having Hashimoto thyroiditis. Such is the reported case of a 67-year-old woman with PTL and with point mutation of the thyroid hormone receptor β gene in exon 10.[22] There have not been many studies which considered TSH as a risk factor for PTL. The main reason is probably inability to have adequate data for patients with highly elevated TSH, who naturally went untreated for hypothyroidism for long periods of time, and hence did not have recorded contacts with health services. There are several studies which reported hypothyroidism in patients with PTL. In these studies 36% to 45% of patients with PTL were also hypothyroid. [11, 23, 24]

Fine-needle aspiration cytology has not been a dependable method for diagnosing PTL, and the main diagnostic method had been ultrasound-guided puncture biopsy or surgical biopsy.[25, 26] Nowadays, with the implementation of immunohistochemical staining of the FNA-biopsied specimens the accuracy of FNA biopsy diagnosis has improved, although frequently additional tissue sampling for subtype confirmation through open biopsy is still required.[27] In our study, we did not evaluate FNA as an independent risk factor for the above mentioned reasons and because the data was available for less than 80% of the patients.

In our study, the median survival time for patients with PTL in relation to the extent of operation did not prove to be significant; thus this reinforces the main role of surgery in patients with PTL. The main role of surgery in the treatment of PTL is to acquire an adequate tissue sample and therefore biopsy is favoured above thyroidectomy.[19] For some subtypes of PTL (especially MALT lymphoma) surgery is beneficial.[28]

The mean overall survival for patients with PTL in our study was over 92 months. Patients with PTL and long-term Hashimoto thyroiditis had a much shorter median survival time (86 months) than patients in the older age group (96 months). Therefore it seems that long standing Hashimoto thyroiditis, apart from being an independent risk factor is also an important factor that affects long-term survival.

There are several limitations when conducting a case-control study. The main limitation of a case-control research is the difficulty to generalize the findings from one case-control study to other settings. Risk of bias may also influence the research and this is especially true when choosing the controls for the case-control study. The choice of the control group in our study was based on previous well established facts and was adequate to examine the association of Hashimoto thyroiditis and PTL, but a different control group could potentially lead to identifying other less known risk factors for PTL. The relatively low number of cases in our study is also a limitation, and could be overcome if a multi-centre study was undertaken with a similar methodology. Naturally, this could further strengthen the findings of our study or lead to new conclusions.

CONCLUSION

Patients older than 55 years with longstanding Hashimoto thyroiditis have a higher risk of developing PTL. Additionally, patients with longstanding Hashimoto thyroiditis have a shorter median survival time compared to other patients with PTL. The main role of surgery in the treatment of PTL is to acquire an adequate tissue sample, and therefore the extent of surgery usually does not affect survival.

Ethical approval: The study was in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments.

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FAKTORI RIZIKA I STOPA PREŽIVLJAVANJA ZA PRIMARNI LIMFOM ŠTITASTE ŽLEZDE: STUDIJA SLUČAJEVA I KONTROLA

Nikola Slijepčević[1, 2], Branislav Oluić[3, 2], Božidar Odalović[1, 4], Milan Grujić[5], Vladan Živaljević[1, 2]

Sažetak

Cilj studije. Cilj studije je bio da se analiziraju demografske i kliničke karakteristike pacijenata sa primarnim tiroidnim limfomom (PTL), identifikuju faktori rizika i utvrdi preživljavanje kod pacijenata sa PTL.

Metodologija. Sprovedena je retrospektivna studija slučaj-kontrola kod pacijenata obolelih od PTL u periodu od 1995. do 2017. godine. U grupu slučajeva su uključeni svi pacijenti sa PTL koji su operisani u datom periodu (ukupno 41). U kontrolnu grupu je uključeno 82 pacijenata sa Hašimoto tiroiditisom bez konkomitatnih oboljenja štitaste žlezde. U statističkoj analizi korišćena je standardna deskriptivna statistika, logistička regresiona analiza, Kaplan-Meier kriva preživljavanja i log-rang test.

Rezultati. U grupi slučajeva je bilo 35 pacijenata sa non-Hodžkin limfomom i šest sa Hodžkin limfomom. Grupa slučajeva i kontrolna grupa su imale predominatno žensku populaciju (>90%). U kontrolnoj grupi skoro 70% pacijenata je bilo mlađe od 55 godina, dok je u grupi slučajeva preko 60% pacijenata bilo starije od 55 godina. Faktori rizika za PTL kod pacijenata sa Hašimoto tiroiditisom su: starije životno doba, dugogodišnji Hašimoto tiroiditis, povišen nivo TSH i sumljiv nalaz aspiracione biopsije. Nezavisni faktori rizika za PTL kod pacijenata sa Hašimoto tiroiditisom su: starije životno doba (>55 godina) i dugogodišnji Hašimoto tiroiditis (>10 godina). Srednje vreme ukupnog preživljavanja kod pacijenata sa PTL je 92.8 meseci. Pacijenti sa dugogodišnjim Hašimoto tiroiditisom imaju kraće vreme preživljavanja (84 meseca).

Zaključak. Pacijenti stariji od 55 godina sa dugogodišnjim Hašimoto tiroiditisom su u povećanom riziku za nastanak PTL. Dodatno, pacijenti sa dugogodišnjim Hašimoto tiroiditisom imaju lošiju prognozu u odnosu na druge pacijente sa PTL.

Ključne reči: primarni tiroidni limfom, Hašimoto tiroiditis, faktori rizika, preživljavanje

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ORIGINAL ARTICLE

CLINICAL AND PATHOHISTOLOGICAL CHARACTERISTICS OF LUPUS NEPHRITIS IN PEDIATRIC AND ADULT POPULATION

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Summary

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease, characterized by abundant production of antibodies, deposits of immune complexes, and activation of the complement system, which disrupts the integrity and function of many organs, including the kidney. Although the frequency of SLE is less common in children, affected children develop lupus nephritis (LN) significantly more often, while in adults with SLE, LN occurs in 23% of cases, more often in males.

Aim: The aim of this study was to analyze clinical parameters (gender, frequency of LN as the first manifestation of SLE, proteinuria, and serum creatinine values) and pathohistological parameters (frequency of LN classes, activity and chronicity index values, immunoglobulin deposit intensity and complement components at immunofluorescence, and blood vessel lesions) in the pediatric and adult populations of LN patients.

Material and methods: The study included 218 biopsy samples of kidney tissue. Patients were divided into two groups: patients under 18 years of age (n=35) and those over 18 years of age (n =183).

Results: Mean values of serum creatinine in pediatric population (71.6±16.4 μmol/l) were statistically significantly lower ($p<0.001$) than in adults (115.5 ±64 μmol/l). Leukocyte interstitial infiltration was statistically significantly higher in the adult group ($p=0.003$). The average value of the chronicity index ($p=0.002$), as well as the tubulointerstitial parameters that determine it (tubular atrophy ($p <0.001$) and interstitial fibrosis ($p=0.011$)) were significantly higher in adults with LN. Leukocyte infiltration ($p=0.003$) and myoelastofibrosis ($p<0.001$) of blood vessels were statistically significantly more common in the adult population.

Conclusions: Serum creatinine values are significantly higher in the adult population of LN. Pathohistological findings indicate that glomerular LN lesions do not differ significantly with regard to activity and chronicity index in pediatric and adult populations, but the degrees of tubulointerstitial lesions are significantly higher, both in terms of activity and in terms of chronicity within the adult groups. Myoelastofibrosis and hyalinization of blood vessels as well as leukocyte infiltration of blood vessels, are statistically significantly more common in the adult population.

Keywords: Systemic lupus erythematosus, Lupus nephritis, pediatric population, adult population

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a distinctive production of large amounts of polyclonal antibodies, deposits of immune complexes, and activation of complement system, which disrupts the integrity and function of many organs, including the kidneys (1). SLE most often occurs in women in the reproductive period, while 10%-20% occur in the pediatric population (2). In the pediatric population, SLE usually occurs between the ages of 12 and 18, rarely before the age of 10, and even less often before the age of 5 (3-5). Lupus nephritis (LN) is usually not the first manifestation of SLE, but as many as 75% of patients with SLE develop some form of kidney lesion during the course of diseases (6). Kidney involvement is crucial in terms of morbidity and mortality due to the possible occurrence of chronic kidney disease (CKD), which in its terminal stage requires dialysis and/or transplantation (7). Although the incidence of SLE is lower in children, they are significantly more likely to develop lupus nephritis (42% of SLE patients), while in adults with SLE, lupus nephritis occurs in 23% of cases, more often in male patients (8-9).

According to the criteria established by the *American College of Rheumatology* (ACR 1997), the occurrence of proteinuria greater than 0.5g per 24 hours, or semi-quantitatively higher than 3+, and/or the presence of cylindruria in patients with SLE can be defined as the development of lupus nephritis (10). However, the gold standard for the diagnosis of LN is a renal biopsy, therefore the World Health Organization in 2018, based on morphological findings (optical-microscopic), classified lupus nephritis (ISN / RSP Code) in 6 classes: Class I - lupus nephritis with minimal lesions, Class II- mesangioproliferative LN, Class III- focal proliferative LN, class IV- diffuse proliferative LN, class V- membranous LN and class VI - advanced sclerosing LN (11). Studies have shown that class IV LN is the most common pathohistological type, both in pediatric and adult populations, with prevalence of about 60% (12-14). Defining the classes of lupus nephritis, as well as determining the presence of active and chronic lesions in the glomeruli and tubulointerstitium, have an important prognostic significance (11). Therefore, in 2018, the activity index and chronicity index were introduced as mandatory parameters in the classification of lupus nephritis (11). These indexes are based on changes in the interstitium (inflammation, tubular atrophy, interstitial fibrosis), and not only changes in the glomeruli (15-17). Studies have shown that the indexes of activity and chronicity of LN increase with class, so classes IV, V, VI of LN could have similar index values. Class II LN has lower index values, and class IV has the highest activity variability (18). In patients with SLE, various alterations of renal vessels can be seen on glomerular capillaries, arterioles and/or renal arteries. Immune deposits are most commonly found in class IV LN (19). In several cases, neutrophils infiltration is present, which may be accompanied by fibrinoid necrosis of glomerular basement membrane (20). In addition to the above mentioned morphological characteristics important for the diagnosis and classification of LN, there is a significant and characteristic finding of immunofluorescence (IF), which in these patients is often characterized by the presence of "*full-house*" phenomenon. This phenomenon implies that all routinely tested antibodies (IgA, IgG, IgM, C3, C1q, kappa, lambda) are positive for IF findings with moderate and/or strong intensity (++/+++), (11).

The aim of this study was to analyze clinical parameters (distribution by gender, frequency of LN as the first manifestation of SLE, values of proteinuria and serum creatine) and pathohistological parameters (frequency of LN classes, activity and chronicity index values, immunoglobulin deposit intensity and complement components immunofluorescence and blood vessel lesions) in pediatric (children and adolescents-younger than 18 years) and adult (adults-older than 18 years) populations of LN.

MATERIAL AND METHODS

The study used material from the Institute of Pathology of the Faculty of Medicine University of Belgrade. The study involved 218 renal biopsies diagnosed between 2003 and 2020. Clinical and laboratory data recorded at the time of biopsy data were collected from medical records. Patients were divided into two age-based groups: patients up to 18 years of age (n=35) and those older than 18 years (n=183).

Activity and chronicity indexes were determined based on the ISN/RPS criteria presented in **Table 1** (11).

Statistical analysis was performed using IBM SPSS software, version 26.0. We used the χ 2 test, Fisher's test, Student's *t*-test, and *Mann-Whitney U* test, and $p < 0.05$ was considered statistically significant.

RESULTS

Clinical parameters

In the group of patients consisting of children and adolescents, the mean age was 13.9±2.6 years (median 14 years, range 6 to 18 years). In the adult population, the mean age was 37.9±13.1 years (median 36 years, range 19 to 74 years).

Details of the clinical characteristics of patients with LN in the adult and pediatric population are shown in **Table 2**. Our study population was composed of 24.3% (n=53) males and 75,7% (n=165) females. Among males, 14.3% (n=5) were patients younger than 18 years, and 26.2% (n=48) were patients older than 18 years. In the female population, 85.7% (n=30) patients belonged to

Table 1. Criteria for determining the index of activity and chronicity of lupus nephritis lesions.

Activity index	**Definition**	**Score**
Endocapillary hypercellularity	Endocapillary hypercellularity in <25% (1+), 25% –50% (2+), or > 50% (3+) of glomeruli	0-3
Neutrophils/karyorrhexis	Neutrophil and/or karyorrhexis infiltration in <25% (1+), 25% –50% (2+), or > 50% (3+) of glomeruli	0-3
Fibrinoid necrosis	Fibrinoid necrosis in <25% (1+), 25% –50% (2+), or > 50% (3+) of glomeruli	(0-3)x2
Hyaline deposits	Wire loop lesions and/or hyaline thrombi in <25% (1+), 25% –50% (2+), or > 50% (3+) of glomeruli	0-3
Cellular/fibrocellular crescents	Cellular and/or fibrocellular crescents in <25% (1+), 25% –50% (2+), or > 50% (3+) of glomeruli	(0-3)x 2
Interstitial inflammation	Interstitial leukocytes in <25% (1+), 25% –50% (2+), or > 50% (3+) in the cortex	0-3
Total		**0-24**
Chronicity index	**Definition**	**Score**
Total glomerular sclerosis	Total glomerular sclerosis in <25% (1+), 25% –50% (2+), or> 50% (3+) glomeruli	0-3
Fibrous crescents	Fibrous crescents in <25% (1+), 25% –50% (2+), or> 50% (3+) glomeruli	0-3
Tubular atrophy	Tubular atrophy in <25% (1+), 25% –50% (2+), or> 50% (3+) glomeruli	0-3
Interstitial fibrosis	Interstitial fibrosis in <25% (1+), 25% –50% (2+), or> 50% (3+) in the cortex	0-3
Total		**0-12**

the pediatric group and 73.8% (n=135) of patients were adults.

It is known that LN may be the first manifestation of SLE. In our study, 34 patients had LN as the primary manifestation, and among them, 16.5% (n=30) were older than 18 years.

Although it was observed that the average value of proteinuria in children and adolescents on average was lower (2.5±2.1g/24h) compared to the average value of proteinuria in the adult population (7.2±6g/24h), the difference was not statistically significant, most likely due to the small number of patients from the group of younger subjects.

Mean values of serum creatinine in pediatric population (71.6±6.4 μmol/l) were statistically significantly lower (p <0.001) than the values in the adult population (115.5±64 μmol/l).

Pathohistological parameters

The distribution of pathohistological parameters is shown in detail in Table 3. Among the examined pathohistological parameters, which determine the LN activity index, glomerular lesions did not differ significantly in pediatric and adult populations, but the value of the intensity of interstitial infiltration by leukocytes was higher in the adult group and covered mainly between 25-50% of the interstitium (p=0.003). The mean values of the chronicity index, as well as the tubulointerstitial parameters that determine it (tubular atrophy and interstitial fibrosis), were statistically significantly higher in adult LN **(Table3)**.

LN was associated with changes in blood vessels in both pediatric and adult populations. It was noticed that leukocyte infiltration and myoelastofibrosis of blood vessels were statistically significantly observed in the adult population. Necrosis of medium size blood vessels was observed only in the adult population **(Table 3)**.

The frequency of classes did not significantly differ in pediatrics and adults. It was noticed that class IV LN was the most common pathohistological form of the disease in both examined populations. On the other hand, the majority of class V LN 21.3 (n=39) was detected in adult population, and one patient with class VI LN belonged to the same group **(Table 3)**.

The absence of a "*full-house*" immunofluorescent finding was observed in 25 patients, of whom 15.6% (21/25) belonged to the adult LN population. On the other hand, a very similar distribution of positive full-house immunofluorescence findings was observed in

Table 2. Clinical parameters in patients with lupus nephritis.

		Children and adolescents	Adults	p
Gender [n (%)]	Male	5 (14.3%)	48 (26.2%)	0.135
	Female	30 (85.7%)	135 (73.8%)	
Primary manifestation [n (%)]	no	30 (88.2%)	152 (83.5%)	0.495
	yes	4 (11.8%)	30 (16.5%)	
Serum creatinine [average value±SD]		71.6±16.4	115.5±64	<0.001*
Proteinuria [average value±SD]		2.5±2.1	7.2±6	0.282

Table 3. Pathohistological parameters in patients with lupus nephritis.

		Pediatrics	Adults	P
		[average value ± SD] *[N (%)]*		
ISN/RPS activity and chronicity indexes				
Activity index		6.1±3	5.7±3	0.407
Endocapillary hypercellularity		1.9±1	1.9±0.7	0.745
Neutrophils/karyorrhexis		1.1±1	1±0.9	0.536
Fibrinoid necrosis		0.9±1.2	0.7±1	0.178
Cellular/fibrocellular crescents		0.6±0.8	0.7±1.3	0.696
Hyaline deposits		0.7±0.9	0.5±0.9	0.187
Interstitial infiltration		0.7±0.9	1.2±0.9	0.003*
Chronicity index		1.1±1.9	2±1.7	0.002*
Total glomerular sclerosis		0.3±0.7	0.5±0.7	0.098
Fibrous crescents		0.1±0.4	0.1±0.3	0.549
Interstitial fibrosis		0.3±0.7	0.7±0.7	0.011*
Tubular atrophy		0.4±0.6	0.9±0.7	<0.001*
Immunofluorescence (IF)				
IgA		2.2±1	1.9±1	0.235
IgG		2.3±0.4	2.2±0.9	0.687
IgM		2±0.9	1.8±0.9	0.229
C1q		2.1±1	1.9±0.9	0.177
C3		2.5±0.8	2.3±0.8	0.185
***"Full-house"* IF findings**	no	4 (14.8%)	21 (15.6%)	0.923
	yes	27 (85.2%)	114 (84.4%)	
Myoelastofibrosis and hyalinization of blood vessels	not present	24 (70.6%)	47 (26.6%)	<0.001*
	present	10 (29.4%)	130 (73.4%)	
Infiltration of blood vessels	not present	27 (82%)	81 (46%)	0.002*
	present	7 (18%)	96 (54%)	
Necrosis of medium-size blood vessels	not present	34 (100%)	167 (94.4%)	0.157
	present	0 (0%)	10 (5.6 %)	
ISN/RPS lupus nephritis classification				
Class I		1 (2.9%)	2 (1.1%)	0.153
Class II		8 (22.9%)	23 (12.6%)	
Class III		6 (17.1%)	19 (10.4%)	
Class IV		18 (51.4%)	99 (54.1%)	
Class V		2 (4.9%)	39 (21.3%)	
Class VI		0 (0%)	1 (0.5%)	

** -statistically significant difference;*

these two groups of subjects, so the differences were not statistically significant p=0.923 **(Table 3)**.

DISCUSSION

Lupus nephritis (LN) can lead to chronic kidney failure (CKF) and is one of the most serious manifestations of systemic lupus erythematosus (SLE) (7). Bearing in mind that SLE is more common in female population, studies indicate that LN is more common in men with SLE (21,22). In a cohort of our subjects consisting of patients with LN, a significantly higher incidence was observed in female patients (male to female ratio was 1:3). Females were more frequent, and the ratio between the genders in the adult population was the same as in the cohort of all patients (1:3), while in the pediatric population the incidence of girls was six times higher than boys (1:6).

It was noticed that LN can be the primary manifestation of SLE up to 80% of cases in the pediatric population (23,24), while in the adult population the primary manifestation of LN in SLE is significantly rarer (35%–50%)

(3). The results of our study indicate a significantly lower incidence of LN as the primary manifestation of SLE in both populations of subjects (12% pediatric and 16% adult).

In our study, the value of serum creatinine was significantly higher in the adult population, but it is generally known that the values of serum creatinine are higher in the population of healthy adult individuals (25), as well as the frequency of comorbidities (hypertension and diabetes) that may contribute to the damage of kidneys and an increase in serum creatinine (26). Serum creatinine is not of great importance in terms of diagnosing LN, but a progressive increase in serum creatinine is associated with a poorer course of the disease (27-29). Also, serum creatinine is used as a parameter in the calculation of eGFR, (30) and it has been shown that lupus nephritis patients have lower eGFR values in the adult population (14). In the lack of data on the body height of pediatric patients, we were unable to determine their eGFR, and statistical analysis was not possible despite the existence of eGFR data in the adult group.

Proteinuria is also an important laboratory parameter in the process of diagnosing LN, although it does not have great specificity. The decline in proteinuria after therapy predicts a good further course of the disease (27-29). In our study, children and adolescents had on average lower values of proteinuria but compared to the values observed in the adult populations, a statistically significant difference was not observed. Our results are in accordance with the previously published data (14).

The histopathological analysis itself is of great importance, because it directly influences the definition of therapy and determines the prognosis (7). Pediatric population is at a higher risk of active LN development, based on activity index parameters (3). Although the values of the activity index and its parameters were not statistically significantly higher, a higher value of the parameters can be observed in the pediatric population. The chronicity index is statistically significantly higher in the adult population, which is due to the higher values of the score of tubular atrophy and interstitial fibrosis. However, studies have shown that the activity index has been shown to be a relatively weak indicator of further impaired renal function because lesions are potentially reversible and patients themselves respond differently to the same therapy (15,17,31,32). Certain studies have shown that the chronicity index correlates with the outcome of the disease only in class III LN (33).

Previous studies have indicated that among LN, class IV has the highest incidence (43% in pediatrics and 67% in adults). Although class II is the rarest in general population, among patients under the age of 18, class II accounted for 25% of all cases, while its frequency is lower in the adults with SLE and accounts for only 2.5%. Classes III and V have the same frequency in total population (15). In pediatric population, class III is present in 10% of cases, and in the adult population in up to 15%, while class V in both populations have the same frequency (14%). This research did not cover classes I and VI (14). In our study, the distribution among classes is similar. Class IV of LN is also the most common class, without differences between populations (51.4% in pediatric LN and 54.1% in adult LN). When it comes to class II LN, our results show a slight difference in frequencies (22.9% in pediatrics and 12.6% in adults). A significant difference was noticed in class V (4.9% in patients younger than 18 and 21.3% in patients older than 18). Class I was three times more common in the pediatric population than in the adult population (2.9% and 1.1%).

In the adult population, vascular lesions are more common (14). We observed that leukocyte infiltration of blood vessels, as well as myoelastofibrosis and hyalinization of blood vessels are statistically significantly more common in the adult population as well. Infiltration of blood vessels occurs in the pediatric population in only 3%, while in the adult population it occurs in 39% of cases. Myoelastofibrosis and hyalinization of blood vessels occur in 62% of cases in adults, and in 5% of cases in pediatric population. Although the data were not statistically significant, fibrinoid necrosis of large blood vessels does not occur in the pediatric population, and in the adult population it occurs in only 6%.

"Full-house" immunofluorescent findings are characteristic for the LN as proven in our results, and the distribution among patient groups was almost identical (85.2% in pediatric and 84.4% in adult).

CONCLUSION

Serum creatinine values are significantly higher in the adult population of lupus nephritis. The histologic finding suggests that the glomerular lesions of LN do not differ significantly in terms of the activity index and chronicity index in the pediatric and adult population, but the tubulointerstitial lesions are significantly higher, both in terms of activity and chronicity in a group of adult patients. Myoelastofibrosis and hyalinization of blood vessels as well as leukocyte infiltration of blood vessels, are significantly more common in the adult population as well.

It is known that tubulointerstitial lesions determine the course and outcome of the disease and directly influence the occurrence of CKD and eventually kidney transplantation, however, for final confirmation of the influence of tubulointerstitial lesions on renal function, it is necessary to perform a follow-up study.

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KLINIČKE I PATOHISTOLOŠKE KARAKTERISTIKE LUPUS NEFRITISA U PEDIJATRIJSKOJ I ADULTNOJ POPULACIJI

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Sažetak

Uvod: Sistemski lupus eritematozus (SLE) predstavlja autoimuno oboljenje koje karakteriše obilna produkcija antitela, imunih kompleksa, kao i aktivacija sistema komplementa, koja remeti normalnu funkciju i rad mnogih organa, uključujući i rad bubrega. Iako je učestalost pojave SLE kod dece retka, jedna od najčešćih manifestacija kod obolele dece je lupus nefritis (LN), dok se kod odraslih obolelih od SLE, lupus nefritis javlja u 23% slučajeva i to češće kod muškaraca.

Cilj rada: Cilj ovog rada je bio da se analiziraju klinički (pol, učestalost LN kao prva manifestacija SLE, proteinurija i vrednosti serumskog kreatinina) i patohistološki parametri (učestalost klasa SLE, indeks aktivnosti i hroničnosti, intenzitet imunofluorescentnog bojenja imunoglobulina i kompleksa komplementa, kao i promene na krvnim sudovima) u pedijatrijskoj i adultnoj populaciji bolesnika sa LN.

Materijal i metode: U studiji je analizirano 218 uzoraka bubrežnog parenhima. Pacijenti su podeljeni u dve grupe: pacijenti mlađi od 18 godina (n=35) i pacijenti stariji od 18 godina (n=183).

Rezultati: Srednje vrednosti serumskog kreatinina u pedijatrijskoj populaciji (71,6±16,4 μmol/l) su bile statistički značajno niže (p<0,001) nego kod odraslih (115,5±64 μmol/l). Intersticijalna infiltracija leukocitima bila je statistički značajno viša u grupi odraslih (p=0,003). Prosečna vrednost indeksa hroničnosti (p=0,002) kao i tubulointersticijski parametri koji ga određuju (tubularna atrofija (p<0,001) i intersticijalna fibroza (p=0,011)) bili su značajno viši kod odraslih sa LN. Infiltracija leukocitima (p=0,003) i mioelastofibroza (p<0,001) krvnih sudova bili su statistički značajno češći u populaciji odraslih.

Zaključak: Vrednosti kreatinina u serumu su značajno više u adultnoj populaciji LN. Patohistološki nalazi ukazuju na to da se glomerularne lezije LN ne razlikuju značajno u pogledu aktivnosti i indeksa hroničnosti u pedijatrijskoj i adultnoj populaciji, ali su stepen tubulointersticijalnih lezija značajno veći, kako u pogledu aktivnosti, tako i u pogledu hroničnosti kod adultnih pacijenata. Mioelastofibroza i hijalinizacija krvnih sudova, kao i leukocitna infiltracija krvnih sudova, statistički su značajno češće u adultnoj populaciji.

Ključne reči: Sistemski lupus eritematozus, Lupus nefritis, pedijatrijska populacija, adultna populacija.

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ORIGINAL ARTICLE

THE SIGNIFICANCE OF 18-FLUORO-DEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY WITH COMPUTED TOMOGRAPHY IN COMPARISON WITH MULTI-SLICE COMPUTED TOMOGRAPHY IN RECURRENT BLADDER CANCER

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Summary

Introduction: Positron emission tomography with computed tomography using 18-fluoro-deoxyglucose (^{18}FDG-PET/CT) is still not applied routinely in clinical practice for the evaluation of recurrent bladder cancer. Recent guidelines recognize the importance of ^{18}FDG-PET/CT, but multi-slice computed tomography (MSCT) is still recommended for monitoring these patients. **Aim:** To determine the agreement between ^{18}FDG-PET/CT and MSCT findings in the categorization of patients into N and M stages of the disease and the agreement of two diagnostic modalities regarding the number of detected lesions.

Material and methods: 31 patients (22 men and 9 women), mean age 61.2 ± 9.2 years, were included in our study after surgical treatment and histopathological confirmation of bladder cancer. Zones of pathological uptake of ^{18}FDG were interpreted visually and semi-quantitatively using the maximum standardized uptake value (SUVmax). The agreement of ^{18}FDG-PET/CT findings was compared to previous MSCT using Cohen's kappa test for interobserver agreement, interpreted based on the Altman's criteria.

Results: The overall agreement between ^{18}FDG-PET/CT and MSCT in N stage of the disease was 77% (κ = 0.54; moderate agreement); in stage N0 68%, N2 77%, N3 29%. In M stage, total agreement was 53% (κ = 0.10; poor agreement); in stage M0 39%, M1a 22%, M1b 44%. ^{18}FDG-PET/CT detected a total of 29 lesions in N stage of the disease, while MSCT detected 16 lesions, with the agreement of 71% (κ = 0.41; moderate agreement). In the M stage of the disease, ^{18}FDG-PET/CT detected 42 lesions and MSCT detected 30 lesions, with overall agreement of 52% (κ = 0.07; poor agreement).

Conclusion: Our results show that there is a moderate agreement between ^{18}FDG-PET/CT and MSCT findings in the categorization of patients and the number of detected lesions in N stage of disease, but that ^{18}FDG-PET/CT detects more lesions. ^{18}FDG-PET/CT also detects a higher number of lesions in M stage, but the agreement with MSCT findings is poor.

Keywords: recurrent bladder cancer, PET/CT, MSCT

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INTRODUCTION

According to the International Agency for Research on Cancer, bladder cancer is the eleventh most common malignant tumor [1]. The incidence increases with age and is more common in males, so that in men over sixty years of age, bladder cancer is in sixth place in frequency [1]. The current age-standardized incidence is higher in men (9.5) than in women (2.4) per 100,000 respondents [1].

Tobacco smoking is one of the most important risk factors for bladder cancer and is the cause of about 43% of all cases in male population, and 26% in female population [2]. The mortality rate is about 2% higher in smokers than in non-smokers, especially in those who start consuming tobacco in adolescence [3]. Other significant risk factors are exposure to certain industrial chemicals, chronic urinary tract infections and urinary tract calculosis [4,5,6].

Bladder cancer belongs to a very heterogeneous group of tumors, but in over 90% of cases the histopathological type is transitional cell (urothelial) cancer, while the remaining ≈10% include urothelial cancers with partial squamous or glandular differentiation, micropapillary cancers, sarcomatoid carcinoma, neuroendocrine tumors, and others [7]. Transitional cell carcinomas are classified into low- and high-grade carcinomas based on the degree of nuclear anaplasia and architectural abnormalities, of which the latter are associated with a poor prognosis [8].

Involvement of the muscle wall of the bladder is a crucial factor in choosing an adequate method of treatment. Over 75% of bladder cancers are not muscle-invasive [9] and are treated surgically with transurethral resection of bladder tumors (TUR). Surgical treatment is usually accompanied by a single intravesical instillation of chemotherapy, which significantly reduces the five-year recurrence rate of the tumor [10], or immunotherapy. Muscle-invasive bladder cancers are initially treated with radical cystectomy, followed by neoadjuvant cisplatin-based chemotherapy, which significantly improves survival rates [11]. The most common sites of bladder cancer metastases are lymph nodes, followed by the liver, bones, and lung parenchyma [12].

Clinical monitoring of patients with bladder cancer without invasion of the muscle wall is most often done by cystoscopy. Recent guidelines of the European Association of Urology for invasive bladder cancer recommend multi-slice computed tomography (MSCT) check-ups every six months for the first three years, and then once a year [13]. Shorter diameter of lymph nodes on MSCT over 8mm and morphological changes in the form of irregular contours are considered significant for suspicion of disease spread [12]. The sensitivity of MSCT in the detection of bladder cancer metastases in lymph nodes is subject to large variations and amounts to 30-75% [14]. The reason for this is that metastases can be present in lymph nodes that are not enlarged, and such, occult metastases, still cannot be reliably detected by available diagnostic modalities.

Positron emission tomography with computed tomography using 18-fluoro-deoxyglucose (^{18}FDG-PET/CT) is based on the fact that malignant tumors show a higher degree of glycolysis than normal cells (Warburg effect), which allows the detection of metastases in lymph nodes and other parts of the body based on increased glucose metabolism. The European Association of Urology recognizes the importance of ^{18}FDG-PET/CT for muscle-invasive bladder cancer, and the fact that its role is still being assessed, but has not yet been recommended as a diagnostic modality of choice in monitoring these patients [13]. The number of studies comparing ^{18}FDG-PET/CT and MSCT findings in patients with bladder cancer is relatively small. With this in mind, the aim of our study was to evaluate the agreement of ^{18}FDG-PET/CT and MSCT findings in categorization of patients in N and M stages of the disease, as well as the agreement between the number of detected lesions in each category.

MATERIAL AND METHODS

Study population. In the period between January 2016 and December 2021, 46 patients with the diagnosis of bladder cancer were referred to our center due to suspicion of disease recurrence. Criteria for inclusion in the study were histopathologically confirmed bladder cancer during surgery, the time between surgery and ^{18}FDG-PET/CT longer than three months, MSCT examination not older than three months before ^{18}FDG-PET/CT examination, the absence of other malignancies and serum glucose level below 11mmol/L on the day of ^{18}FDG-PET/CT examination. According to the aforementioned criteria, 15 patients were excluded from the study. The remaining 31 patients (22 men and 9 women), mean age 61.2 ± 9.2 years, were included in the study. All included patients gave informed consent for the research and the study was approved by the Ethics Committee of the University Clinical Center of Serbia (number 668/6).

Acquisition and interpretation of ^{18}FDG-PET/CT findings. Whole body ^{18}FDG-PET/CT imaging was performed on all patients using 64-slice hybrid PET/CT (Biograph, TruePoint64, Siemens Medical Solutions, Inc. USA) in our center. Patients did not consume food or sweetened drinks at least 6 to 8 hours prior to the examination. 5.5MBq of ^{18}FDG per kilogram of body weight was administered intravenously, after which patients lay down to rest in quiet and darkned room for at least 60 minutes before acquisition. Patients were instructed to void before imaging. Low dose CT (120kV, slice thickness 5mm) and three-dimensional PET/CT images were acquired from mid-thigh to skull base. Corrected and uncorrected ^{18}FDG-PET/CT and CT images were in-

Table 1. Patient characteristic

Characteristics	Number
Total number of patients (n)	31
Age (years)	
Mean ± standard deviation	61.2 ± 9.2
Surgical treatment, n (%)	
Radical cystectomy	15 (48.4%)
Transurethral resection of the tumor	16 (51.6%)
Histopathological type of tumor, n (%)	
Transitional cell cancer	28 (90.3%)
a) Low grade	4 (12.9%)
b) High grade	24 (77.4%)
Adenocarcinoma	3 (9.7%)
Chemotherapy / radiation therapy, n (%)	
Chemotherapy	12 (38.7%)
Radiation therapy	3 (9.6%)

terpreted on *Syngo Multimodality* workstations (*Siemens AG*) by two nuclear medicine specialists. After excluding physiological accumulation of ^{18}FDG and those attributed to benign lesions, the zones of increased ^{18}FDG uptake were assessed visually and semi-quantitatively by using maximum standardized uptake value (SUVmax). The obtained results of ^{18}FDG-PET/CT findings were compared to previous MSCT findings.

Statistical analysis. Patient demographics are presented as mean ± standard deviation and as percentage values. To compare the agreement between the results of ^{18}FDG-PET/CT and MSCT, Cohen's kappa coefficient (κ) was used for N0, N2, N3, M1a and M1b stages of bladder cancer as well as for the number of detected lesions via the mentioned diagnostic modalities. Interpretation of the Cohen's kappa coefficient for interobserver agreement between two diagnostic modalities was performed based on Altman's criteria (κ value < 0.20 poor; 0.21-0.40 poor; 0.41-0.60 moderate; 0.61-0.80 good: 0.80-1.0 very good agreement). Results are presented in cross-distribution tables that show agreement and disagreement between ^{18}FDG-PET/CT and MSCT.

Table 2. Frequency distribution of patients in nodal (N) stage of the disease based on ^{18}FDG-PET/CT and MSCT findings (category agreement marked in bold and underlined).

		MSCT			
		N0	N2	N3	Total
^{18}FDG-PET/CT	N0	**15**	1	0	16
	N2	1	**7**	0	8
	N3	5	0	**2**	7
	Total	21	8	2	31

RESULTS

Patient characteristics. Patients' characteristics included in our study are presented in Table 1. All patients were treated surgically, and the most common histopathological type of tumor (over 90%) was transitional cell carcinoma, while the remaining 9.7% of cases were attributed to adenocarcinoma. 12/31 patients (38.7%) received chemotherapy and 3/31 patients (9.6%) received radiation therapy.

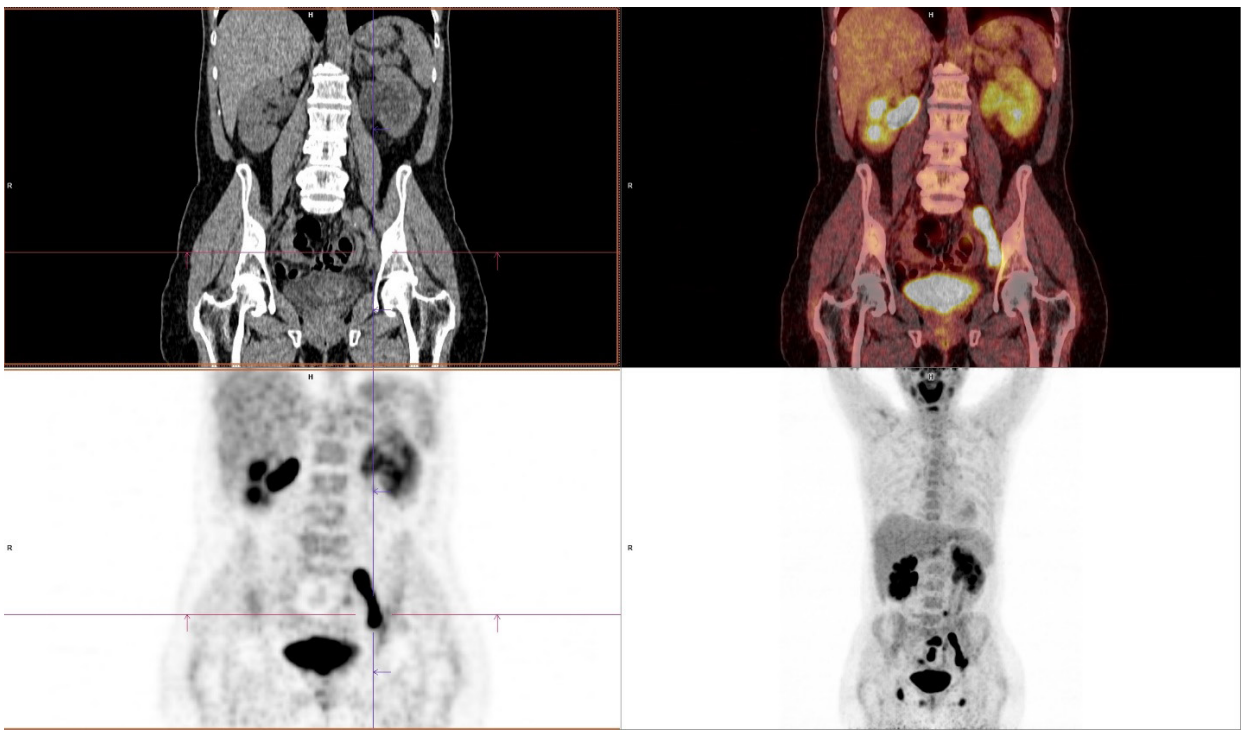

Figure 1. Coronal plane of unenhanced low dose CT, PET, fused PET/CT and MIP (maximal intensity projection). Increased uptake in left iliac lymph nodes and in regional bones.

Table 3. Frequency distribution of patients in metastatic (M) stage of the disease based on ^{18}FDG-PET/CT and MSCT findings (category agreement marked in bold and underlined).

		MSCT			
		M0	M1a	M1b	Total
^{18}FDG-PET/CT	M0	**7**	1	4	12
	M1a	3	**2**	0	5
	M1b	3	3	**8**	14
	Total	13	6	12	31

^{18}FDG-PET/CT and MSCT results. The distribution of patients in N stage of the disease is shown in Table 2. No patient showed N1 stage of the disease (metastases in a single pelvic lymph node) detected by analyzed diagnostic modalities, so it was excluded from further statistical evaluation. Total agreement between ^{18}FDG-PET/CT and MSCT for N stage of the disease was 77% (observed κ = 0.54; moderate agreement). The agreement for N0 stage was 68%, 77% for N2 stage (metastases in two or more pelvic lymph nodes), while for N3 stage of the disease (metastases along the common iliac blood vessels) the calculated agreement was 29%.

Observed κ = 0.54, which shows moderate agreement of 77% in N stage of the disease between the analyzed diagnostic modalities.

Distribution of patients in metastatic (M) stage of the disease is shown in **Table 3**. Total agreement between ^{18}FDG-PET/CT and MSCT for M stage of the disease was 53% (observed κ = 0.10; poor agreement). The agreement for M0 stage was 39%, 22% for M1a stage (metastases in distant lymph nodes), and 44% for M1b stage (distant metastases in other parts of the body).

Observed κ = 0.10, which shows poor agreement of 53% in M stage of the disease between the analyzed diagnostic modalities.

Apart from ^{18}FDG-PET/CT and MSCT agreement in N and M stages of the disease, we also compared agreement on the number of detected lesions in N and M categories. In N category ^{18}FDG-PET/CT detected 29 lesions, while MSCT detected 16 lesions. Total agreement in the number of detected lesions in N category was 71% (κ = 0.41; moderate agreement), as shown in **Table 4**.

Observed κ = 0.41, which shows moderate agreement of 71% in the number of detected lesions in N stage of the disease between the analyzed diagnostic modalities.

In M category, ^{18}FDG-PET/CT detected 42 lesions, while 30 lesions were detected by MSCT. Total agreement in the number of detected lesions in M category was 52% (κ = 0.07; poor agreement). The distribution of detected lesions in M stage is shown in **Table 5**.

Observed κ = 0.07, which shows poor agreement of 52% in the number of detected lesions in M stage of the disease between the analyzed diagnostic modalities.

DISCUSSION

In our study we analyzed the agreement between ^{18}FDG-PET/CT and MSCT in N and M stages of the disease, as well as the agreement in the number of detected lesions

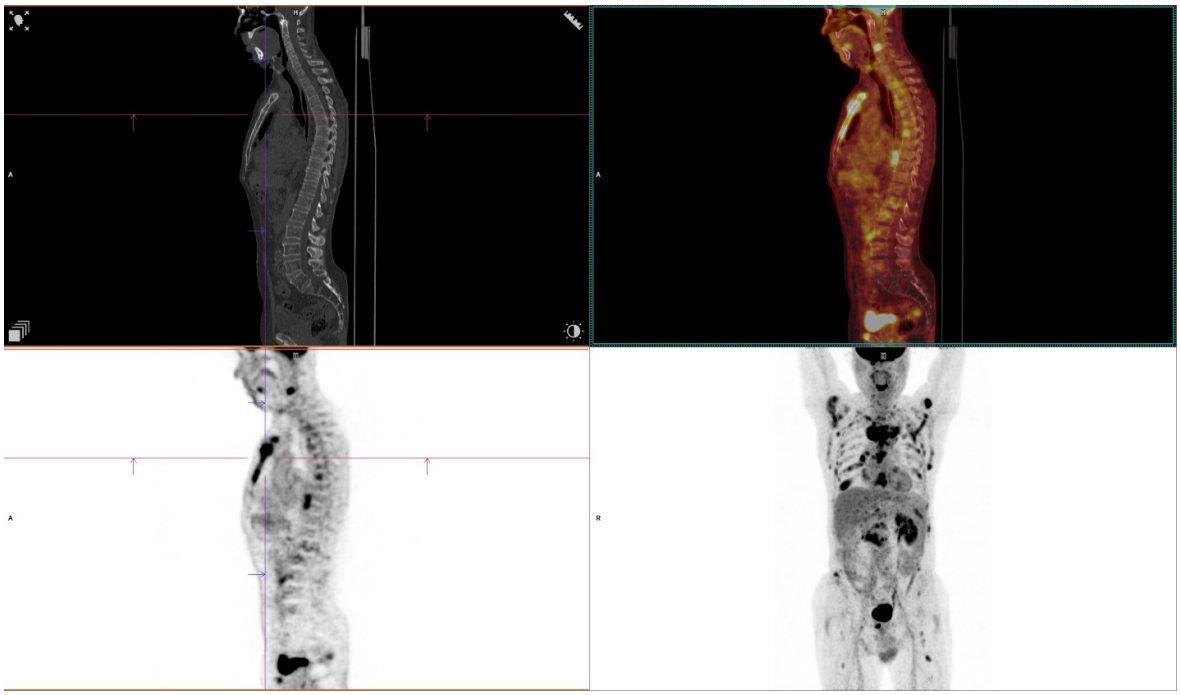

Figure 2. Sagittal plane of unenhanced low dose CT, PET, fused PET/CT and MIP (maximal intensity projection). Multiple zones of increased uptake in bones and lymph nodes.

Table 4. Frequency distribution of the number of detected lesions in nodal (N) stage of the disease based on ^{18}FDG-PET/CT and MSCT findings (category agreement marked in bold and underlined).

		MSCT					
		0	1	2	3	>3	Total
	0	**15**	1	0	0	0	16
	1	3	**2**	0	0	0	5
^{18}FDG-PET/CT	2	1	1	**4**	0	0	6
	3	2	1	0	**1**	0	4
	>3	0	0	0	0	**0**	0
	Total	21	5	4	1	0	31

in patients with recurrent bladder cancer. Our results show that ^{18}FDG-PET/CT classifies a higher number of patients in both N and M stages of the disease and detects a higher number of lesions compared to MSCT.

There are few studies in literature that evaluate comparison of ^{18}FDG-PET/CT and MSCT for patients with recurrent bladder cancer, which is confirmed by the fact that ^{18}FDG-PET/CT has not been recommended yet for follow-up of these patients following the guidelines of the European Association of Urology [13]. While searching literature, we only found one paper that compared ^{18}FDG-PET/CT with other conventional diagnostic modalities. In this paper, by Zattoni et al. (2018), a comparison was made regarding urothelial cancers, but unlike our research, it was not limited to bladder cancers, but it also included upper urinary tract cancers [15]. In addition, the comparison of ^{18}FDG-PET/CT was done not only with MSCT, but also with MRI, so it is expected that the results obtained between our and the above mentioned study will differ. However, the calculated Cohen's kappa coefficient in Zattoni et al.'s (2018) research was 0.43, which based on Altman's criteria indicates moderate agreement between the compared diagnostic modalities. Our study also showed a moderate agreement between ^{18}FDG-PET/CT and MSCT in the N stage of the disease (κ = 0.54, 77% agreement) and a moderate agreement of 71% (κ = 0.41) in the number of detected lesions in N stage. On the other hand, our results show poor agreement between ^{18}FDG-PET/CT and MSCT in M stage of the disease (κ = 0.10, 53% agreement), especially in M1a stage where the agreement was only 22%. This can be explained by the fact that as the disease spreads in lymph nodes outside of the pelvis (retroperitoneal, mesenteric, mediastinal, as well as in other lymph node groups), the chance of metastases that have not yet caused morphological changes in lymph nodes used as a criteria for MSCT assessment increases. In our study, this was especially true for the mediastinal group of lymph nodes where ^{18}FDG-PET/CT successfully detected bladder cancer metastases in lymph nodes whose short axis was as low as 7mm.

Aljabery et al. (2015) obtained data showing that ^{18}FDG-PET/CT did not contribute significantly to the detection of regional lymph node metastases [16]. Our results partially agree with Aljabery et al.'s data (2015); although the agreement between ^{18}FDG-PET/CT and MSCT in N stage of the disease is over 70%, ^{18}FDG-PET/CT upstaged N disease from N0 to N2 in one patient, and from N0 to N3 stage in 5 patients, which effectively rep-

Table 5. Frequency distribution of the number of detected lesions in metastatic (M) stage of the disease based on ^{18}FDG-PET/CT and MSCT findings (category agreement marked in bold and underlined).

		MSCT					
		0	1	2	3	>3	Total
	0	**9**	2	1	0	1	13
	1	4	**5**	0	0	0	9
^{18}FDG-PET/CT	2	1	0	**1**	0	0	2
	3	1	1	1	**1**	0	4
	>3	0	1	1	1	**0**	3
	Total	15	9	4	2	1	31

resents 19% change in the choice of adequate treatment, while MSCT upstaged one patient from N0 to N2 stage.

In the meta-analysis conducted by Xue et al. (2020), the overall sensitivity and specificity of ^{18}FDG-PET/CT in detection of recurrent or residual bladder cancer were 94% and 92% respectively [17]. However, the data in literature are still heterogeneous. A review of literature by Einerhand et al. (2020), indicates that there is a larger number of studies showing that ^{18}FDG-PET/CT is more sensitive than MSCT for the detection of lymph node metastases, with similar specificities [18]. For the detection of metastatic disease in the above mentioned literature review by Einerhand et al. (2020), it was found that while ^{18}FDG-PET/CT was diagnostically accurate, there were still not enough papers comparing it with MSCT [18]. These data show that more research is needed on the role of ^{18}FDG-PET/CT in relation to conventional diagnostic modalities in order to obtain the accurate data on the possible contribution of ^{18}FDG-PET/CT in the management of bladder cancer patients.

Our study has certain limitations. The number of patients included in the study is relatively small, given the number of categories analyzed by ^{18}FDG-PET/CT and MSCT, with the evaluation of the number of lesions by category. Furthermore, the data would probably be more accurate if the time between ^{18}FDG-PET/CT and MSCT imaging was as short as possible, and in our study it is 46±9.2 days, but this is difficult to achieve in our country given the number of available PET scanners.

CONCLUSION

Our results show that there is a moderate agreement between ^{18}FDG-PET/CT and MSCT findings for N stage of the disease, but that ^{18}FDG-PET/CT detects a higher number of lesions. ^{18}FDG-PET/CT also detects a greater number of lesions in the M stage of the disease, but the agreement with MSCT is poor, especially in M0 group. Further research is necessary on a larger number of patients in order to obtain more precise data on the agreement between the two diagnostic methods.

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ZNAČAJ POZITRONSKE EMISIONE TOMOGRAFIJE SA KOMPJUTERIZOVANOM TOMOGRAFIJOM 18-FLUORO-DEOKSIGLUKOZOM U ODNOSU NA MULTI-SLAJSNU KOMPJUTERIZOVANU TOMOGRAFIJU U REKURENTNOM KARCINOMU MOKRAĆNE BEŠIKE

Slobodanka Beatović [1,2], Miloš Veljković [2], Isidora Grozdić–Milojević [1,2], Jelena Petrović [1,2], Strahinja Odalović [1,2], Milica Stojiljković [1,2], Vera M. Artiko [1,2], Dragana P. Šobić-Šaranović [1,2]

Sažetak

Uvod: Pozitronska emisiona tomografija sa kompjuterizovanom tomografijom 18-fluoro-deoksiglukozom (^{18}FDG-PET/CT) još uvek se ne koristi u svakodnevnoj kliničkoj praksi za evaluaciju rekurentnog karcinoma mokraćne bešike. Savremeni vodiči prepoznaju značaj ^{18}FDG-PET/CT, ali se još uvek preporučuje kompjuterizovana tomografija (MSCT) za praćenje ovih pacijenata.

Cilj: Određivanje slaganja između ^{18}FDG-PET/CT i MSCT nalaza u kategorizaciji pacijenata u N i M stadijume bolesti, kao i slaganje navedenih dijagnostičkih metoda u broju detektovanih lezija.

Materijal i metode: 31 pacijent (22 muškaraca i 9 žena) sa dijagnozom karcinoma mokraćne bešike, prosečne starosti 61.2 **± 9.2** godine, uključen je u našu studiju. Zone patološkog nakupljanja ^{18}FDG su interpretirane vizuelno i semi-kvantitativno koristeći maksimalnu standardizovanu vrednost preuzimanja radiofarmaka (SUVmax). Proučavano je slaganje dobijenih nalaza sa prethodnim nalazima MSCT koristeći Kohenov kappa test slaganja, interpretiranog na osnovu Altmanovog kriterijuma.

Rezultati: Ukupno slaganje između ^{18}FDG-PET/CT i MSCT za N stadijum bolesti je iznosilo 77% (κ = 0.54 ; umereno slaganje), za N0 stadijum 68%, N2 77%, N3 29%. Za M stadijum, ukupno slaganje je iznosilo 53% (κ = 0.10 ; minimalno slaganje), za M0 39%, M1a 22%, M1b 44%. ^{18}FDG-PET/CT je u N stadijumu bolesti detektovao ukupno 29 lezija, a MSCT 16 lezija, sa slaganjem od 71%, (κ = 0.41 ; umereno slaganje). U M stadijumu bolesti, ^{18}FDG-PET/CT je detektovao 42 lezije, a MSCT 30 lezija, ali slaganje iznosi 52% (κ = 0.07 ; minimalno slaganje).

Zaključak: Naši rezultati pokazuju da postoji umereno slaganje između ^{18}FDG-PET/CT i MSCT nalaza u kategorizaciji pacijenata i broju detektovanih lezija u N stadijum bolesti, ali da ^{18}FDG-PET/CT detektuje veći broj lezija. ^{18}FDG-PET/CT takođe detektuje veći broj lezija u M stadijumu bolesti, ali je slaganje sa nalazima MSCT minimalno.

Ključne reči: rekurentni karcinom mokraćne bešike, PET/CT, MSCT

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REVIEW

ANTENATAL CORTICOSTEROIDS - BETWEEN BEING A USEFUL DRUG AND POSING A RISK FOR FETAL AND ADULT DEVELOPMENT

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Summary

Antenatal corticosteroid therapy (ACST) is very important in reducing the sequelae of prematurity, respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), and necrotizing enterocolitis (NEC). This therapy has short-term and long-term neonatal consequences that range from reduced neonatal body weight, brain growth, hypertension, hypoglycemia and obesity to delayed neurological development. In addition to undeniable importance this type of therapy has on fetal maturation, it may also impact programming of fetuses future development and health during childhood and adulthood. ACST must be personalized, as a single course, and determined by indications and assessment of the expected time of delivery, so that the exposure time of the fetus to the effects of endogenous and exogenous steroids is shortened.

Keywords: antenatal corticosteroid therapy, programming, prematurity, fetus, neonate

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INTRODUCTION

Generally, preterm delivery is the most important and unresolved problem in perinatal medicine. The global prevalence rate of preterm birth ranges from 5% to 13.3% (1). In Serbia, since 1998 the preterm birth rate of 8 % of all births has declined to 6.8% (2022), which confirmed our country in the group of countries with stable parameters of neonatal statistics (2). If a birth occurs before the completed period of gestation (37 weeks), it is defined as preterm.

According to the gestational age, there are sub-categories of preterm birth: extremely preterm – below 28 gestational weeks; very preterm – 28 to 32 weeks; and late preterm birth – after 32 to 37 weeks (3).

Preterm delivery has multifactorial etiology, and nowadays, we use the name "preterm delivery syndrome" (2). Regardless of the definition, preterm birth is related to increased maternal and perinatal morbidity and mortality as well as to the consequences on development until adulthood.

In 1972, a randomized controlled trial regarding maternal administration of betamethasone was carried out by Liggins and Howie. The results were published in a groundbreaking article that documented a decrease in cases of respiratory distress syndrome (RDS) in preterm infants and neonatal mortality. The incidence of RDS and neonatal mortality dropped from 15.6% to 10% and 11% to 6.0%, respectively (4).

Crowley and colleagues published a meta-analysis of 12 randomized controlled trials of ACST in 1990. This meta-analysis demonstrated that the ACST therapy significantly reduced not only RDS, neonatal intraventricular hemorrhage (IVH) and necrotizing enterocolitis (NEC), but also the overall neonatal mortality (5). In 2021, the WHO Executive Guideline Steering Group defined recommendations for sustainable implementation of antenatal corticosteroid therapy (ACST) as one of the therapies that improved preterm birth outcomes (6) **(Table 1)**.

Today, antenatal corticosteroid administration is one of the most important and beneficial treatments used in perinatal medicine.

Regardless of the therapeutic benefits in the improvement of preterm perinatal outcomes, corticosteroids are very potent medications with a still questionable impact on fetal development and with long-term consequences related to the occurrence of chronic diseases in adulthood.

ANTENATAL CORTICOSTEROIDS FROM FETAL AND NEONATAL DEVELOPEMENT TO ADULTHOOD – *PROS AND CONS*

A) ACST and placenta: The levels of corticotropin-relising hormone (CRH) of placental origin increase during the second and third trimester. Placenta is an alternative source of adrenocorticotropin hormone (ACTH). As a result, this placental synthesis of ACTH can lead to a two- or tri-fold increase in its concentration. Total and free maternal ACTH concentrations rise gradually during pregnancy.

Placental villi are the target tissue affected by ACST in single or increased doses. There are many debates about the placental function after ACST regarding the optimal dose and interval between doses, especially when it comes to repeated doses. Some studies of increased doses of ACST demonstrated contradictory results related to placental weights, abnormalities of villous development, placental infarcts, or decreased vascular reactivity.

Um-Bergstorm et al. (2018) confirmed the association between multiple courses of ACST and accelerated villous maturation and preterm labor (7). Accelerated villous maturation is also observed in pregnancies complicated by chronic hypoxia. These mechanisms are very different. Wallace and Baker presumed that single-dose ACST improved villous as well as umbilical circulation (8). The possibly increased villous velocities are related to an increase in larger number of villi as well as placental perfusion and its diffusion capacity longer after the administration of ACST (9). Consequently, a single–dose of ACST improves perinatal and neonatal outcomes in preterm labor (10). Babovic et al. (2016) did not confirm the relation between a single-dose of ACST and umbilical circulation (RiAU) (11). In contrast, repeated doses of ACST are related to decreased placental weight as well as to adverse fetal or neonatal outcomes (12). Chronic hypoxic environment stimulates placental villi to secrete soluble fms-like tyrosine kinase-1 (sFlt-1). Overexpression of sFlt-1 has been linked to accelerated villous maturation, for example in preeclampsia. ACST does not change the level of sFlt-1 (13).

Finally, Leavely et al . (2017) showed that on the molecular level the accelerated villi are of advanced age, indicating that those placentas could be affected by premature activation of a normal maturational program. However, further studies that include gene expression and investigate the mechanisms and pathogenesis of preterm accelerated villous maturation are necessary(14).

B) Fetal corticosteroids Recent studies confirmed functional pituitary-adrenal axis (significant adrenal secretion of dehydroepiandrostenedion-sulphate (DHEAS) at 8-10 weeks of gestation. The fetal zone (comprising most of the adrenal cortex) is the major site of steroidogenesis before birth. Precursors of fetal steroids are placental 5-pregnenolone and 4-progesterone. Fetal cortisol is critical for placental synthesis of estrogens, and its concentrations are 5-10 times lower than maternal concentrations (14). Two placental mechanisms maintain the low glucocorticoid environment during gestation: 11β-hydroxysteroid dehydrogenase (11β-HSD)-2 conversion of active cortisol and corticos-

Table 1: WHO 2022 recommendations on antenatal corticosteroid therapy for improving preterm birth outcomes

1.0 Antenatal corticosteroid therapy is recommended for women with a high likelihood of preterm birth from 24 weeks to 34 weeks of gestation when the following conditions are met: Gestational age assessment can be accurately undertaken. There is a high likelihood of preterm birth within 7 days of starting the therapy. There is no clinical evidence of maternal infection. Adequate childbirth care is available (including capacity to recognize and safely manage preterm labor and birth) The preterm newborn can receive adequate care (including resuscitation, kangaroo mother care, thermal care, feeding support, infection treatment and respiratory support including continuous positive airway pressure [CPAP] as needed)	Context-specific recommendation
1.1 Antenatal corticosteroid therapy should be administered to women with a high likelihood of giving birth preterm in the followingt 7 days, even if it is anticipated that the full course of corticosteroids may not be completed.	Context-specific recommendation
1.2 Antenatal corticosteroid therapy is recommended for women with a high likelihood of preterm birth, irrespective of whether single or multiple birth is anticipated.	Context-specific recommendation
1.3 Antenatal corticosteroid therapy is recommended for women with preterm prelabour rupture of membranes and no clinical signs of infection	Context-specific recommendation
1.4 Antenatal corticosteroid therapy is not recommended for women with chorioamnionitis, who are likely to give birth preterm.	Not recomended
1.5 Antenatal corticosteroid therapy is not recommended for women undergoing a planned cesarean section at 34 weeks 0 days to 36 weeks 6 days.	Not recommended
1.6 Antenatal corticosteroid therapy is recommended for women with hypertensive disorders in pregnancy, who have a high likelihood of preterm birth.	Context-specific recommendation
1.7 Antenatal corticosteroid therapy is recommended for women with a high likelihood of preterm birth of a growth-restricted fetus	Context-specific recommendation
1.8 Antenatal corticosteroid therapy is recommended for women with pre-gestational and gestational diabetes when there is a high likelihood of preterm birth, and this should be accompanied by interventions to optimize maternal blood glucose control.	Context-specific recommendation
1.9 **Either intramuscular (IM) dexamethasone or (IM) betamethasone (total 24 mg in divided doses) is recommended as the antenatal corticosteroid of choice.**	Recommended
1.10 A single repeat course of antenatal corticosteroids is recommended for women who have received a single course of antenatal corticosteroids at least 7 days before and, on clinical assessment, have a high likelihood of giving birth preterm in the next 7 days.	Recommended

terone into their intrinsically inert 11-keto-metabolites, and P-glycoprotein-mediated active retrograde transport of glucocorticoids from fetus to mother, maintaining the feto–maternal glucocorticoid gradient in both endogenous and exogenous glucocorticoids (15, 16). 11β-HSD2 is present in a higher concentration especially in the fetal brain during early-to-mid-gestation (17). In late gestation, there is an increased endogenous synthesis of glucocorticoids in both the mother and the fetus, while the synthesis of glucocorticoids in the placenta decreases due to a lower activity of placental 11β-HSD-2. Increased concentrations of endogenous glucocorticoids, which act via the glucocorticoid receptor (GR) to mature the fetal lungs and other organs, are important for survival in the extrauterine environment after birth (18). Finally, it is the main etiological factor of fetal programming and human development.

C) *ACST-- drugs and treatment:* Two main synthetic corticosteroids used in clinical practice are betamethasone-acetate and dexamethasone sodium phosphate (Table 1). They are not inactivated by endogenous dehydrogenase enzymes. These agents have minimal mineralocorticoid and weak immunosuppressive activity with short-term administration. However, betamethasone is more potent than dexamethasone (19). ACST has been administered in a variety of ways including orally, intramuscularly, intravenously, intraamniotically, and as a direct intramuscular injection into the fetus. The most common way of administration is the Liggins method – intramuscular injection into the mother's muscle tissue (4). ACST treatment includes two 12-mg doses of betamethasone given i.m 24 hours/four 6-mg doses of dexamethasone administered i.m every 12 hours (Table 1). The benefit of ACST administration is greatest 2–7 days after the initial dose.

D) *Fetal lung and ACST* Endogenous as well as exogenous glucocorticoids induce the production of lipid and protein surfactant components. The production of lipid surfactant components include the induction of lipogenic enzymes (phosphocholine transferase, methyl transferase) necessary for phospholipid synthesis, and the conversion of unsaturated to disaturated phosphatidylcholine. Glucocorticoids via thyroid transcription factor-1 (TTF-1), which is vital for normal lung development and is primarily expressed by type II alveolar epithelial cells in the fetal lung at term and in postnatal life, induce pro-

tein surfactant production. TTF-1 plays a role in the synthesis of SP-A, B and C proteins at gene level. Pulmonary surfactant is produced by type II pneumocytes (20). It decreases the surface tension of terminal alveoli, promotes lung expansion during inspiration, prevents alveolar collapse and loss of lung volume at the end of expiration, and facilitates the recruitment of the collapsed alveoli. Finally, more than 20 years ago, Ballard (1995) documented that antenatal corticosteroids increase tissue and alveolar surfactant production, lung compliance, clearance of fluid from the lungs, maturation of parenchymal structure, and they reduce vascular permeability (21).

E) Fetal brain, behavior and ACST Miller SR et al. observed increased cerebral vascular resistance, decreased fetal cerebral blood flow and oxygen delivery, and impaired hippocampus region-specific cerebral oxygen delivery (22). In contrast, Vafaei et al. (2021) documented that antenatal corticosteroid administration increased fetal middle cerebral artery pulsatile index (MCA PI), as an autoregulation mechanism (to stabilize cerebral perfusion) (23). Finally, Babovic et al. observed no changes in resistant indices in cerebral circulation after direct fetal intramuscular corticosteroid therapy (23). These findings depend on the mode of corticosteroid administration. The CST receptors are expressed in the hippocampus. Exposure to antenatal glucocorticoids in utero, via CST receptors, has widespread acute effects on neuronal structure and synapses that induce alteration morphology and the function of the hippocampal area. These effects may induce neuronal death. Treatment with antenatal dexamethasone caused dose-dependent neuronal degeneration of hippocampal neurons and reduced hippocampal volume. These effects persisted in rhesus monkeys until they were about 20 months old. Fetuses receiving multiple lower-dose ACST injections were more severely affected than those receiving a single large injection. As Seckl cited, human and animal studies have demonstrated that disrupting normal patterns of myelinization, reduction of neuronal cell number, and interneuronal signal pathways in the fetal brain may be associated with several consequences on fetal behavior and neonatal memory (24). Our study demonstrated that maternal corticosteroid therapy interfered with the diurnal rhythm in fetal movements, with ~50% decreased movements for 24-48 hours as well as a decreased number of breathing movements (11). On the other hand, direct fetal intramuscular corticosteroid therapy induces increased fetal breathing movements and fetal biophysical profile (25).

F) Fetal cardiovascular system and ACST Glucocorticoids act via intracellular receptors in fetal myocard: the glucocorticoid receptor (GR or type II receptor) and the high-affinity mineralocorticoid receptor (MR or type I receptor). These receptors are activitated by ligand- binding, which further triggers the transcription factors of specific genes in the cellular nucleus (26). Endogenous glucocorticoids, through receptors, promote structural, functional, and metabolic remodeling in fetal cardiomyocytes. The systolic and diastolic ratio (E/A wave ratio), as a marker of cardiac maturity, is reduced. Our study did not confirm significant changes in the systolic function of the fetal heart after ACST (11). Early systolic and diastolic function is impaired, similar to the preterm neonates. This functional immaturity is related to the structural immaturity of the fetal heart: as shortened and disorganized myofibrils, a lack of cardiomyocyte alignment, and biochemical immaturity (in terms of calcium handling) (27). Recent studies observed that ACST reduces fetal capillary length and volume as well as placental vascularisation (28,29). These observations are associated with increased resistance in placental and umbilical circulation. In support of this observation, ACST can induce abnormal cardiac maturation, with impaired heart function in late gestation in the third trimester of pregnancy (30). After ACST, the transient increase in peripheral vascular resistance raises blood pressure resulting in increased human fetal cardiac output. Dexamethasone rapidly induces the regulation of cardiac mitochondrial capacity as a marker for the functional and metabolic maturation of the fetal heart in both in-vivo and in-vitro studies. The key point of that regulation is the abolition of glucocorticoid effects on mitochondrial oxygen consumption and its relation to the maturation of myofibril structure (27). Dexamethasone alone is ineffective in the induction of human maturation of cardiomyocytes. But when dexamethasone acts together with the thyroid hormone (T3), it induces maturation and improves the contractile force of cardiomyocytes (31) This inter-dependent relationship of T3 and glucocorticoids in cardiac maturation is an important confirmation of ACST, where only glucocorticoid is administered. If glucocorticoid receptors are activated before the HPA axis has started to produce circulating fetal T3 in any substantial amount, the maturation of fetal organs induced by glucocorticoids could be limited. ACST decreases or increases the basal fetal heart rate (BFHR), but all changes are less than 10 bpm. The changes are suggestive of sympathetic suppression (11). Long-term heart rate variability is reduced with fewer accelerations (32). The transient deterioration in the heart rate pattern may lead to a misdiagnosis of fetal distress (11).

ANTENATAL CORTICOSTEROIDS PROGRAMMING NEONATAL DEVELOPMENT AND ADULTHOOD

The concept of early-life physiological 'programming' or 'imprinting' implies the relationship between prenatal environmental events, altered fetal growth and development, and later pathophysiology (33). Exposure to antenatal corticosteroid therapy by single or repeated courses in utero at critical developmental stages can alter the functioning of many organ systems that extend into adult life.

A) Short-term neonatal effects of a single-course ACST are *transient neonatal tachypnea/apnea after 34 gw, hypoglycemia, reduced risk of neonatal RDS, reduced risk of neonatal intraventricular hemorrhage (IVH), and reduced risk of necrotizing enterocolitis (NEC)* (6).

B) **Long-term neonatal effects** are principally related to repeated ACST courses:

- ***decreased birth weight*** Bloom et al. observed the link between low neonatal birth weight and repeated maternal ACST course in the third trimester of gestation. (34). This birth weight reduction presumably reflected the catabolic actions of exogenous steroids (33).
- ***decreased neonatal length*** Rodriguez et al. (2019) observed a significant correlation between decreased neonatal birth length and adult height as well as a risk of death from several major chronic diseases in adulthood (35).
- ***decreased brain growth*** The majority of cells in the developing brain originate from the neural stem or progenitor cells. Many nuclear receptors, including thyroid hormone receptors and the glucocorticoid receptor (GR) that are expressed in these cells, are pivotal for the development of the brain (36). In the developing brain, the classical genomic GR signaling pathway is important for the stabilization of vascular endothelial cells. The stabilization of vascular endothelial cells by GR plays an important role in decreasing the risk of IVH after preterm birth. Neurogenesis, gliogenesis as well as the production of neural stem or progenitor cells all depend on whether ACST was given in a single dose or repeatedly. This process indirectly contributes to the cognitive and behavioural impairments observed in infants exposed to ACST in utero. (37) This hypothesis explains that the timing of neurogenesis and gliogenesis is controlled and regulated by the formation of oligodendrocytes and astrocytes before and after birth in humans (38).
- ***deleterious effects on the fetal/neonatal cerebral myelination*** Aszatols et al. (2013) observed a spectrum of neurodevelopmental disabilities such as neuromotor (nonambulatory cerebral palsy), neurosensory (blindness, deafness, or need for visual or hearing aids), or neurocognitive or neurobehavioral function (abnormal attention, memory, or behavior) of 2141 preterm survival children at 18-24 months of age, linked to maternal exposure to multiple courses of corticosteroids. She suggests that neuromotor disability is a consequence of interference in the developing brain or nerve and potential exposure to antenatal corticosteroids. It is extremely important that the preterm fetus is exposed not only to the exogenous corticosteroid treatment, but also to the natural endogenous fetal surge of cortisol in late pregnancy, which is critical for normal fetal growth and development (39). In contrast, our study investigates the fact that a single course of ACST could impact neurological conditions, as assessed through the muscular tone of 82 prematurely born infants (31-33 gw) over the first 12 months after birth. During this period, a significantly greater number of infants from the ACST group had good muscular tone when compared to those from the control group without therapy (40).
- ***increased blood pressure and human newborns*** Mudler (2004) observed increased blood pressure within 24 hours after maternal administration of one course of betamethasone (41). This was ascribed to the baroreceptor reflex, in which an increase in blood pressure very rapidly triggers a decrease in heart rate, causing blood pressure to fall, but not in very early fetuses. Babovic et al. (2016) did not support these findings after maternal administration of one course of dexamethasone (11). Excess endogenous or exogenous cortisol directly elevates blood pressure at birth in humans. But, ACST affects fetal/neonatal blood pressure during organogenesis, growth, or maturation depending on gestational age and the stage of organ development at the time of fetal exposure to glucocorticoids (42). Exposure to glucocorticoids during the final week of pregnancy in rats is sufficient to produce permanent adult hypertension. This effect may be primarily due to the complex species-specific patterns of expression of glucocorticoid receptors and specific enzyme 11b-HSD, which regulates maternal glucocorticoid transfer to the fetus and modulates glucocorticoid action in individual tissues (43).

CONCLUSION

Antenatal corticosteroid therapy is a gold standard in the prevention of short-term and long-term sequels of prematurity. Our knowledge is limited concerning the relation between an excess of exogenous steroids and fetal, neonatal, and childhood development. In addition to the undeniable importance of fetal maturation, this type of therapy also implies the influence on the programming of future development and health during childhood and adulthood. ACST must be personalized, as a single course, determined by the indications and the assessment of the expected time of delivery, so as to shorten the exposure time of the fetus to the effects of endogenous and exogenous steroids.

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Authors' contributions

IB, RS, MJ, and NKO contributed to the conceptualisation. IB, JB,VP and JP contributed to the methodology and investigation. JB and JM contributed to the resources. JM contributed to data curation. IB, JB and JM contributed to writing and original draft preparation. RS contributed to writing, reviewing and editing. VP, JP and MA contributed to visualisation. IB contributed to supervision. IB and NKO contributed to project administration.

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ANTENATALNI KORTIKOSTEROIDI - IZMEĐU LEKA I RIZIKA ZA RAZVOJ FETUSA I PROGRAMIRANJA ODRASTANJA

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Sažetak

Antenatalna kortikosteroidna terapija (ACST) je veoma značajna u redukciji sekvela prematuriteta respiratornog distres sindroma (RDS), intraventrikularne hemoragije (IVH) kao i nekrotizirajućeg enterokolitisa (NEC). Ova terapija ima kratkotrajne i dugotrajne neonatalne posledice, počev od smanjenja telesne mase neonatusa na rođenju, rasta mozga, hipertenzije i hipoglikemije i gojaznosti, do odloženog neurološkog razvoja. Pored nesumnjivog značaja u maturaciji ploda, ova vrsta terapije podrazumeva i uticaj na programiranje budućeg razvoja i zdravlja tokom detinjstva i zrelog doba. ACST mora biti personalizovana, u vidu jednog kursa, određena indikacijama i procenom očekivanog vremena završavanja porođaja, kako bi se skratilo vreme ekspozicije fetusa delovanju endogenih i egzogenih steroida.

Ključne reči: antenatalna kortikosteroidna terapija, programiranje, prematuritet, fetus, neonatus

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REVIEW

HIPPOCAMPAL SCLEROSIS

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Summary

Hippocampal sclerosis is one of the most common causes of focal epilepsy. At the same time, hippocampal sclerosis is the most common surgical substrate in focal pharmacoresistant epilepsies. The hippocampus has a specific anatomical structure consisting of a total of four sectors. In a physiological context, the hippocampus is essential in many neuropsychological processes, so hippocampal sclerosis (an entity recognized and associated with epilepsy as early as the 19th century) is very interesting in terms of research. The pathohistological pattern of hippocampal sclerosis is now very precisely represented, which helps uniform recognition. The causes of hippocampal sclerosis are not known, but so far, numerous factors have been identified that are associated with the occurrence of this pathological process. There is no doubt that excitotoxicity, along with changes in the redox system, is the most essential pathophysiological mechanism. Hippocampal sclerosis is clinically very recognizable. Epilepsy patients whose basis is hippocampal sclerosis have very typical epileptic seizures consisting of an epigastric aura followed by a focal epileptic seizure characterized by confusion of consciousness and oroalimentary automatisms. Today, thanks to modern neuroimaging (primarily magnetic resonance imaging), the detection of this pathological pattern is exact and unambiguous.

Keywords: hippocampal sclerosis, epilepsy, magnetic resonance imaging

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INTRODUCTION

Hippocampal sclerosis (HS) is recognized in modern epileptology as one of the most common causes of focal epilepsy (1). It is present in 10% of newly diagnosed focal epilepsies (2). One third of pathological findings in surgical series of pharmacoresistant epilepsy in adults belong to this etiological entity (3). On the other hand, the facts that the hippocampus has a physiological role in the formation of memory and that it probably possesses extraordinary neural plasticity in the central nervous system, which is shown by the presence of neurogenesis in the dentate gyrus (4), make HS, in addition to the "title" of the carrier of a specific epileptic syndrome also interesting in many ways as a research model. This is also why HS is a research model of pervasive study, both by epileptologists and other disciplines in neuroscience.

ANATOMY OF THE HIPPOCAMPUS

The name of this brain structure was given by the Venetian anatomist Aranzi (Julius Caesar Aranzi) in the 16th century due to its resemblance to a seahorse (lat. hippocampus from Greek ἵππος - horse and κάμπος - seahorse). In the 18th century, the Danish anatomist Winsløw (Jacob Winsløw) proposed the name "cornu Amomonis" - the horns of (the Egyptian god) Amon. This name remained for a sector of the hippocampus – CA1-4. Anatomically, the hippocampus is the folded edge of the cerebral cortex in the S-shape and is essentially a bilaminar structure, i.e., the hippocampus formation consisting of the "real" hippocampus and the dentate gyrus (both structures maintain a mutual relationship along the entire length). The hippocampus belongs to the limbic cortex (lat. limbus - border), as is the case with cingulate and olfactory cortex and amygdala. In the structural sense, it is a most straightforward cortical organization - the allocortex (or archeocortex). This brain structure is arched around the mesencephalon and is divided into three parts: the head (anterior segment), where the elevations (hippocampal digitations) can be seen; the body (middle segment); and the tail of the hippocampus (posterior segment) which gradually narrows and disappears below the splenium. The hippocampus has a very complex geography, and one of the consequences is that slices through the hippocampus can show different shapes depending on the angle and location of the slice.

Nevertheless, four fields of the "true" hippocampus are recorded in the coronary section (5): CA 1 – starts from the subiculum, and its pyramidal neurons are triangular, small, and scattered; CA 2 –- continues from CA 1 and consists of large, ovoid and densely packed neurons; CA 3 – continues from CA 2 and its neurons are similar but rarer and are characterized by the presence of unmyelinated mossy fibers originating from the dentate gyrus; CA 4 – right above the dentate gyrus and consists of sparsely distributed ovoid and large neurons and myelinated mossy fibers originating from the dentate gyrus. In the coronary section, the dentate gyrus is a narrow and dorsally concave lamina that envelops the CA 4 segment. In the histological sense, the "real" hippocampus has six layers (from outside to inside – alveus, stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum, stratum moleculare), and the dentate gyrus has three layers (stratum granulosum, stratum moleculare and polymorphic layer).

HIPPOCAMPAL SCLEROSIS IN THE MIRROR OF TIME

The pathological change of the hippocampus in epilepsy was recognized in 1825 in a research by Camille Bouchet and Jean-Baptiste Cazauvielh who attempted to determine the cause of epilepsy (l'épilepsie) and madness (l'aliénation mentale), when they described a macroscopic abnormality of the hippocampus in 5 out of 31 post-mortem patients with epilepsy. In the description of "crazy epileptic number 10", the authors documented a clinical picture that correspond to the syndrome of mesial temporal lobe epilepsy (MTLE) where "a 31-year-old patient becomes restless before the attacks, which are accompanied by vomiting and stomach irritation... and at autopsy, there is a small mass of greyish, hard and resistant matter contained in the horn of Ammon on the left side" (6) **(Figure 1)**. However, Bushe and Cazauvieilh did not establish an interdependent relationship between epilepsy and sclerotic hippocampus. It was only in 1880 that Karl Wilhelm Sommer examined 90 autopsied patients with epilepsy and concluded that "there is no doubt that epileptic symptoms are often associated with

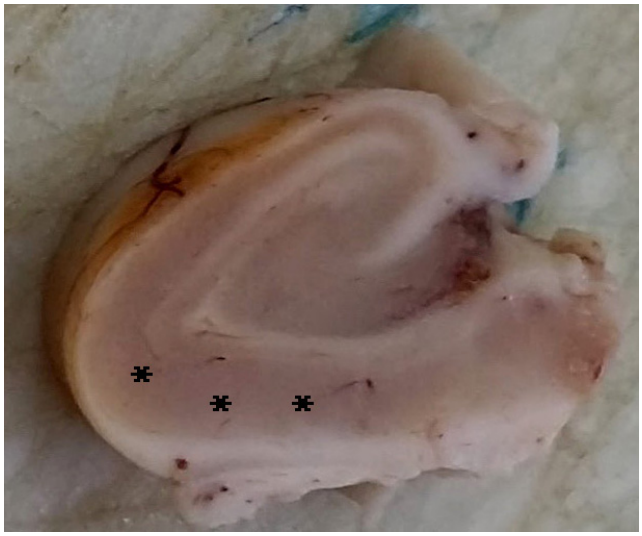

Figure 1. Macroscopical features of hippocampal sclerosis (type 2). * CA1 sector – absence of the clear boundaries between gray and white matter (sample from the patient with mesial temporal lobe epilepsy operated at the Clinic for Neurosurgery, University Clinical Center of Serbia).

diseases of the horn of Ammon and its surroundings." In a very detailed analysis, Sommer described the basic microscopic features of HS in the case of a 25-year-old patient with complex focal seizures - gliosis and a loss of pyramidal cells primarily in the CA1 sector, which was later called Sommer's sector (7) **(Figure 2)**. At the very end of the 19th century, Emil Bratz published a study of microscopic analysis of the hippocampus in patients with epilepsy where he concluded that there was a region between the hilus and Sommer's sector (in which the hippocampal pyramidal cells are very resistant and which is nowadays known as the CA2 sector) and that HS was not a consequence of vascular processes, but a pathological entity with a unique pathogenesis (8). These early understandings were confirmed in the new momentum of research epileptology, this time in the Anglo-Saxon area, in the works of Wilder Penfield and Jasper in the 1960s, and then in the literature that we know as modern. However, most of the observations published by Sommer or Bratz are challenging to reconcile with today's experiences. For example, as many as 60 out of 90 HS cases in Sommer's study were described as dementia patients. Of course, we should keep in mind that the perception Sommer had when anticonvulsant therapy was not available and when most patients with epilepsy died early in hospitals is hard to imagine nowadays.

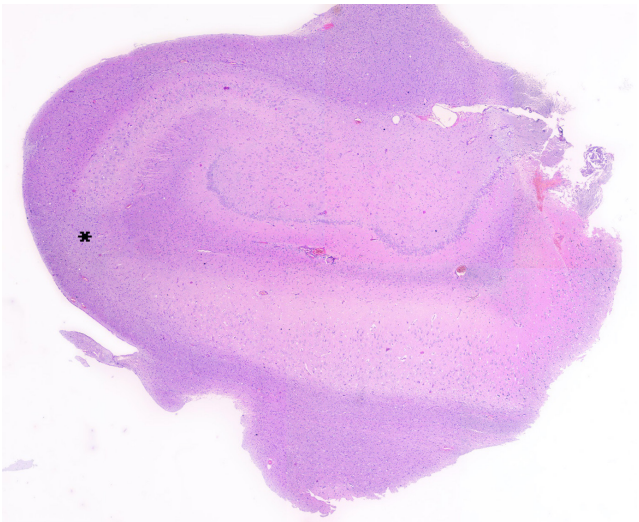

Figure 2. Microscopical features of hippocampal sclerosis (type 2). * CA1 sector – loss of the pyramidal cells (sample from the patient with mesial temporal lobe epilepsy operated at the Clinic for Neurosurgery, University Clinical Center of Serbia)

CAUSES OF HIPPOCAMPAL SCLEROSIS

When and how HS occurs, and which are the predisposing factors responsible for its occurrence are central issues in the current research efforts in epileptology. First of all, identifying susceptible individuals could lead to the application of prophylaxis in prevention of the onset or further progression of HS.

Generalized tonic-clonic seizures (GTCS) and status epilepticus (SE) are the earliest recognized causes of HS. In the same year when Sommer pathohistologically defined HS, Ludwig Pfleger described his 25 cases of macroscopically diagnosed HS. In one patient who died due to SE, he determined hemorrhagic lesions in the mesial temporal lobe as a result of "metabolic disorders" that occurred during the attack (9). Although in modern epileptology this causality has become generally accepted, in a study of postmortem samples of the hippocampus of patients with refractory epilepsy, it was shown that SE did not inevitably result in HS (in one patient, HS was not detected even after 30 episodes of ES) (10). Unfortunately, the characteristics that make patients with prolonged GTCS or SE more susceptible to developing HS are still unknown.

Significant brain insult or initial precipitating injury (IPI), such as, e.g., febrile convulsions, meningitis, or prolonged epileptic seizure, are often associated with HS (up to 50% in retrospective studies). The hypothesis supporting such observations argues that IPIs irreversibly damage (or change) the hippocampus, resulting in a process that finally leads to HS after a "latent period." The theoretical assumption that there is a "narrow age window" for this type of process (up to 4 or 5 years of age) in which the hippocampus is highly vulnerable is also significant. The effects of epileptic seizures in IPI are rather complex and include synaptogenesis, neurogenesis in the dentate gyrus, and neuronal loss (11). Direct evidence supporting this concept comes from neuroimaging studies which have shown that HS occurs after prolonged febrile convulsions. About 10% of children in whom febrile convulsions are >30 minutes have increased signal intensity on the T2 weighted MRI sequence in the hippocampus region, which later evolves into HS (11). However, it remains unknown why a systemic event, such as elevated body temperature, results in significant asymmetry (or exclusive unilaterality) in the involvement of the hippocampus, while this finding can be maintained throughout life. A partial explanation lies in the possible existence of subtle structural abnormalities of the hippocampus, shown in a neuroimaging study of blood relatives with familial aggregation of febrile convulsions, where a significant difference in hippocampal volume was determined (12). However, this observation's histological or genetic background has not been shown to date.

If accompanied by non-convulsive SE, brain injury can rarely result in HS (13). However, HS associated with vascular anomalies (e.g., cavernous angioma), severe malformations of cortical development, or low-grade tumors associated with epilepsy (dysembryoplastic neuroepithelial tumor - DNET or ganglioglioma) is far more significant and common. Moreover, it has been estimated that as many as 15% of patients with lesional epilepsy have associated hippocampal sclerosis (the so-called "dual pathology") (14). The hypothesis of this association suggests that seizures are first generated from the immediate vicinity of the lesion and then spread to the hippocampus. Long-term exposure of the hippo-

campus to discharges from the primary epileptic center leads secondarily to HS. This connection forms an inextricable epileptic network. This conclusion stems from the observation that in the case of surgical treatment in patients with "dual pathology," it is necessary to remove the hippocampus in addition to the lesion (15). There are, however, opinions that this rule excludes epilepsy of the "posterior quadrant," where the pathology of the hippocampus is temporally synchronous with the pathology of the occipitoparietal cortex (e.g., prenatal insult) because, in these patients, resection in the posterior part of the brain was sufficient to induce a complete remission (16). However, this study dealt with hippocampal atrophy without clear radiological signs of sclerosis.

Recently, there has been research interest in the relative contribution of factors other than epileptic seizures (including genetic, environmental, and developmental factors) in the development of HS. Thus, the professional public was sporadically informed that there is a possible association of HS with genes encoding inflammatory cytokines (including interleukin 1β) or with the ApoE ε4 genotype (10). In addition, some research groups have reported that environmental factors may play a role in the development of HS, such as herpes virus infection (17). However, such results have not been consistently replicated in subsequent studies. In the most recent works, the research is concentrated on the association of HS with autoimmune encephalitis. In this context, the association of HS with autoimmune limbic encephalitis with antibodies to voltage-dependent potassium channels is best documented. Typically, these antibodies lead to acute or subacute encephalitis with enlargement of mesiotemporal structures with increased signal intensity on T2-weighted MRI. In the sequel, HS occurs in the same place (18). This suggests the importance of intensive early autoimmune encephalitis treatment.

The number of HS is decreasing in surgical series in most major surgical centers worldwide (19). The explanation of this observation is not uniform so far. One belief is that the number of smaller centers that have "taken over" the surgical treatment of cases with HS as "easily solved" has increased. Because of this, the number of patients with MTLE associated with HS in large surgical centers has decreased. However, the more likely hypothesis is that the number of operated patients with MTLE associated with HS is decreasing because the epidemiology of drug-resistant epilepsy is changing. More precisely, better treatment of risk factors for MTLE associated with HS, such as infections and complex febrile convulsions with anti-inflammatory drugs, is the essence of such observations. This belief is based on the observation that although the reduction in the number of cases of MTLE associated with HS is visible in all age categories, this phenomenon is particularly noticeable in the younger population.

Mechanisms of damage to hippocampal neurons

The question whether HS is a cause or effect of epileptic seizures seems to have been around for 100 years - without a good answer. Nevertheless (and not in order to absolve the research failure), it is fair to state that HS is a process that is far more complex than the simplistic consideration of simple neuronal loss. Insights into the mechanisms underlying hippocampal damage are mainly obtained from animal models of SE. These studies showed that although some degree of neural damage results from hypoxia, hypoglycemia, or hypotension, most nerve cell deterioration occurs independently of these factors (11). For now, it seems likely that excitotoxicity is stimulated by epileptic activity that mediates cell death through glutamate receptors.

Complementary and parallel examinations of changes in the human tissue of patients with MTLE associated with HS and in experimental animal models (the most commonly used kainate model) can provide more insight into the pathological process. There is enough evidence for the correctness of such conclusions, but one observation is particularly striking. In 1987, 14 people were poisoned by mussels containing the biotoxin domoic acid on the Prince Edward Islands in Canada. Domoic acid has an excitotoxic property similar to kainic acid owing to its structural similarity to glutamate. Neuropathological findings in those who died from poisoning showed necrosis and neuronal loss in the hippocampus and amygdalae (similar to the kainate model). In an 84-year-old man who had non-convulsive status epilepticus as part of the clinical picture of poisoning, MTLE developed after a "latent" period of one year. This patient died of pneumonia three years and three months after poisoning, and bilateral HS was found at autopsy (20).

In contrast to knowledge derived from human samples, which mainly reflect the late stages of epilepsy, animal models offer the potential to analyze cellular and structural changes through time dynamics. IPIs can significantly alter neurotransmitter receptors' and ion channels' expression and distribution in hippocampal neurons. It has been shown, especially in animal models, that acquired channelopathies significantly change the excitability of different neuronal hippocampal populations, such as, for example, altered expression of GABAA receptor subunits in the dentate gyrus (21). Some channelopathies can even render neurons susceptible to degeneration in selective populations. Deleting the calcium channel subunit, Cav3.2, prevents the loss of IPI-exposed neurons, as seen in HS (22). In addition, chemoanatomical studies show that "vulnerable" sectors of the hippocampus are rich in kainate (endfolium and sector CA3) and NMDA receptors (sector CA1) (21).

In the context of inflammatory mechanisms (autoimmune encephalitis), significant heterogeneity of the cascade of pathophysiological events was also demonstrated.

In the cascade where the antigens are intracellular (anti-Hu, anti-GAD), the process is mediated by a cytotoxic T-lymphocyte reaction targeting hippocampal neurons. On the other hand, in the case of encephalitis with antibodies to the complex of voltage-dependent potassium channels expressed on the cell surface, the essence of the mechanism lies in the increase of the frequency of spontaneous depolarization of neurons.

A genetic predisposition to HS is suggested by the frequency of positive family history in patients with HS and the occurrence of familial MTLE associated with HS. Unfortunately, the specific gene in which the genetic abnormality would be directly and essentially related to the pathophysiological process has not been identified.

Although oxidative stress is undoubtedly at play in HS, it is unclear whether this disorder is a cause or effect in epileptogenesis. Our group was the first in the scientific community to show that the antioxidant system functions abnormally in the tissue of operated patients with MTLE associated with HS. More precisely, numerous enzymes of this process (catalase, glutathione peroxidase, glutathione reductase, manganese superoxide dismutase, and copper-zinc superoxide dismutase) show altered activity (23). In the context of our research efforts, the concentration of metals and electrolytes was also studied. It was found that the hippocampi of patients with epilepsy have reduced copper, manganese, and potassium values compared to controls (24). Moreover, it was found that metals (such as zinc, copper, or manganese) have a different distribution in the sectors of the hippocampus in patients with MTLE associated with HS compared to controls (25), which could indirectly indicate that metabolism metal has a specific role in the mechanism of epileptogenesis.

So far, only one thing is clear. HS is a multifactorial disorder; no single pathogenic factor is necessary or sufficient to generate this intriguing histopathological process.

Hippocampal sclerosis - more than one entity

Just as HS can have several recognized causes, the neuropathological concept is diverse. A new one replaced the earlier system of grading the severity of neuronal loss. In addition to the devastation of neurons, the pattern of nerve cell loss was also considered **(Table 1)** (26). The current classification correlates with the postoperative outcome and, to some extent, with the nature of the precipitating events. Unfortunately, although brain MRI can determine the severity of HS, currently, it cannot identify the pathohistological types of HS.

CLINICAL EXPRESSION OF HIPPOCAMPAL SCLEROSIS

Different causality, quite logically, can also cause different clinical pictures. There are three clinical entities in which HS is present. Without exception, the most important entity is the MTLE syndrome, in which HS is the central etiological substrate. In MTLE associated with HS, cell loss combined with synaptic reorganization in the hippocampus leads to electrophysiological changes that generate epileptic seizures with typical clinical phenomenology.

In a somewhat rarer epileptological entity-dual pathology, loss of hippocampal neurons occurs secondary to "kindling" by epileptic seizures originating from an epileptogenic lesion localized out of the hippocampus. In terms of the clinical manifestation of epileptic seizures, there are significant overlaps between patients with MTLE and those with dual pathology. Of course, the clinical picture of focal epilepsy primarily depends on the localization of the epileptic network. For this reason, MTLE has the most consistent clinical manifestation, while in the case of dual pathology, it also depends on extrahippocampal changes.

The third and rare clinical entity consists of elderly patients with HS, in whom epilepsy is not part of the clinical picture. In these patients, the crucial clinical presentation is cognitive impairment (so-called dementia with HS). Recent data indicate that this entity may represent part of the spectrum of tauopathy or even frontotemporal dementia (27).

The characteristic clinical syndrome of MTLE is mainly an expression of the fact that HS is a highly ep-

Table 1. The International League Against Epilepsy (ILAE) Classification of Hippocampal Sclerosis

Type 1	CA 1: > 80% cell loss CA 2: 30 - 50% cell loss CA 3: 30 - 90% cell loss CA 4: 40 - 90% cell loss The dentate gyrus (DG) is usually affected by 50–60% granule cell loss: 50 - 60%
Type 2	This type presents histopathologically with predominant neuronal loss in CA1, affecting almost 80% of pyramidal cells. All other sectors show mild cell loss barely visible by qualitative microscopic inspection, that is, in CA2 < 20%, in CA3 < 20%, and in CA4 < 25% of principal cells.
Type 3	This type 3 shows predominant cell loss in CA4 (approximately 50% cell loss) and the dentate gyrus (35% cell loss), whereas CA3 (<30%), CA2 (<25%), and CA1 (<20%) are only moderately affected
Type 4	Histopathologically do not show significant neuronal cell loss with only reactive gliosis

ileptogenic lesion that selectively affects one region of the brain, i.e., the hippocampus and immediate cortical structures. More specifically, no other epileptogenic lesion consistently affects a single anatomical region. The significant uniformity of the clinical manifestation of MTLE with HS was defined earlier (28), which allowed this syndrome to be viewed today as a paradigm of focal epilepsy. The typical clinical picture of MTLE with HS has several phases, some of which may be absent in individual patients: 1) febrile attacks in early childhood (<5 years) occur in up to 80% of patients. In a smaller percentage (about 30%), symptomatic epileptic seizures occur due to a CNS infection. Febrile attacks are atypical, i.e., complicated, with a duration longer than 30 minutes or occurring in a series of attacks in one febrile episode. Focal signs in atypical febrile attacks, such as Todd's paresis, are common but, unfortunately, rarely documented; 2) the latent period (in which antiepileptic therapy is often discontinued) until the onset of afebrile attacks lasts on average around 7.5 years (from 1 month to 31 years); 3) initial afebrile secondary GTCS rarely occur (28%) and are usually well controlled with lower doses of antiepileptic drugs. As a rule, after the cessation of such attacks, focal attacks occur in which full pharmacoresistance is manifested (almost 90%) (29). Secondary GTCS are rarely continuously present during the disease (12%), and even in about half of the patients, this type of attack will never occur; 4) initial afebrile focal seizures with typical semiology that occur after a latent period are refractory to high doses of antiepileptic drugs and occur in about 2/3 of patients. The average frequency of such attacks is about 13 per month.

Abdominal (epigastric) aura, which is one of the most typical initial clinical signs of epileptic seizures in MTLE syndrome associated with HS, is determined by electrical activation of the insula, not the hippocampus, which is best proven by registration from deep electrodes placed in both of these structures (Patrick Chauvel, personal communication). Electrical stimulation of the hippocampus with deep electrodes did not lead to any manifestation. Therefore, stimulation of the anteroinferior part of the insula consistently produced a specific sensation in the epigastrium region, which then had an ascending flow. Also, the very common affective, psychic aura of fear is most likely produced in the amygdala. The gustatory aura, in case of MTLE, is produced by an electrical discharge in the anterior or middle part of the insular cortex, where neurons sensitive to gustatory impulses are typically located (30).

On the other hand, the olfactory aura is produced by activation in the anteromedial temporal region (uncus, piriform cortex, or amygdala), proximal to the olfactory bulb in the orbitofrontal cortex or the region of the anterior insula. Apart from the mentioned auras, one of the most typical objective signs in the manifestation of epileptic attacks in MTLE are oroalimentary automatisms (OAA). They represent stereotypic actions involving the mouth, tongue, and throat and can mimic normal behaviors such as chewing, swallowing, or smacking. Data from deep electrode studies show that the appearance of OAA in attacks originating from the anterior and medial temporal lobes depends on their spread to insulo-opercular areas, especially their anterior part (e.g., anterior insula and frontal operculum). OAAs were not observed when the electrical spread was limited to the medial structures of the temporal lobe but became visible only after the spread involved the insulo-opercular regions bilaterally (31). The typical semiology of focal seizures in MTLE

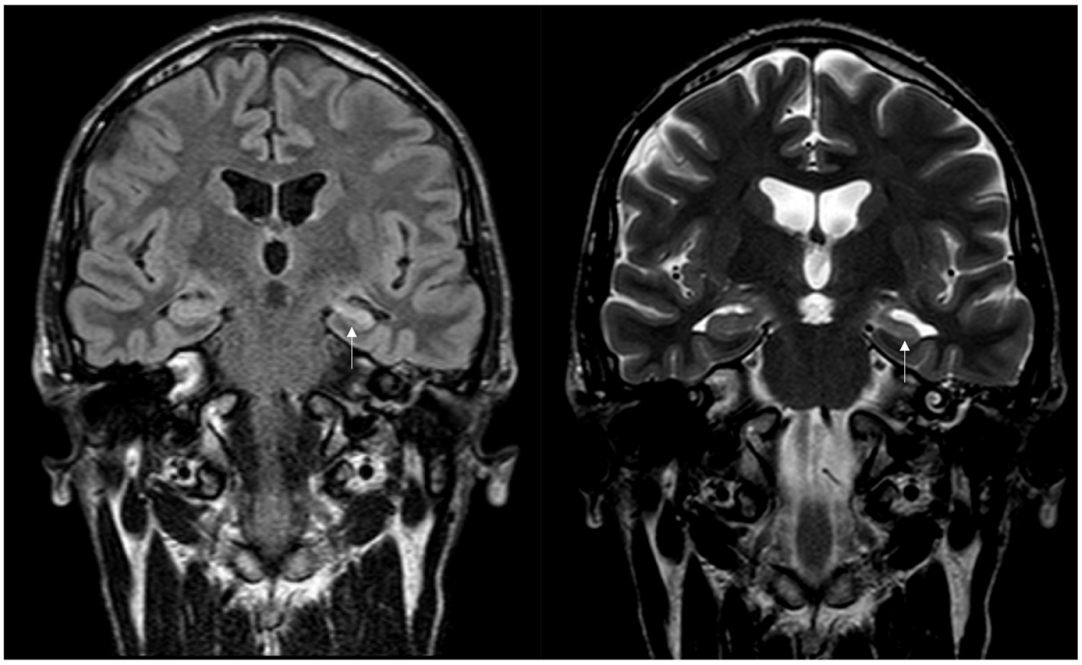

Figure 3. Brain MRI showing left hippocampal sclerosis (white arrow) on FLAIR and T2-weighed sequences on coronal slices.

associated with HS also includes a dystonic hand position (seen in almost all patients with this syndrome) (32, 33). It occurs contralateral to the epileptogenic zone and automatisms (usually hands and, less often, the legs) that are ipsilateral to the epileptogenic zone (34). Dystonia is defined as forced and prolonged, unnatural positioning of an arm or leg on one side of the body, either in flexion or extension, proximally or, more often, distally and usually with a rotatory component. Automatisms are characterized by stereotyped and purposeless involuntary movements more dominantly manifest distally. Although the lateralizing and localizing value of the described signs is only moderate if they occur individually, it is important to note that their combined manifestation - dystonia contralateral to the epileptic focus and automatism ipsilateral to the epileptic focus - is a reliable sign of MTLE associated with HS that carries a significant lateralization value (34).

DIAGNOSTIC METHODS

Magnetic resonance (MRI) enables the detection of HS in vivo, without which diagnosing the syndrome of MTLE associated with HS is unthinkable. Initial MRI examinations of patients with epilepsy during the early 80s did not detect HS. However, improvements in scanning techniques have enabled highly reliable detection of HS in the following years. This primarily refers to a special protocol for patients with MTLE. Such a protocol includes coronary sections perpendicular to the longitudinal axis of the hippocampus, the use of thin sections (e.g., 1 to 3 mm), and the application of IR (Inversion Recovery) and FLAIR (Fluid Attenuated Inversion Recovery) in addition to standard T1 and T2 sequences. MRI features of HS include hippocampal atrophy (from 90 to 95%), increased signal intensity on T2 and FLAIR sequences (80 to 85%), loss of internal structure with loss of hippocampal digitations predominantly seen on T2 sequences (60 to 95%) and signal reduction on T1 sequence (10 to 95%) **(Figure 3)** (35). Associated MRI features seen in HS are: 1) enlarged temporal horn of the lateral ventricle, 2) hypotrophy of the ipsilateral temporal lobe, 3) hypotrophy and increased T2 signal intensity in the amygdala, and 4) reduction of the gray and white matter demarcation of the temporal lobe (especially the temporal apex). In addition to qualitative analysis, HS can be detected by quantitative analysis, the so-called hippocampal volumetry. Although volumetrics can also be done by visual analysis, today, very advanced and precise software techniques exist (36). Positron emission tomography (PET) enables the examination of cerebral glucose metabolism in vivo. PET in patients with epilepsy mainly uses fluoro-2-deoxyglucose (FDG) as a ligand, and rarely 11C-flumazenil (FMZ), which binds to the central benzodiazepine receptor, is used. Brain MRI mainly limited the use of PET in patients with MTLE to rare cases in which HS was not recorded by MRI examination. FDG-PET shows multiregional hypometabolism of ipsilateral medial/lateral temporal lobe (90 to 95%), contralateral medial/lateral temporal lobe (10 to 40%), ipsilateral thalamus (60 to 80%), ipsilateral basal ganglia (40 to 50%), ipsilateral insula (40 to 60%), ipsilateral basal frontal lobe (20 to 30%), ipsilateral parietal lobe (20 to 30%), and ipsilateral occipital lobe (0 to 4%) (35). However, this proportion of hypometabolism regions in the brain is related to MTLE associated with HS and to "non-lesional" MTLE, MTLE associated with vascular changes or cortical organization disorder, or neocortical temporal lobe epilepsy. FMZ binds reduced to the hippocampus and, to a lesser extent, to the ipsilateral insula and thalamus. Areas of reduced FMZ binding on FMZ-PET are usually smaller than glucose hypometabolism detected on FDG-PET.

CONCLUSION

Current neurological science considers HS as both the cause and the consequence of epilepsy. It symbolizes much more than neuronal extinction restricted to the hippocampus. HS is the most frequently encountered cause of refractory temporal lobe epilepsy. MTLE due to HS is a surgically remediable focal epilepsy syndrome. HS is often associated with complicated febrile convulsions early in life, less commonly following non-febrile status epilepticus in infancy, but also as a consequence of CNS infections. The lower prevalence of HS in early childhood indicates that HS is part of a multistage/progressive condition (with an early initial injury followed by a long-lasting latent period prior to the development of chronic refractory epilepsy). Typical brain MRI features of a sclerotic hippocampus are a high signal on FLAIR and T2-weighted images, a low signal on T1-weighted images, and atrophy (volume loss). HS is characterized by neuronal death and alterations in neuronal connectivity and network behavior that underlie the development of chronic epilepsy and memory deficits.

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HIPOKAMPUSNA SKLEROZA

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Sažetak

Hipokampusna skleroza je jedna od najčešćih uzroka fokalne epilepsije. U isto vreme hipokampusna skleroza je i najčešći hirurški supstrat kod fokalnih farmakorezistentih epilepsija. Hipokampus poseduje specifičnu anatomsku strukturu koja se sastoji od ukupno četiri sektora. U fiziološkom konteksu hipokampus je veoma važan u nizu neuropsiholoških procesa, pa je hipokampusna skleroza (entitet koji je prepoznat i povezan sa epilepsijom još u XIX veku) veoma zanimljiva u istraživačkom smislu. Patohistološki obrazac hipokampusne skleroze danas je vrlo precizno predstavljen, što pomaže u uniformnom prepoznavanju. Uzroci hipokampusne skleroze nisu poznati ali su do sada prepoznati brojni faktori koji su udruženi sa nastankom ovog patološkog procesa. Nema nikakve sumnje da je ekscitotoksičnost ujedno sa izmenama u redoks sistemu najvažniji patofiziološki mehanizam. Hipokampusna skleroza je klinički veoma prepoznatljiva. Oboleli od epilepsije u čijoj je osnovi hipokampusna skleroza ima veoma tipične epileptične napade koji se sastoje od epigastrične aure iza koje sledi fokalni epileptični napad kojeg karakterišu pomućenje svesti i oroalimentarni automatizmi. Danas je zahvaljujući modernom neuroimidžingu (prvenstveno magnetnoj rezonanci) detekcija ovog patološkog obrasca veoma precizna i nedvosmislena.

Ključne reči: hipokampusna skleroza, epilepsija, magnetna rezonanca

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REVIEW

COMPLEX KARYOTYPE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Summary

Chronic lymphocytic leukemia (CLL) is a genetically heterogeneous disease with chromosomal and genomic aberrations found in more than 80% of patients, either by conventional or by molecular cytogenetics. Complex karyotype (CK) is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of CLL malignant cell and is considered a potential prognostic parameter in CLL. The detection of CK in CLL patients can potentially affect prognosis and treatment, considering that CK is associated with the progression of HLL and a worse prognosis, as well as with a higher risk of developing Richter transformation. This review will assess the complexity of karyotype analysis in CLL and its prognostic importance and implications.

Key words: chronic lymphocytic leukemia, complex karyotype, chromosomal aberrations

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disease of typically CD5 positive B cells in the blood, bone marrow, lymph nodes, or spleen, and it represents the most common form of leukemia that affects the elderly population (1–3). CLL is a genetically heterogeneous disease with chromosomal and genomic aberrations found in more than 80% of patients, either by conventional or by molecular cytogenetics (1,4). Most common aberrations found in CLL patients are deletion in chromosome 13q (del(13q)), deletion in chromosome 11q (del(11q)), deletion in chromosome 17p (del(17p)), or trisomy 12 (1–9). Deletion of 6q, 2p gain, 8q gain, deletion of 14q, deletion of 15q, trisomy 18, trisomy 19, and others are found, but less frequently (6).

Genetic diversity of CLL reflects clinical heterogeneity, with significant variation in clinical course among patients. To enhance our understanding of CLL and develop effective prognostic models, multiple prognostic factors have been identified and studied (10). These factors include various genetic and clinical parameters that aid clinicians in predicting a patient's clinical course and risk profile. As it was shown during the COVID-19 pandemic, due to compromised immune function and increased susceptibility to severe infectious complications, CLL patients may be particularly vulnerable, which is why, it is an imperative to identify CLL patients who exhibit poor prognostic factors (11).

TP53 mutation (del(17p)) and mutational status of immunoglobulin heavy variable gene (IGHV) are widely recognized as standardized prognostic factors in CLL, with significant clinical implications for predicting disease progression and overall survival (1, 6, 10). These factors have been incorporated into clinical and research guidelines for the management of CLL patients and are particularly relevant in determining the most appropriate treatment strategy, such as choosing between chemoimmunotherapy and targeted therapy.

While previously mentioned established prognostic factors have been proven to be clinically valuable, they are not without limitations. New and more accurate prognostic factors may be needed to capture the heterogeneity of CLL, provide more accurate predictions, and guide personalized treatment decisions (12-13). Advances in genomics and other technologies have made it possible to identify new prognostic factors, such as complex karyotype (CK), tumor microenvironment, epigenetic changes, and others (12-15).

Complex karyotype in CLL is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of a CLL malignant cell (4,5). Since CK occurs in approximately 20% of untreated CLL patients, the question of its prognostic value is becoming more important (5). This review will assess the complexity of karyotype analysis in CLL and its prognostic importance and implications.

DETECTION OF CHROMOSOMAL ABERRATIONS

The fluorescence in situ hybridization (FISH) analysis has become a standard diagnostic procedure in patients with CLL, carried out to detect the four most common aberrations, including the del(17p), one of the main prognostic parameters in CLL. Still, FISH, which is mainly used for the detection of chromosomal aberrations, cannot provide a complete overview of the cytogenetic landscape of CLL (2, 3, 16).

Conventional cytogenetic methods, such as chromosome banding analysis (CBA), offer an assessment of a malignant CLL clone. CBA provides single-cell analysis, detection of balanced chromosomal rearrangement, and detection of clonal evolution. Understanding clone characteristics can potentially affect prognosis and treatment (3, 16). However, CBA was not introduced in routine practice with respect to CLL, unlike in the case of acute leukemias and myelodysplastic syndromes, primarily due to insufficient in vitro proliferative capacity of CLL cells, resulting in poor sensitivity of this method with regard to the detection of abnormal clones (16). After the issue of CLL culture growth had been successfully overcome, the use of conventional cytogenetics expanded and potentiated better detection of aberrations.

Although FISH has overcome the limitations of CBA, these methods complement each other, since there are prognostically significant aberrations that cannot be identified using a single technique. The use of chromosome microarray analysis (CMA) in CLL also provides the whole genome scan but cannot identify balanced chromosome rearrangements (17). Recent recommendations by Jondreville et al. for karyotype and FISH analysis in CLL are shown in **Table 1**.

Recent CLL guidelines suggest FISH, TP53 gene analysis, and IGHV mutational status in general practice.

Table 1. Recent recommendations for karyotype and FISH analysis in CLL

	Clinical practice	Clinical trials
On diagnosis		
Karyotype	recommended	mandatory
FISH – 4-probe[1]	recommended	mandatory
FISH – other probes	depending on karyotype[2]	depending on the purpose[3]
Before treatment		
Karyotype	mandatory	mandatory
FISH – 4-probe[1]	mandatory	mandatory
FISH – other probes	depending on karyotype[2]	depending on the purpose[3]

[1] Detection of del(13)(q14)(D13S319), +12, del(11)(q22)(ATM) and del(17)(p13)(TP53)

[2] Detection/confirmation of other chromosomal abnormalities (within CK or not) with a prognostic impact (e.g., 2p gain, 8q gain, 8p deletion)

[3] Other probes depending on the chromosomal abnormality of interest in clinical trial

CBA analysis is recommended only in the clinical trials setting, since the significance of the CK is still under investigation.

A CLOSER LOOK AT CONVENTIONAL CYTOGENETIC METHODS

In the past, most CLL cases had a very low mitotic index, even in the presence of B-cell mitogens (polyclonal B-cell activators including Epstein-Barr Virus (EBV), lipopolysaccharide from *E. coli* (LPS), pokeweed, CD-40 ligand, and/or different interleukins). Therefore, the use of metaphase (conventional) cytogenetics was very limited. However, those results led to the discovery of recurrent cytogenetic aberrations in CLL. Those findings were implemented in a much simpler method for analyzing genetic aberrations in CLL – interphase FISH.

A significant improvement in conventional cytogenetics in CLL was the introduction of immunostimulatory CpG oligonucleotide DSP30 in combination with interleukin -2 (IL-2). This combination induces cell cycle progression of CLL cells *in vitro* and provides sufficient mitoses for conventional cytogenetics in more than 80% of CLL patients (18). Dicker et al. used 10^7 peripheral blood mononuclear cells that were cultured in 5 mL RPMI 1640 medium (Gibco) with 20% fetal calf serum, DSP30 (2 μM) (TIB MolBiol) and IL-2 (200U/ml) for metaphase induction. After 48 hours, colcemid (Sigma) at a concentration level of 0.15 g/mL was added for another 24 hours before chromosome preparation. Chromosome preparation and staining was done according to standard protocols. Chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN). In three cases, peripheral blood and bone marrow were available from the same patients, which showed that cell culture with DSP30/IL-2 resulted in the detection of the same aberrations on metaphases from different sources. Therefore, peripheral blood was shown to be an adequate sample for conventional cytogenetic analysis, with the procedure being more comfortable for patients.

Baliakas et al. tested protocols used for metaphase induction based on either phorbol-12-myristate-13-acetate (TPA) or immunostimulatory CpG-oligonucleotide DSP30 plus IL-2 following standard procedure and concluded that no difference regarding the number of obtained metaphases was observed between the two protocols (3).

According to the latest recommendation of the ERIC (European Research Initiative on CLL) from 2022, the most appropriate source of tumor cells for conventional cytogenetics is peripheral blood on heparin, as it usually has a high CLL cell fraction. A total of 2×10^6 leukocytes/mL medium are cultured in medium with 20% fetal calf serum and mitogens. It is recommended that 2 parallel cultures with different cell mitogens be set up for each patient, one with 12-O-tetradecanoly-phorpol-13-acetate (TPO), and the other with IL-2 plus DSP30. CLL cells remain in culture for 72 hours, after which antimitotic colcemid is added to the media to obtain metaphases. Upon incubation, harvesting of the cultures is performed following standard cytogenetic procedures: hypotonic solution and fixation with Carnoy's solution (3: 1 = methanol:acetic acid). Finally, a cell suspension is obtained, adjusted to an optimal cell concentration, and slides are prepared. After that, banding and staining is carried out using trypsin and Giemsa. Metaphases should be screened with a microscope or captured using a metaphase finder. A minimum of 20 metaphases should be analyzed in cases with a normal karyotype. Ten metaphases should be fully analyzed, with additional 10 analyzed or counted and scored, for relevant structural chromosomal aberrations (16).

THE COMPLEX KARYOTYPE – DEFINITION

The CK in CLL is defined as the presence of ≥ 3 structural or numerical aberrations in the same clone of a CLL malignant cell, found in 2 out of 20 cells. The presence of ≥ 5 abnormalities is considered to be high CK (3,12). Nonetheless, cytogenetic analysis interpretation can be challenging; therefore, it is recommended that cytogeneticists count aberrations in order to enable clinicians to draw a clear conclusion. Guidelines for counting aberrations in karyotype suggest counting every aberration in every clone and subclone. A single change should be counted only once if it is present in more than one clone. Additionally, special interest should be devoted to distinguishing between the CK with 3, 4, and ≥ 5 aberrations (6).

Based on the existing data, it is evident that CLL heterogeneity also exists within the CK group, and that not all CKs have the same level of significance (3, 5, 9). The number and type of abnormalities, as well as the effects of clonal selection resulting from the treatment, are some of the factors that seem to influence the clinical relevance of CK in CLL. Therefore, it is important to consider a specific CK profile of each individual patient rather than just the number of abnormalities when assessing the patients' prognosis and making treatment decisions.

The number of chromosomal aberrations in CK signals different prognoses in CLL. More specifically, patients with ≥ 5 abnormalities (high-CK) have a very poor outcome, with a median overall survival of 3.1 years. This is independent of clinical stage, TP53 aberrations, and IGHV gene somatic hypermutation status (3, 9). Patients with 3 or 4 aberrations (low-CK and intermediate-CK, respectively) have a shorter survival (median OS of 4.3 years) only when accompanied by TP53 aberrations (3, 9). Furthermore, there are patients with ultra complex karyotypes, having ≥ 10 or ≥ 15 abnormalities, who have particularly poor survival (20).

Besides the number, a type of chromosomal aberrations in CK also affects its prognostic features. An example of this is patients with +12 and +19 aberrations that fulfil criteria for CK but are characterized by an extremely indolent course with prolonged time to the first treatment (TTFT) and OS which is longer than any of the other CK cases or cases without CK, including other M-CLL (3, 9, 16). Interestingly, they have peculiar clinical features (i.e., female predominance, young age at diagnosis, etc.) and comprise nearly 10% of all CK ≥ 3 cases. CK may also reflect clonal complexity, i.e., the presence of subclones. In one study, co-occurrence of CK and clonal aberrations was found in 74% cases, which significantly affects the outcome in CLL patients (22, 23). The effect of various disease characteristics and treatment options on the impact of CK in CLL are shown in **Table 2**. Disease features as well as different treatment options may either aggravate the negative impact of complex karyotype (listed in the second column, or have a neutral effect (listed in the third column). (26)

CK may occur at the time of diagnosis, in relapse, or in the progression of the disease. CK has been observed in up to 20% treatment-naive patients and in up to 40% patients with R/R CLL (5, 20, 24). There is a scarcity of research on sequential karyotype analysis in patients with CLL, but one study has found that the analysis can reveal clonal evolution by means of chromosome analysis in nearly half the patients (45.8%) who remain untreated for 24 months (20, 24). Moreover, patients who exhibit clonal evolution are at a higher risk of disease progression, which underscores the importance of monitoring chromosomal changes over time in CLL patients.

The exact cause of CK in CLL is uncertain. In patients with TP53 mutation/deletion, genome instability leads to clone evolution and detection of CK in progression or in relapse (4). In U-CLL, it has been suggested that the origin of CK development lies in an enhanced lymphocyte response to antigens, which leads to the stimulation of intracellular B-cell-receptor (BCR) signaling and proliferation. Next, during each cell division, telomeres shorten, promoting the development of genetic lesions. Genes implicated in the DNA repair (e.g., TP53, ATM), in ubiquitin-mediated degradation of oncoproteins (e.g., FBXW7), and in the inflammatory pathway (e.g., MYD88) could be affected as well, which further increases the risk of chromosome breaks (26).

PROGNOSTIC VALUE OF THE CK IN CLL

Identification of complex karyotype in CLL patients may assist in risk stratification, based on the results of studies that have evaluated the prognostic value of CK, including its association with other poor outcome prognostic factors, as well as its value as a single factor of adverse prognosis. These studies have shown that unfavorable prognostic factors, such as unmutated IGHV status, del(17p), TP53, and del(11q), are commonly seen in CLL patients with CK (3, 5, 6, 17, 27-29). Apparently, the interaction between CK, TP53 mutation, and IGHV mutational status is very complex.

An association between complex karyotype and unmutated IGHV status in CLL patients has been demonstrated in some studies, with the latter being indicative of poorer prognosis relative to mutated IGHV status (3, 5, 6, 17, 27, 29, 30). A comprehensive study by Baliakas et al. involving 5290 CLL patients found that 72% of patients with complex karyotype (CK) had unmutated IGHV status, while only 28% of patients with a normal karyotype had unmutated IGHV status (3). Biological mechanisms underlying this relationship, however, remain incompletely understood. It is hypothesized that genetic alterations leading to a complex karyotype may disrupt the signaling pathways involved in IGHV gene expression and somatic hypermutation, thereby contributing to the development of an unmutated IGHV status. On the other hand, in M-IGHV patients without any CK subtypes at diagnosis the disease is characteristically very indolent, with a median TTFT of 19 years, and with more than 90% of patients being alive 10 years upon the diagnosis (3, 29).

The presence of a complex karyotype in CLL patients is strongly associated with TP53 aberrations, with up to 80% of patients exhibiting both abnormalities (3, 5, 17, 27-29). This co-occurrence is of significant clinical concern, as it is associated with a very poor prognosis and limited treatment options. It has been observed that CK CLL patients with TP53 abnormalities have unsatisfactory

Table 2. The clinical significance of complex karyotype in CLL

Effect on impact of CK	Negative	Neutral
Disease features / type of treatment	High CK	CK with +12, +19
	TP53 aberrations in low - CK and intermediate - CK	low - CK and intermediate – CK without TP53 aberrations
	U – CLL	M – CLL ?
	Chemotherapy	Novel agents ?

Disease features as well as different treatment options may either aggravate the negative impact of complex karyotype (listed in the second column) or have a neutral effect (listed in the third column).
CK, complex karyotype, U – CLL, unmutated chronic lymphocytic leukemia, M – CLL , mutated chronic lymohocytic leukemia (adopted from Baliakas et al, Hemasphere 2022)

responses to chemotherapy, and that their disease is typically more aggressive, with only 40% alive after 10-year follow-up (29). The underlying biological mechanisms linking complex karyotype and TP53 aberrations in CLL likely relate to their shared association with genomic instability and genetic damage in CLL cells (27, 29).

The genomic landscape of aberrations in CLL is characterized by heterogeneity and diversity, which can even differ within the same case, defining subclones of the disease. Even so, there seem to be some "order in chaos", as certain genomic aberrations are more common in some subgroups of CLL – those which are defined by the characteristics of their BcR IG expression (3, 31, 32). This suggests that some connections may exist between specific antigenic triggers and distinct pathways of genomic evolution in CLL. The observed phenomenon also applies to CK, as high CK is often accompanied by TP53 aberrations and U-CLL, pointing to intense cell proliferation (3, 31).

Essentially, CK also emerged as a potentially independent prognostic factor in CLL. An earlier study showed an association between CK and a shorter time to the first treatment, especially in cases with more than five abnormalities ($p < 0.001$). CK with more than 5 abnormalities retained its significance for the time to the first treatment even in multivariate analysis, along with mutational status of IGHV genes and an advanced clinical stage ($p < 0.05$) (28).

In a large retrospective study on CLL by the ERIC (3), CK was detected in 15% of 5290 patients. Advanced clinical stage and previously mentioned negative prognostic factors were statistically significantly more frequent in these patients than in patients without CK ($p < 0.008$). In addition, shorter overall survival (OS) was found in patients with CK (6.9 years, 2.5–18.2 years, $p < 0.0001$). CK retained its significance regarding shorter OS even in multivariable analysis along with other negative parameters. The value of CK as a prognostic parameter was shown in patients with a normal FISH analysis, because the patients with CK and normal FISH experienced significantly shorter OS compared to patients with a normal FISH and without CK (median OS of 7.88 years vs. median OS of 13.7 years, $p < 0.002$). Patients with CK needed the treatment sooner comparing to those without CK (3).

In a study that included 644 untreated patients with CLL, the correlation between CK and OS was examined. CK was detected in 12.3% of patients, on diagnosis or before treatment, and in those patients, OS appeared shorter than in a group without CK (77 months vs. 115 months $p < 0.0001$). In the same study, the impact of known negative prognostic parameters (TP53 and ATM deletions) and CK on OS was assessed. Patients with both CK and TP53 deletion proved to have shorter OS in comparison with patients who only had TP53 deletion ($p < 0.001$) (4).

The previously cited large ERIC study has also demonstrated survival disparity between patients with CK regarding the number of chromosomal aberrations, grading them into three subgroups: low-CK (3 aberrations), intermediate-CK (4 aberrations), and high-CK (≥ 5 aberrations). The TP53 dysfunction in patients with low and intermediate CK was associated with unfavorable outcome, whilst in patients with high-CK the unfavorable prognosis was observed even in the absence of TP53. The difference among patients with CK was found in those with +12 and +19, because in those patients the disease displays an indolent clinical course, confirming that patients with +12 and +19 form a distinctive group of CLL patients (3).

The risk of developing Richter transformation in patients with CLL and CK is unknown, but considering the negative prognostic value of CK, this association was assessed as plausible in several studies. A retrospective study that included 540 treatment-naïve patients with CLL revealed that CK was significantly more common in patients who developed Richter syndrome than in those who did not, with a seven-fold higher risk of developing Richter transformation in patients with high-CK (18). The analysis of four studies on ibrutinib-treated patients with CLL showed that in patients with CK this transformation is more probable than in those without CK ($p = 0.008$) (33).

CK has not been evaluated as part of a prognostic index yet, mostly because there is a lack of cytogenetic data for most patients included in such studies. It is unclear whether incorporating CK into the development of prognostic indices could enhance their usefulness, since this has not been explored yet.

PREDICTIVE VALUE OF CK IN CLL

The use of CK in treatment decisioning process poses a challenge because of the potential predictive value of CK, especially in the era of personalized therapy (6). The presence of complex karyotype (CK) has been identified as an unfavorable predictive marker in patients with CLL who undergo chemo(immuno)therapy (CIT) (27, 34, 35). This observation suggests that CK may be associated with a worse prognosis and a lower likelihood of a positive treatment outcome. Since this is so, screening for CK prior to treatment initiation may play a vital role in predicting treatment response and selecting appropriate treatment options for patients with CLL. It is important to acknowledge that the precise role of complex karyotype (CK) as an independent predictor in CLL patients undergoing chemo(immuno)therapy remains unclear (3, 5, 27). This knowledge gap arises partly due to the limited inclusion of comprehensive CK assessments in clinical trials evaluating CLL treatments. Consequently, it is uncertain whether the observed association of CK with poor treatment outcomes is solely due to its own impact, or it results from the co-occurrence of other unfavorable

biomarkers, such as TP53 aberrations and U-CLL. To elucidate the ambiguity concerning the independent predictive value of CK in CLL patients undergoing chemo(immuno)therapy, a series of investigations has been undertaken in the context of clinical trials, which will be mentioned hereinafter. These investigations aim to appraise the clinical utility of CK as a potential predictor of treatment outcomes in CLL patients. Through the evaluation of the impact of CK in conjunction with other adverse biomarkers, these studies strive to provide additional insights into the independent prognostic value of CK and its prospective usefulness in predicting treatment response in CLL patients.

In CLL patients treated with chlorambucil-based regimens as first line therapy, as indicated in a prospective study, the interconnection between CK and shorter OS is clear ($p = 0.004$), in spite of the confounding factors. The worst prognosis, as expected, was noted in patients with both CK and TP53 abnormalities ($p < 0.001$) (34).

Regarding CIT based treatments, in a study that included 34.5% of patients with CK treated with rituximab in combination with fludarabine and cyclophosphamide, shorter progression-free survival (PFS) and OS were observed in comparison with patients without CK ($p = 0.005$ and $p = 0.03$, respectively) (27). Similarly, another research proved a relationship between shorter PFS and OS in patients with CK ($p < 0.001$ and $p = 0.02$, respectively) (35).

Based on the previously mentioned studies, CK appears as a negative predictor in patients who are treated with chemotherapy or immunochemotherapy.

According to the latest guidelines, treatment with targeted therapy, such as BTK inhibitors, is strongly recommended. In relapsed/refractory (R/R) CLL patients treated with ibrutinib-based regimens, Thompson et al. suggest that complex karyotype is a stronger predictor than del(17p) of inferior outcome (36). The study showed that R/R CLL with CK treated with ibrutinib had a shorter event-free survival (EFS) and overall survival (OS) compared to those without CK. Specifically, the association between the presence of CK and shorter EFS was statistically significant ($p = 0.006$), as was the association with shorter OS ($p = 0.008$) However, co-existence of del(17p) and CK seemed to have a significant impact on these results, because of 21 patients with CK, 17 had del(17p) (~80%).

In RESONATE study, a prospective study on previously treated patients with CLL, the prognosis for the patients with CK CLL treated with BTK inhibitors was also one of the subjects. In that research no significant differences in PFS and OS were found in patients with CK in comparison to those without CK (≤ 2 cytogenetic aberrations) in median follow-up of 19 months. On the other hand, in this study, in patients with CK who received ofatumumab, there were significantly lower ORR and PFS compared to those without CK (37). After a long follow-up (44 months) median PFS in patients with CK was 40.8 months, as opposed to those without CK, in whom median PFS was not reached (33).

The study Alliance A041202 analyzed the effects of ibrutinib on CLL with CK in individuals who had not received any prior treatment (38). Interestingly, in this study the existence of initial karyotype complexity did not indicate a greater likelihood of progression or mortality in patients treated with ibrutinib, which leads to speculation on whether baseline CK holds the same biological significance as CK resulting from selective clonal expansion following chemotherapy.

Regarding idelalisib regimens, a study which assessed efficacy of idelalisib with rituximab in relapsed CLL patients with significant comorbidities showed longer median OS in the CK group treated with idelalisib (28.3 months), compared to the patients who did not receive target therapy (9.2 months) (39). But there was no difference between ORR in CK and non-CK groups (81% vs. 89%) in patients who received idelalisib. So, it is worth mentioning that this therapy could overcome the bad prognosis of CK, although with limited evidence.

The predictive impact of CK in venetoclax-based regimens was demonstrated in MURANO study, in R/R patients treated with venetoclax-rituximab or bendamustin-rituximab (40). The researchers divided patients into low-, intermediate-, and high-CK, based on genomic complexity. The patients without CK showed better progression-free survival (PFS) compared to those with low-CK or high-CK status (with hazard ratios of 2.0 and 2.9, respectively), and statistically significant differences were observed (with p-values of 0.025 and 0.0057, respectively). Furthermore, patients who had more genomic aberrations exhibited a tendency towards inferior progression-free survival (PFS) compared to those with fewer abnormalities.

In CLL14 trial, the presence of CK among treatment-naïve patients did not significantly affect the outcome of venetoclax-obinutuzumab (VenG) therapy, with ORR 82.4 and 87.3% for patients with CK and non-CK, respectively. Also, the rates of undetectable minimal residual disease (uMRD) were high in both CK and non-CK groups treated with VenG, and there was no significant difference in OS and PFS between these patients (41).

According to a multicenter study on relapsed/refractory CLL patients treated with acalabrutinib, patients with CK, as well as patients with del(17p) had significantly shorter PFS (median PFS 36 and 33 months, respectively), compared to the rest of the cohort (median PFS not reached) (42).

In the context of patients with CLL who undergo stem cell transplantation, a group of investigators made a score that predicts an outcome for these patients. The presence of ≥ 5 chromosomal abnormalities was found to be a prognostic indicator of PFS outcomes, suggesting that karyotypic complexity may be an important factor

to consider in CLL patients undergoing stem cell transplantation (43).

To conclude, when it comes to clinical trials with target therapy, there is limited information on the predictive impact of CK because the number of cases included is very small, and as a result, any conclusions drawn from it are uncertain. More studies are necessary to obtain robust findings on the predictive significance of CK. Based on the available evidence, CLL patients with a complex karyotype may be less responsive to certain treatments, such as chemotherapy. Therefore, some experts have suggested that targeted therapies, such as B-cell receptor signaling inhibitors and BCL-2 inhibitors, may be more effective in this patient population. However, the optimal treatment approach for CLL patients with a complex karyotype is still an area of active research and debate, and individualized treatment decisions should be based on several factors, including the patient's age, overall health status, and the presence of other genetic and molecular markers.

THE FUTURE IMPLICATIONS OF CK IN CLL

CK is a newly identified potential biomarker in CLL that appears to have a prognostic value, and even more importantly, a predictive value. The identification and characterization of complex karyotype in CLL has important future implications. It has a potential to improve risk stratification and personalized treatment selection for CLL patients, especially in the age of new therapies. Understanding the specific genetic aberrations that contribute to a complex karyotype may lead to the development of targeted therapies that can address these abnormalities. Additionally, further research on complex karyotype in CLL may provide insights in the mechanisms of disease progression and therapy resistance. Ultimately, a better understanding of clinical implications of complex karyotype in CLL may lead to improved outcomes for patients with this disease.

CONCLUSION

In order to improve treatment decision-making in patients with CLL along with the TP53 mutation presence, mutational status of IGHV, and FISH analysis, conventional cytogenetics should also be evaluated. Conventional cytogenetics can reveal aberrations that are not detected with FISH analysis and confirm the presence of CK. CK in patients with CLL is associated with the progression of the disease and a worse prognosis. In addition, CLL patients with CK should be closely monitored for the Richter transformation during the follow-up period.

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KOMPLEKSNI KARIOTIP U HRONIČNOJ LIMFOCITNOJ LEUKEMIJI

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Sažetak

Hronična limfocitna leukemija (HLL) je genetski heterogeno oboljenje u kojem se metodama konvencionalne ili molekularne citogenetike registruju hromozomske aberacije u više od 80% pacijenata. Kompleksni kariotip (CK) se definiše kao prisustvo ≥ 3 strukturne ili numeričke aberacije u istom klonu maligne HLL ćelije, i smatra se mogućim prognostičkim parametrom u HLL. Detekcija CK kod pacijenata sa HLL potencijalno može uticati na prognozu i odabir terapijskog modaliteta, uzimajući u obzir povezanost CK sa lošijom prognozom i progresijom HLL, kao i sa povećanim rizikom od razvoja Rihterove transformacije. U ovom preglednom radu biće razmotrena kompleksnost analize kariotipa u HLL i njegov prognostički i klinički značaj.

Ključne reči: hronična limfocitna leukemija, kompleksni kariotip, hromozomske aberacije

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CASE REPORT

NON-CLASSIC FORM OF CONGENITAL ADRENAL HYPERPLASIA

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Summary

Introduction: Congenital adrenal hyperplasia is an autosomal recessive disease caused by gene mutation resulting in 21α-hydroxylase deficiency and a consequent reduction in adrenal steroidogenesis. The disease could present as classic and non-classic form. The frequency of non-classic form is 0.1% in general population, the most common clinical presentation is premature adrenarche, and the diagnosis is made by determining the concentration of 17-hydroxyprogesterone. The recommended treatment is hydrocortisone with close growth monitoring.

Case report: A 7.5-year-old girl was admitted due to premature puberty, accelerated bone maturation and tall stature. The clinical exam revealed hypertrichosis, normal blood pressure and normal ultrasound of internal reproductive organs. The karyotype was 46 XX, the basal and stimulated levels of 17-hydroxyprogesterone were elevated. Clinical and laboratory regression with stagnation of bone and body growth after starting treatment with hydrocortisone confirmed the diagnosis of non-classic congenital adrenal hyperplasia.

Conclusion: Early diagnosis and therapy provide a better quality of life, reaching the target height in adulthood and avoiding the development of complications.

Key words: non-classic congenital adrenal hyperplasia, 17-hydroxyprogesterone, hydrocortisone, children, hyperandrogenism.

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an inherited autosomal recessive genetic disease with inadequate synthesis of the enzyme 21α-hydroxylase and a consequent reduction in the production of adrenal steroid hormones. In 90% of cases, it is caused by the mutation of the CYP21A2 gene. According to the clinical picture and the level of residual activity of the enzyme 21α-hydroxylase, the disease is divided into classic and non-classic forms (1). The non-classic form of congenital adrenal hyperplasia (NCCAH) is characterized by a higher frequency (0.1-0.2%) in general population, a later onset of symptoms, a milder clinical presentation and increased activity of steroidogenesis enzymes compared to the classic form of the disease (2, 3). The non-classic form of the disease is more often diagnosed among women, due to the difficult-to-recognize signs of androgen excess in men (4). The most common mutation detected in NCCAH is p.V281L, which in the Western European population has the highest mutation carrier frequency (7.5%) among Spaniards (5).

The clinical presentation of NCCAH in childhood is premature adrenarche as a result of excess androgens, in adolescence: hirsutism, acne, clitoromegaly, irregular menstrual cycles, oligomenorrhea, tall stature and accelerated growth, and in adulthood: infertility and short stature. Differences in the phenotype of patients depend on their age, sex, and level of residual activity of the missing enzyme. If patients are compound heterozygotes of the classic enzyme mutation, NCCAH is usually detected at an earlier age and is characterized by a more severe clinical presentation (1). NCCAH is rarely diagnosed by neonatal screening due to its failures. If there is suspicion, the level of 17-hydroxyprogesterone (17-OHP) is determined by taking an early morning blood sample or during the follicular phase in girls with the menstrual cycle. Values of 17-OHP concentrations that are up to 2 ng/ml are considered physiological, those from 2-10 ng/ml require an additional ACTH stimulation test, and all values higher than 10 ng/ml are sufficient for the diagnosis of CAH (6). In NCCAH, basal values of 17-OHP are often elevated to more than 15 nmol/L (5 ng/ml), while values below 6 nmol/l (<2 ng/ml) generally rule out NCCAH. A stimulated value, after the ACTH stimulation test, above 30 nmol/l (>10 ng/ml) is sufficient for the diagnosis of NCCAH. The dilemmas surrounding the value of 17-OHP could be resolved by genetic testing (6, 1). Patients with NCCAH generally have adequate basal cortisol reserves and therefore do not require increasing the hydrocortisone dose in stressful situations, compared to the classical form of the disease. Determining the concentration of cortisol is not necessary for the diagnosis of NCCAH. Its diminished response could be detected by the ACTH stimulation test, when its concentration is lower than 18 μg/dl 60 minutes later (7).

Unlike the classic form of the disease, the decision to treat NCCAH is mainly reserved for symptomatic patients who present with hyperandrogenism. Glucocorticoid therapy in the pediatric age is limited to hydrocortisone (10-15 mg/m^2) due to its diminished negative impact on growth. The use of hydrocortisone preparations with extended release and continuous intravenous subcutaneous infusions have been described in literature. Mineralocorticoid therapy, such as fludrocortisone is rarely used in NCCAH, except for reducing the administered dose of glucocorticoids (1).

CASE PRESENTATION

A 7.5-year-old girl was examined at the Department of endocrinology of the University Children's Hospital in Belgrade due to premature pubic hair, accelerated bone maturation and tall stature. The history revealed that she is the second child from the second uneventful pregnancy, born at term, by Caesarean section. Birth measurements are 3500g/50cm/9, she had regular psychomotor development, no complaints, menarche absent. In the family history, the mother body height (BH) 163 cm is obese, the father (BH 185 cm) is healthy. On examination, the girl is tall (BH 141 cm; SDS +3.21; P >99), with high growth velocity (high velocity-HV 6.43 cm/yr.; SDS +0.75; P 77.47), obese (BMI 20.37; SDS +1.92; P >97), increased hair on the extremities, normal blood pressure (BP), Tanner staging A (axillary hair) 1, B (breast size) 1, Ph (pubic hair) 3, without clitoral enlargement, the rest of physical exam was unremarkable, **Table 1**.

Diagnostic procedures included measuring the basal values of 17-OHP, which were elevated, so ACTH stimulation test was performed (corticosyntropin in a dose of 0.25 mg), while the basal values of cortisol, estradiol, testosterone, FSH, LH were in the physiological range for the age (8), which is shown in **Table 2**.

Table 1. Auxological characteristics

	BW (kg)	BH (cm)	HV (cm/yr)	BMI (kg/m^2)	BS (m^2)	TH (cm)	BP (s) mmHg	BP (d) mmHg
	40.5	141	6.43	20.37	1.25	167.5	100	60
SDS	2.71	3.21	0.75	1.92	/	0.61	/	/
P	> 97	99.93	77.47	97.22	/	73.06	40	46

BW-body weight; **BH**–body height; **HV**-High Velocity; **BMI**-body mass index; **BS**- body surface; **TH**-target height; **BP (s)**-systolic blood pressure; **BP (d)**-diastolic blood pressure; **SDS**-standard deviation; **P**-percentile

Table 2. Hormone values

hormone	17OHP (ng/ml)	S 17OHP 30 min	S 17OHP 60 min	C (0 8 h) (ug/dl)	S C (ug/dl)	estradiol (pmol/L)	T (nmol/L)	FSH (U/L)	LH (U/L)
patient values	11.7	18.9	22.3	12.6	15.2	142.5	0.7	1.5	0
normal values (7,8)	< 2	<10	<10	5-23	<18	26-220	0.52-1.21	1.5-12.8	0.10-12.0

17-OHP-17-hydroxyprogesterone;**S 17OHP**- stimulated17-hydroxyprogesterone; **C**-cortisol; **SC**-stimulated cortisol; **T**-testosterone; **FSH**-follicle-stimulating hormone; **LH**-luteinizing hormone.

Abdominal ultrasound visualized normal internal female reproductive organs, without significant enlargement of the adrenal glands. Bone age corresponded to the age of 11 years and showed advanced bone maturation of +3.5 years (11 years - 7.5 years = 3.5 years). Karyotype is normal female, 46XX.

Based on the clinical picture, anthropometric parameters (BH+3SDS), hormone concentration (increased basal and stimulated 17-OHP), accelerated bone age in relation to chronological age (+3.5 years), it was concluded that the girl had NCCAH. Hydrocortisone treatment was started orally in the dose of 10+10 mg per day, 16 mg/m^2. The observed response to therapy after five months was the improvement of metabolic and clinical parameters. Basal values of 17-OHP (11.7 ng/ml to 6.54 ng/ml) and growth rate (6.43 cm/y to 2.54 cm/y) were reduced, and further bone acceleration was ceased at 11 years. Due to a significant increase in BW (+10kg), which caused a change in body surface area, further correction of the administered dose of hydrocortisone was required, with the continuation of the comparison of metabolic parameters.

DISCUSSION

The classic form of CAH with complete or partial enzyme activity results in a more severe clinical presentation due to cortisol deficiency and androgen excess. The non-classic form of CAH is characterized by 20-70% enzyme activity, which results in mild cortisol deficiency and reduced feedback inhibition of the pituitary gland. The consequence is increased synthesis of ACTH and enlargement of the adrenal gland, which is visualized by ultrasound examination of the abdomen. Partial inactivity of the enzyme 21α-hydroxylase causes the accumulation of metabolic precursors above the site of its activity, which results in elevated concentrations of 17-OHP, the level of which is determined in diagnostics (1). Neonatal screening rarely detects NCCAH, as concentrations of 17-OHP during the first two weeks are in the physiological range, and their increase occurs only after this period with no possibility of detecting these patients at that stage (9). In the earliest years of childhood, an excess amount of androgens has no effect on growth rate, but later in childhood their effect becomes apparent. Higher concentrations of androgens and 17-OHP are converted to estrogen, which causes an advance in bone maturity with consequent accelerated pubertal growth. A radiological image of the left hand in children is a gold standard for the estimation of the acceleration of bone maturation in relation to the chronological age. Premature fusion of the epiphyses stops the further growth of the child, and without therapy patients are at risk of not reaching the target height of their parents. Long-term complications of NCCAH include cardiovascular diseases, insulin resistance, obesity, hypertension, diabetes, and psychiatric diseases (1).

Tumors that produce androgens, different exposure to androgens, premature adrenarche or cortisone reductase enzyme deficiency should be considered in differential diagnosis (10).

Treatment aims to suppress accelerated pubertal growth, with minimal risks that the therapy itself carries. In order to avoid possible complications, it is advised to monitor the following: anthropometric parameters (height, weight), signs of sexual development, bone maturity by radiography of the left hand, as well as the measurement of androgen levels (11). New forms of treatment are focused on gene and cell therapy in order to replace glucocorticoid therapy, which will certainly contribute to a better outcome for the patient (12).

CONCLUSION

In patients with a non-classic form of congenital adrenal hyperplasia, early detection of the disease with adequate therapy enables a better and longer life expectancy, while reaching the target height in adulthood. Avoiding the side effects of therapy and minimizing the accompanying complications of the disease requires adequate and constant supervision by a pediatric endocrinologist.

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NEKLASIČAN OBLIK KONGENITALNE ADRENALNE HIPERPLAZIJE

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Sažetak

Uvod: Kongenitalna adrenalna hiperplazija je autozomno recesivno oboljenje gde mutacija gena najčešće izaziva deficit 21α-hidroksilaze i posledično smanjenje steroidogeneze nadbubrega. Bolest se deli na klasičnu i neklasičnu formu. Učestalost neklasične forme je 0,1% u opštoj populaciji, kliničku sliku karakteriše prevremena adrenarha, a dijagnoza se postavlja određivanjem koncentracije 17-hidroksiprogesterona. Lečenje dece je hidrokortizonom uz praćenje njegovog negativnog uticaja na rast.

Prikaz slučaja: Devojčica uzrasta 7,5 godina hospitalizovana je zbog prerane pubarhe, ubrzanog koštanog sazrevanja i visokog rasta. Uočena je pojačana maljavosti ekstremiteta, povišene bazalne i stimulisane vrednosti 17-hidroksiprogesterona, normotenzija, uredan ženski kariotip i ultrazvučni pregled unutrašnjih ženskih reproduktivnih organa. Klinička i laboratorijska regresija uz stagnaciju koštanog i telesnog rasta po započinjanju lečenja hidrokortizonom, navode na dijagnozu neklasične kongenitalne adrenalne hiperplazije.

Zaključak: Rana dijagnoza i terapija omogućavaju kvalitetniji životni vek, dostizanje ciljne visine u odraslom dobu i izbegavanje razvoja komplikacija.

Ključne reči: neklasična kongenitalna adrenalna hiperplazija, 17-hidroksiprogesteron, hidrokortizon, deca, hiperandrogenizam

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CASE REPORT

DYSGERMINOMA IN PREGNANCY

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Summary

Introduction: Malignant germ cell tumors (MGCTs), as a subtype of rare non-epithelial ovarian cancers (NOEC), are most commonly found in pregnancy. Of all MGCTs, 38% are dysgerminoma. Considering the rarity of these entities, the aim of this paper is to show a rare case of ovarian dysgerminoma presented in pregnancy and its influence on course and outcome of the pregnancy.

Patient Review: Patient aged 26, gravida 2, para 1, with one vaginal delivery five years before, was admitted to the Clinic for Gynecology and obstetrics in term pregnancy because of uterine contractions accompanied by left thigh pain and tingling sensation in the left leg. Solid hypoechogenic mass with regular borders, 125x90 mm in diameter adjacent to the left side of the uterus was seen by ultrasound, without free fluid in pelvic cavity. Since regular uterine contractions started, the decision was made to terminate pregnancy by Caesarean section (CS) because of tumor previa. Histopathological examination confirmed ovarian dysgerminoma, but after staging operation which was performed two months after CS, following imaging diagnostics, ovarian dysgerminoma was confirmed with FIGO stage IA, meaning that patient's specific oncological treatment was finished.

Conclusion: Diagnosis of ovarian dysgerminoma is in general challenging since up to 50% are asymptomatic or symptoms are non-specific. The management of ovarian cancer in pregnancy should be multidisciplinary and individualized in the best interest of the mother and the fetus. The overall five-year survival rate for ovarian dysgerminoma is favorable in more than 90% of cases. Women diagnosed with dysgerminoma in pregnancy are young and in general have good feto-maternal outcome.

Key words: Ovarian dysgerminoma, feto-maternal outcome, dysgerminoma in pregnancy, ovarian cancer, cancer in pregnancy

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INTRODUCTION

The incidence of cancer in pregnancy is approximated to be 1 in 1000 deliveries [1]. Gynecological cancers are among the most frequently diagnosed cancers during pregnancy with ovarian malignancies complicating 1% to 6% of pregnancies [1,2]. Malignant germ cell tumors (MGCTs), as a subtype of rare non-epithelial ovarian cancers (NOEC), are most commonly found in pregnancy. Of all MGCTs, 38% are dysgerminoma [3].

In general, MGCTs present as unilateral rapidly growing tumors in adolescence and early adulthood [4,5]. Most of them are diagnosed at an early stage and have very high survival rate. Considering young age of patients, fertility sparing surgery is treatment of choice [6].

Adnexal mass in pregnancy is not only a challenge for diagnosis and treatment, but can also lead to feto-maternal complications [7]. Hence, each patient demands individualized approach in order to achieve an adequate and timely diagnosis and provide appropriate treatment both for the mother and the fetus.

Since this group of ovarian cancers are rare entities, especially in pregnancy, the aim of this paper is to show a rare case of ovarian dysgerminoma presented in pregnancy and its influence on the course and outcome of the pregnancy.

CASE REPORT

Patient aged 26, gravida 2, para 1, with one vaginal delivery five years before, was admitted to the Clinic for Gynecology and obstetrics in term pregnancy because of uterine contractions accompanied by left thigh pain and tingling sensation in the left leg. At presentation, abdominal and ultrasound examination were performed, as well as basic laboratory tests. The uterine fundus height was 33 cm and abdominal circumference was 95 cm. A firm painless mass along the left isthmic side of the uterus with reduced mobility was palpated. Ultrasound examination revealed a vital term fetus with cephalic presentation, adequate amount of amniotic fluid for gestational age and normal placental insertion. Solid hypoechogenic mass with regular borders, 125x90 mm in diameter adjacent to the left side of the uterus was seen by ultrasound, without free fluid in the pelvic cavity. Basic laboratory test results were within normal range for term pregnancy.

Upon admission, the patient also reported that on initial prenatal ultrasound at 13 weeks of gestation hypoechoic lesion measuring 5 cm on the left side was found and that the finding was highly suspected to be fibroid. She also reported that this finding persisted only with minor size enlargement throughout trimesters. Tumor markers (CA-125, HE4, CEA, CA 19-9) were evaluated during the first trimester of pregnancy and were within normal range. Amniocentesis proved normal fetal male karyotype. This procedure was performed in the second trimester because the mother has 47, XX karyotype with small supernumerary marker chromosomes (sSMC). Otherwise normal course of pregnancy was noted with adequate fetal development.

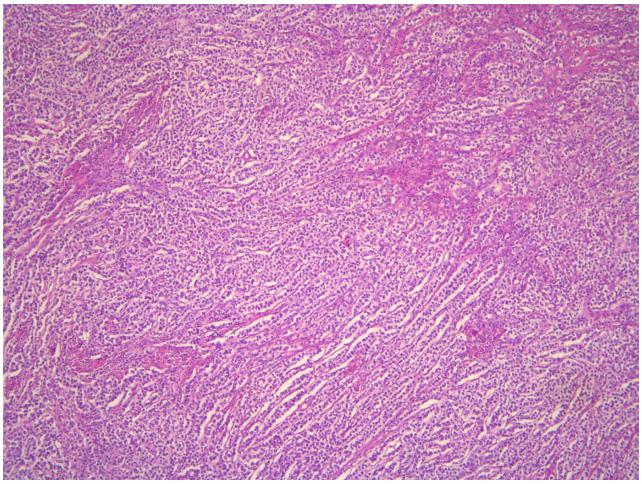

Figure 1. Background lymphocytes surrounding sheets of tumor cells.

Since regular uterine contractions started, the decision was made to terminate pregnancy by Caesarean section because of tumor previa. The patient delivered healthy baby with Apgar score 9 in the fifth minute. Intraoperatively, the left ovary was transformed into a large (140x95x80 mm) homogeneous tumor, with yellowish-white intact capsule and softer consistency. There were no signs of disease spread or free fluid in the abdominal and pelvic cavity. Microscopically cut section showed tumor composed of solid, confluent beaches and bands of large, polygonal, indistinct border cells with large nucleus and light pink, watery plasma **(Figure 1 and 2)**. A scant lymphoid infiltrate and sparse fibrous stroma were present. Final histopathological examination confirmed ovarian dysgerminoma, possibly FIGO IA stage. Imaging was done postoperatively. Neither abdominal and pelvic MRI nor chest X-ray showed signs of disease spread. All the analyzed tumor markers (CA-125, CA 19-9, CEA,

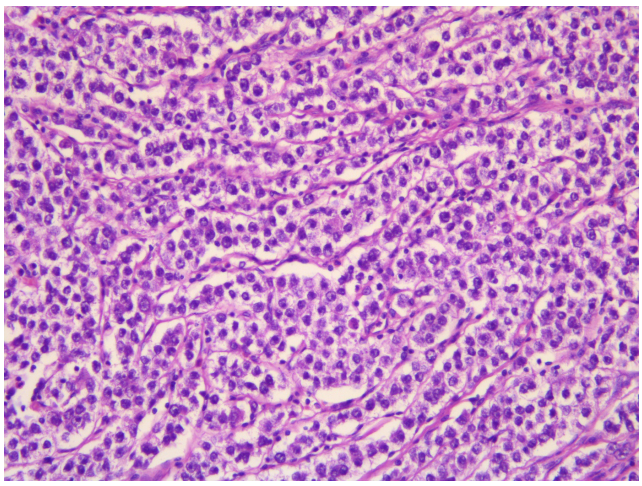

Figure 2. Tumor cells with large clear cytoplasm

LDH, beta hCG) were within normal range. Staging operation was performed two months after CS confirming ovarian dysgerminoma FIGO stage IA, meaning that patient's specific oncological treatment was finished. During the seven-year follow-up with regular pelvic ultrasound, MRI imaging and tumor markers no sign of recurrence has been noticed. The patient was discharged from hospital with a guideline for regular follow-ups. After eight-year follow-up, there has been no sign of disease relapse. Also, normal psychomotor and social development of the child has been reported.

DISCUSSION

This paper presents a case of pregnancy terminated by term CS and complicated by a large adnexal mass that was later proven to be ovarian dysgerminoma.

Although malignant germ cell tumors are rare, their peak incidence is in women of reproductive age [7]. Because of this, one fifth of all ovarian malignancies in pregnancy are MGCT and ovarian dysgerminoma prevail with almost 40% of cases [7]. Overall incidence of ovarian dysgerminoma is less than 1 per 100 000 pregnancies, so most of the literature data are based on case reports and small case series [8].

Generally, diagnosis of ovarian dysgerminoma is challenging as in up to 50% of cases it is asymptomatic or its symptoms are non-specific [9]. Diagnosing these malignancies in pregnancy is even harder because common symptoms of MGST such as abdominal pain and distension may occur in normal pregnancy leading to misdiagnosis. Also, as pregnancy progresses, the growing uterus with fetus interferes with adnexal or uterine masses [7]. This is the reason why ovarian dysgerminoma happens to be an incidental finding during CS as was our case [9].

Although diagnosis is usually made by ultrasound, data show that up to 40% of cases could be missed on ultrasound examination [7]. The numbers are probably higher in the second and the third trimester, meaning that the first trimester exam is important not only for fetal anatomy scan but also for the pelvic inspection. Second line examination is MRI of the pelvis which is safe throughout pregnancy and is more sophisticated in adnexal mass diagnosis and provides more information that ultrasound.

Although in this case adnexal mass was ultrasonographically seen in the first trimester, it was mistaken for fibroid. Dysgerminoma is most commonly misdiagnosed as uterine fibroid, especially pedunculated uterine fibroid with focal cystic degeneration [10]. Beside ultrasound and pelvic MRI, additional diagnostic tool that could be helpful in differentiation of adnexal mass are elevated tumor markers such as serum lactic dehydrogenase and AFP which could be elevated in up to 86% cases, as well as serum beta hCG [9], but these tumor markers are also elevated in pregnancy itself. Histopathology stays the gold standard of ovarian malignancy diagnosis.

The management of ovarian cancer in pregnancy should be multidisciplinary and individualized in the best interest of the mother and the fetus. When making a decision, patients age, gestational age, parity, stage of the tumor, desire for present pregnancy, and future fertility should be taken into consideration [9]. For most early-stage ovarian cancers, unilateral oophorectomy or adnexectomy with appropriate staging should be the surgery of choice and it is safest to perform in the second trimester [6]. Ovarian dysgerminoma is highly chemosensitive to platin-based chemotherapy and is reserved as adjuvant therapy for patients except stage IA [6,11].

The overall five-year survival rate for ovarian dysgerminoma is favorable in more than 90% of cases [10]. Recurrence rate for stage IA is approximately 20% [10].

The possible rate of complications of adnexal mass in pregnancy such as torsion, incarceration, hemorrhage and rupture has increased [9]. Intrauterine growth restriction (IUGR) is the most common (22,8%) complication in neonates [9].

According to a systematic review of literature by Kodama el al. which included 102 ovarian MGCT-complicated pregnancies, the majority of cases resulted in live birth (77.5%) at term (56.6%) via Cesarean section. IUGR was present in 22.8%. During the pregnancy course, obstructed labor, tumor rupture and torsion occurred in 2.9%, 8.8%, and 1.0%, [12].

In our case we had a normal pregnancy course without influence on the neonate and mechanical symptoms of tumor that manifested at the term of delivery. Histopathological analysis did not show necrosis or hemorrhage of the tumor. After eight-year follow-up, our oncological, perinatal and pediatric outcome is good.

CONCLUSION

Women diagnosed with dysgerminoma in pregnancy are young and in general have good feto-maternal outcome. Fertility sparing surgery can be offered in women desirous of pregnancy. The treatment strategy must be discussed and structured individually.

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DISGERMINOM U TRUDNOĆI: PRIKAZ SLUČAJA

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Sažetak

Uvod: Maligni tumori germinativnih ćelija (MGCT), kao podtip retkih neepitelijalnih karcinoma jajnika (NOEC), su najčešći maligni karcinomi jajnika koji se mogu javiti u trudnoći a od kojih se disgerminom javlja u svega 38%. Obzirom na retkost ovih entiteta, cilj ovog rada je da prikaže redak slučaj disgerminoma jajnika koji se javio tokom trudnoće, kao i njegov uticaj na tok i ishod trudnoće.

Prikaz slučaja: Pacijentkinja starosti 26 godina, drugorotka, primljena je na Kliniku za ginekologiju i akušerstvo u terminu trudnoće zbog bolova po tipu kontrakcija, koji su praćeni bolom u levoj butini sa širenjem ka levoj nozi. Ultrazvukom je uočena čvrsta hipoehogena masa pravilnh kontura, prečnika 125x90 mm uz levu stranu materice, bez slobodne tečnosti u maloj karlici. S obzirom na spontano započinjanje porođaja, doneta je odluka da se trudnoća završi carskim rezom zbog pridružećeg tumora. Histopatološkim pregledom potvrđen je disgerminom jajnika, ali nakon operacije preduzete da se utvrdi stadijum tumora koja je obavljena dva meseca nakon carskog reza, i prethodne imidžing dijagnostike, disgerminom jajnika je potvrđen kao FIGO stadijum IA, što znači da je specifično onkološko lečenje pacijentkinje završeno.

Zaključak: Disgerminom jajnika u trudnoći predstavlja veliki izazov u dijagnostici jer je u do 50% slučajeva asimptomatski ili su simptomi nespecifični. Lečenje karcinoma jajnika u trudnoći treba da bude multidisciplinarno i individualizovano, te u najboljem interesu majke i ploda. Ukupna petogodišnja stopa preživljavanja kod disgerminoma jajnika je povoljna u više od 90% slučajeva. Žene sa dijagnozom disgerminoma u trudnoći su mlade i generalno imaju dobar feto-maternalni ishod.

Ključne reči: Disgerminom jajnika, feto-maternalni ishod, disgerminom u trudnoći, karcinom jajnika, karcinom u trudnoći

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CASE REPORT

RARE CASE OF PLEOMORPHIC ADENOMA PRESENTING AS PERITONSILAR TUMOR

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Summary

Pleomorphic adenoma, which is considered to be the most common benign neoplasm of small salivary glands, occurs mainly in the region of the hard palate with mild predilection for women and the peak incidence between the third and sixth decades of life. We present the case of a fifty-one-year-old patient with a left peritonsillar region tumor that the patient has been familiar with for several years. Clinical and radiological examination (computed tomography) indicated a clearly limited, encapsulated tumor change in the left peritonsillar region, 2.5 x 2.5 cm in diameter. The change was completely surgically removed transorally and pathohistologically verified as a pleomorphic adenoma of the small salivary gland. Computed tomography and correct pathohistological diagnosis are essential for the decision on surgical treatment in order to completely remove the lesion. Although it is a benign tumor, early detection is important for a timely decision for surgical treatment. Complete excision of tumor is necessary to prevent regrowth and possible malignant transformation.

KEY WORDS: pleomorphic adenoma, asymptomatic tumor, peritonsillar swelling

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INTRODUCTION

According to the World Health Organization (WHO), salivary gland tumors account for 3 to 6% of all head and neck tumors (1). Pleomorphic adenoma (PA) is the most common benign neoplasm of the salivary glands. It is also known as a benign mixed tumor (BMT), due to its dual origin from epithelial and myoepithelial elements (2, 3, 4). It represents 45-75% of all salivary gland tumors with an annual incidence of 2 to 3.5 cases per 100,000 inhabitants. It occurs in people of all ages. It is most common between the third and sixth decades of life. The frequency of PA is slightly higher in women than in men – ratio 2:1 (5).

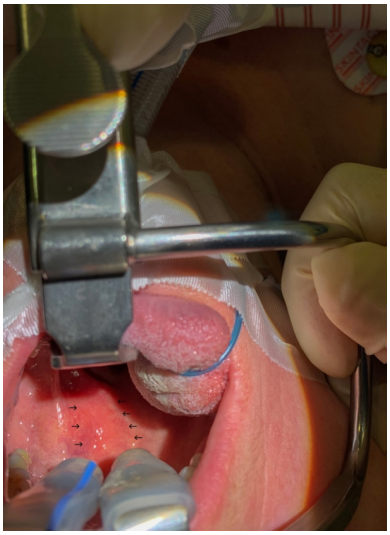

Figure 1. Oropharyngoscopic examination – peritonsillar submucosal swelling (arrows) covered with healthy mucosa

In 80% of cases, salivary gland neoplasms are benign, but they have a potential to become malignant (6, 7). Therefore, early and accurate diagnosis and adequate treatment are necessary.

CASE REPORT

A 51-year-old woman came to the Otorhinolaryngology (ENT) clinic of our institution due to a growth in the region of the left tonsil. She stated that she had noticed the change several years before, but since it had not caused her any problems, the patient did not show up for an examination. In the past six months, she noticed an increase in swelling, so she decided to consult a doctor. She stated that she did not have any complaints in the sense of difficult or painful swallowing, pain in the ear region and that she did not notice a change in the color of her voice. A clinical oropharyngoscopic examination revealed a painless, mobile nodular lesion on the left peritonsillar region, about 2.5 x 2.5 cm in diameter, with firm consistency, covered by a healthy mucosa, without ulceration and surrounding inflammation **(Figure 1)**.

Blood count analysis did not show increased inflammation parameters – leukocyte and CRP values were normal. Computed tomography (CT) of the oropharynx and neck with i.v. contrast showed an encapsulated spherical formation on the soft palate, in the left tonsil region **(Figure 2)**.

The tumor was removed under general endotracheal anesthesia, surgically, through a transoral approach, with a wide local excision **(Figure 3, 4)**.

Pathohistological analysis resulted in the diagnosis of pleomorphic adenoma (PA) of the small salivary gland and the tumor was completely removed **(Figure 5)**.

After 4 days, the patient was discharged from hospital treatment and was regularly monitored. At the latest check-up, two years after surgical removal of the lesion, no recurrence of the disease was noted.

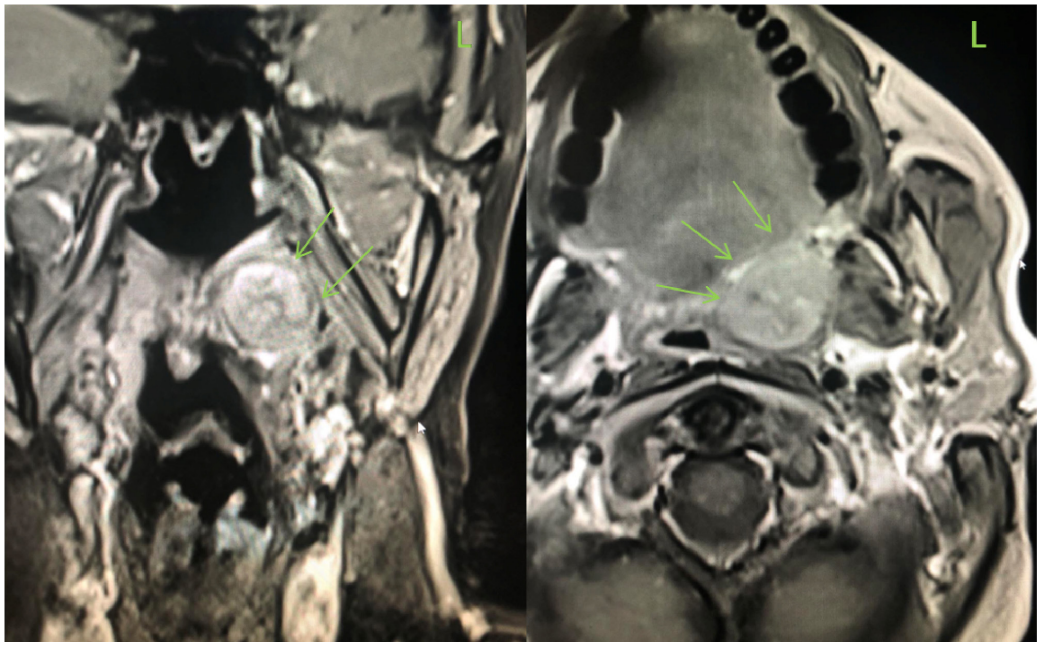

Figure 2. Neck MRI findings – encapsulated tumor in the region of the soft palate on the left (arrows)

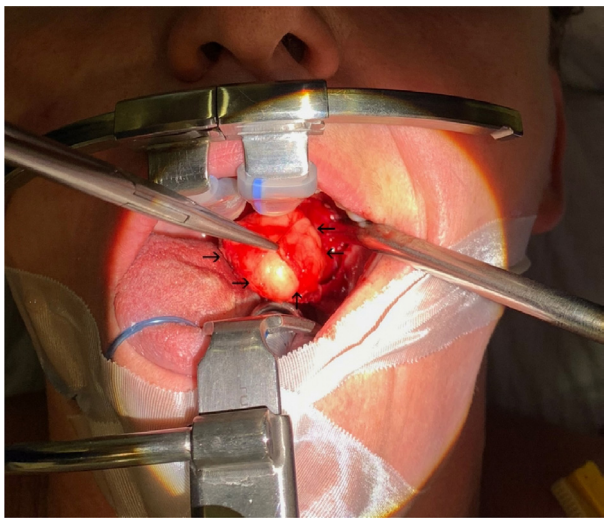

Figure 3. Intraoperative findings – surgical removal of the tumor through a transoral approach

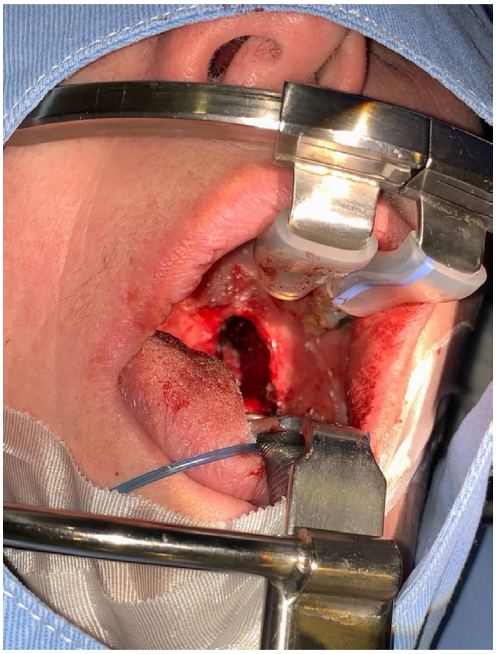

Figure 4. Oropharyngoscopic status immediately after removal of the tumor

DISCUSSION

Pleomorphic adenoma was first named by Willis (8). Previously, it was called mixed tumor, enclave, branchia, endothelium, or enchondroma (9). PA is most often localized in the parotid gland – 85% (and more often originates from its superficial lobe), smaller salivary glands – 10% and submandibular gland – 5%. The most common intraoral localization is the palate, followed by the upper lip, buccal mucosa, tongue and the floor of the mouth (10).

The histogenesis of PA salivary glands is a controversial issue that explains the existence of different theories about the origin of tumors today (11).

The source of salivary gland PA can be epithelial cells lining the secretory cysts and excretory ducts, as well as myoepithelial cells containing secretory elements in the cytoplasm. Development of this tumor from stromal elements is also possible (12). The phenomenon of epithelial-mesenchymal transformation plays an important role in the histogenesis of these tumors (13). It is known that PA of the salivary glands can develop in four variants. The classic version is characterized by the same content of epithelial and mesenchymal components. The other three types of tumors are: mesenchymal, epithelial and myoepithelial (14). Tumors, like healthy tissues, need nutrients and oxygen to live, as well as the removal of metabolic products by carbon dioxide. Tumors meet these needs by neovascularization (15). It is already known that active processes of angiogenesis in a tumor contribute to its malignant potential, process progression and increase the likelihood of recurrence (1). It is known that benign tumors are characterized by the presence of a well-defined connective tissue capsule as a barrier to tumor propagation. Encapsulated tumors are less aggressive than non-encapsulated ones (15).

The etiology of PA is unknown, but the incidence of this tumor has been increasing in the last 15-20 years in relation to radiation exposure. One study suggests that an

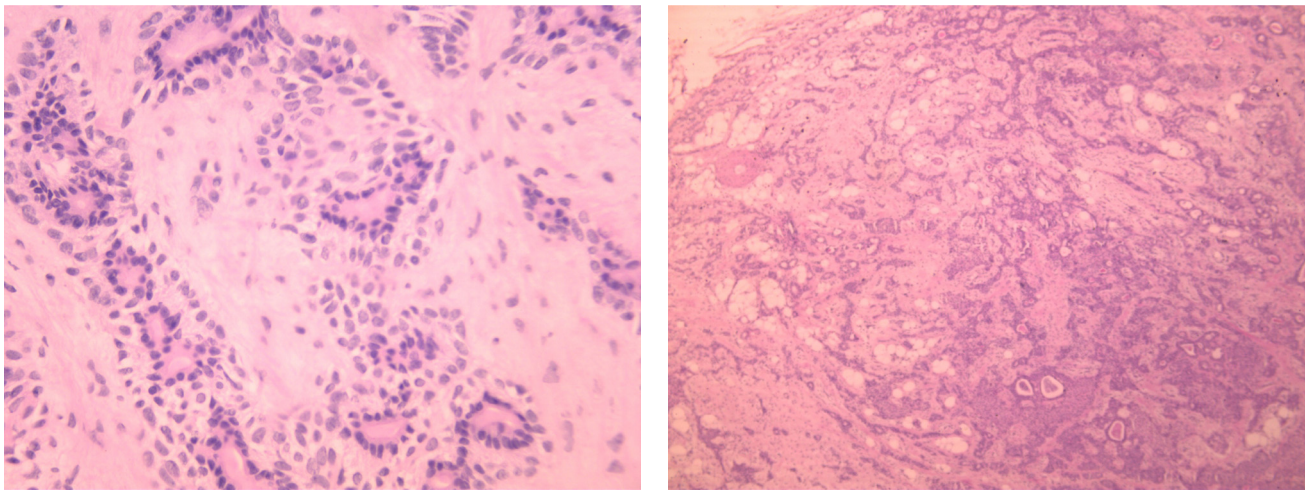

Figure 5. Pathohistological preparation of pleomorphic adenoma: epithelial (ductal) cells containing homogeneous eosinophilic material; myoepithelial cells with an outer layer of cysts and tubules in myxoid stroma; stromal components from myxoid, chondroid and myxochondroid tissue; metaplastic changes: fatty tissue, squamous epithelial metaplasia. Histopathological analysis reported clear margins of resection.

oncogenic simian virus (SV40) may play a role in the onset or progression of PA. Previous irradiation of the head and neck is also a risk factor for the development of these tumors (16).

The diagnosis is made on the basis of anamnestic data, clinical examination and imaging of the head and neck. The main clinical indicator is an increase in volume in the area of the palate (17), as shown in our case.

PA is generally an asymptomatic, solitary, mobile, painless, slow-growing mass, which may be present for many years. Symptoms and signs mainly depend on the size, localization and malignant potential. Rapid enlargement of the tumor nodule should raise concern for suspected malignant change (17). In the parotid gland, signs of involvement of the facial nerve appear when the tumor is large and compresses the nerve or if the tumor has changed malignantly. PA in the deep lobe of the parotid gland can present as an oral, retrotonsillar, or parapharyngeal mass that is visible to the naked eye or palpable. Smaller salivary gland tumors can present with a variety of symptoms, including dysphagia, hoarseness, dyspnea, difficulty chewing, and epistaxis depending on the location of the tumor (18).

CT has become a mandatory diagnostic method to detect the exact localization, size and extent of the lesion (18, 19). Contemporary literature considers it superior to nuclear magnetic resonance (MRI) in relation to the characteristics of these neoplasms. The final diagnosis is determined by histopathological analysis (19).

On CT examination, PA usually appears as a smooth marginal or lobular homogeneous globular mass of soft-tissue density. Necrosis can present in larger masses. The presence of several foci of calcification is common. Smaller tumors show pronounced homogenous amplification early, while in the case of larger tumors the amplification is less pronounced and delayed (17).

An MRI image is similar to a CT scan. Smaller masses appear well circumscribed and homogeneous, while larger tumors present as heterogeneous masses (17).

From the perspective of differential diagnosis, the following can be considered: infection (peritonsillar abscess, dental infection), congenital anomalies (20) and neoplasms: Vartin's tumor, metastases in the parotid gland, schwannomas of the facial nerve, myoepitheliomas, mucoepidermoid and adenoid cystic carcinoma and a large number of other neoplasms not specific to the salivary glands. Histopathological examination (analysis) remains the golden standard in diagnosis and differentiation of these neoplasms (17).

Surgical technique is a relevant factor in order to avoid tumor recurrence. Smaller benign salivary gland tumors localized on the palate are removed by wide local excision within the palatal mucosa with margins of 5 to 10 mm and preservation of the pseudocapsule. As these tumors do not penetrate the periosteum, the bone does not need to be resected. The exposed palatal bone is left to heal secondarily or is resected and reconstructed with a soft tissue flap (21). Traditionally, the most common form of treatment in the literature is wide local excision with removal of the periosteum or bone if these tissues are involved, while preserving the adjacent nerve. Previously performed simple enucleation procedures are associated with a high recurrence rate and should be avoided (22).

The prognosis for PA is good, with a cure rate of 95%. When relapse occurs, PAs show significant resistance to treatment, with options including observation alone, surgery and radiotherapy. PAs carry a low risk of malignant transformation. It is observed that the malignant potential is proportional to the time of the lesion in situ (1.5% in the first 5 years, 9.5% after 15 years). This leads to the conclusion that excision is justified in almost all cases. Other risk factors for malignant transformation include advanced age, radiation therapy, especially to the head and neck region, large tumor size, and recurrent lesions (23). In the patient shown, two years after the surgical removal of the tumor, no recurrence of the disease was observed.

CONCLUSION

Pleomorphic adenoma is a benign lesion that, due to its long asymptomatic evolution, is discovered relatively late. Minor, non-specific complaints or asymptomatic cases are detected during clinical examination and other diagnostic procedures from other indications. Although it is a benign tumor, early detection is important for a timely decision for surgical treatment. Complete tumor excision is necessary to prevent regrowth and possible malignant transformation.

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RETKA PREZENTACIJA PLEOMORFNOG ADENOMA KAO PERITONZILARNI TUMOR, PRIKAZ SLUČAJA

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Sažetak

Pleomorfni adenom, koji se smatra najčešćom benignom neoplazmom malih pljuvačnih žlezda, javlja se uglavnom u predelu tvrdog nepca sa blagom predilekcijom kod žena i vrhuncem incidencije između treće i šeste decenije života. Prikazujemo slučaj pedesetjednogodišnje pacijentkinje sa tumefaktom leve peritonzilarne regije za koji pacijentkinja zna unazad nekoliko godina. Klinički i radiološki pregledi ukazali su na jasno ograničenu, inkapsuliranu tumorsku promenu leve peritonzilarne regije, promera 2.5 x 2.5 cm. Promena je u celini hirurški odstranjena transoralnim putem i patohistološki verifikovana kao pleomorfni adenom male pljuvačne žlezde. Kompjuterizovana tomografija i ispravna patohistološka dijagnoza su od suštinskog značaja za odluku o hirurškom lečenju u cilju potpunog uklanjanja lezije. Iako se radi o benignom tumoru, rano otkrivanje je važno zbog pravovremene odluke vezane za hirurški tretman. Kompletna ekscizija tumora neophodna je zbog sprečavanja ponovnog rasta i eventualne maligne transformacije.

Ključne reči: pleomorfni adenom, asimptomatski tumor, peritonzilarni otok

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CASE REPORT

PEMPHIGUS VEGETANS HALLOPEAU WITH NAIL CHANGES AND NO ORAL INVOLVEMENT

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Summary

Introduction: Pemphigus vegetans (PVeg) is the rarest form of autoimmune pemphigus. Lesions are primarily flexural and mucosal, although they may occur at any site. Oral involvement is common. Two subtypes are recognized – the Neumann type and the less common Hallopeau type.

Patient review: We present a Hallopeau-type PVeg with no oral lesions and with uncommon nail changes. In the follow-up period of ten years, the patient experienced several flares but no changes in the oral cavity.

Conclusion: This case is interesting due to the disease's rarity, atypic clinical presentation, and an association with multiple pregnancies as a possible precipitating factor.

Key words: pemphigus, Hallopeau, vegetations, pustules

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INTRODUCTION

PVeg is the rarest form of pemphigus vulgaris, and it constitutes up to 1-2% of all pemphigus cases (1). The two subtypes – Neumann and Hallopeau – are differentiated based on clinical presentation, course, and response to treatment (2). Neumann type is characterized by bullae that extend and coalesce, evolving into vegetating masses (3). Hallopeau-type PVeg is characterized by a polycyclic eruption of pustules forming firm pink papilloma that progressively flatten, with a benign course and few relapses (3,4). PVeg usually affects younger adults or middle-aged females (5). Oral involvement is present in nearly all PVeg cases (5,6).

CASE REPORT

A 40-year-old healthy Caucasian woman had a 4-month history of pustular lesions affecting the scalp and, a month later, the genital area. The patient had a history of nine pregnancies (six children, the youngest child being 11 months old, and three artificial abortions). She had been previously treated with antibiotics with little or no improvement.

On examination, firm and eroded plaques were present on the scalp and vulvar region, coated with pustules **(Figures 1a and b)**. Oral mucosa was not affected. There was discoloration of her fingernails and periungual swelling, erythema, and pustules **(Figure 1c)**. Cervical and inguinal lymph nodes were enlarged.

Laboratory findings revealed blood eosinophilia up to $6.15x10^9$/L (normal range 0-0.4) with mild leukocytosis (up to $14.4x10^9$/L). All other routine laboratory studies were within normal range. The gynecological examination was normal except for the vulvar lesions; there were no lesions on the genital mucosa.

Circulating pemphigus antibodies binding to the monkey esophagus were found at a titer of 1:640. Enzyme-linked immunosorbent assay showed positive anti-Dsg3 antibodies and anti-Dsg1 negative (62.3 RU/ml and 3.3 RU/ml, respectively; cut off < 20 RU/ml). The direct immunofluorescent test revealed IgG deposits in intercellular spaces of the epidermis. The patient initially

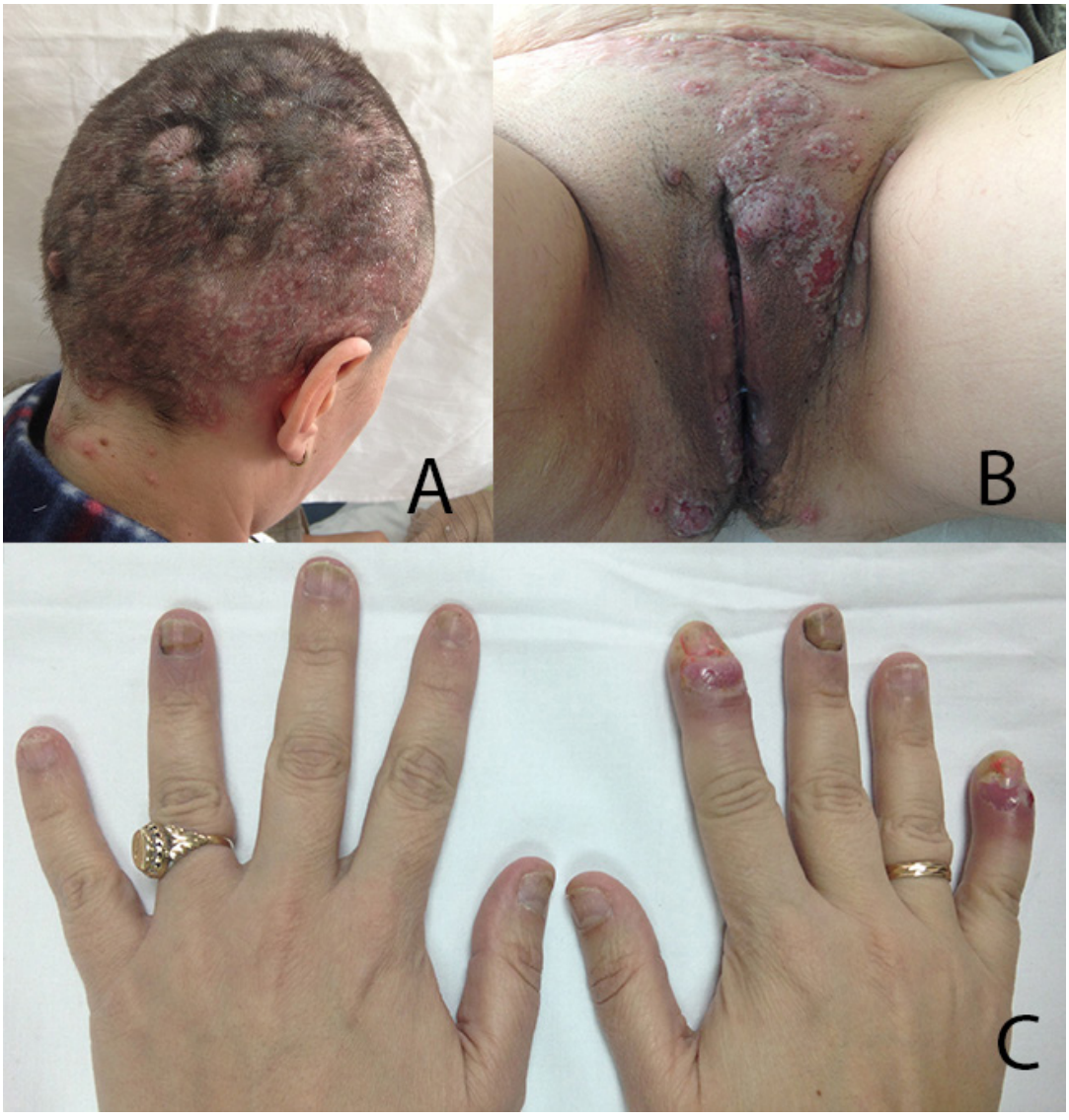

Figure 1. Skin lesions on admission: A. Scalp, B. Vulvar region, C. Nails

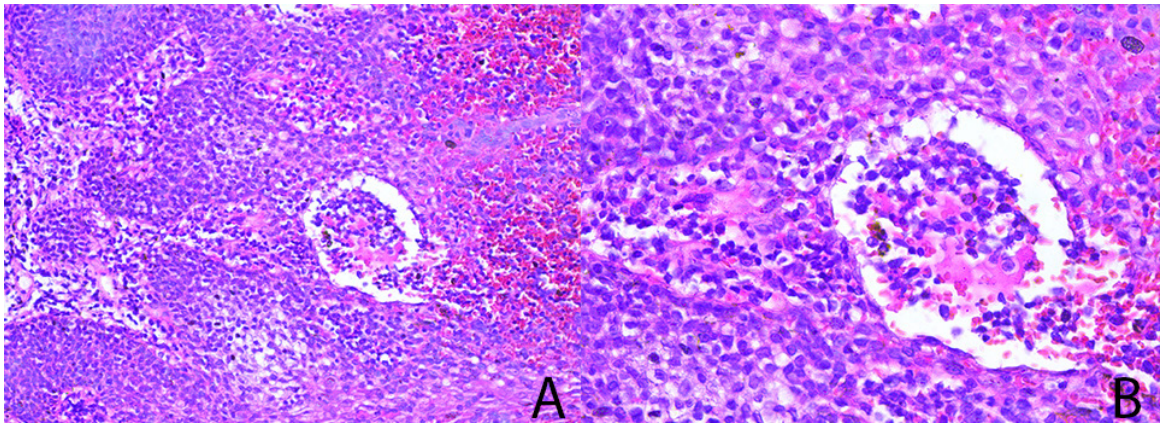

Figure 2. Histopathology finding, H&E

refused another biopsy, which was postponed for three weeks. Skin biopsy showed suprabasal acantholysis and a few microabscesses with eosinophils, neutrophils, and acantholytic cells. **(Figure 2a and 2b)**

The patient was treated with oral prednisone (0.8 mg/kg body weight), reduced to 0.2 mg/kg after 1.5 months, and discontinued after three months; azathioprine, 2 mg/kg body weight daily (discontinued after three weeks due to elevated liver enzymes). All lesions resolved. **(Figures 3a, 3b, and 3c)**. She was disease-free for 19 months when a mild relapse on the scalp occurred and fissures in the lip angles, without lesions in the oral cavity. Cyclosporine A was introduced (3.5 mg/kg body weight). After a 2-month therapy, the lesions healed. She continued with a lower dose of Cyclosporine A (2 mg/kg body weight for two months and 1.5 mg/kg t for another three months). In the next 20 months, no new lesions occurred; after that period, new lesions occasionally occurred, almost always preceded by infection (pharyngitis, urinary infections, scabies, and head lice). Cyclosporine A was reintroduced (3.5 mg/kg body weight), with low dose prednisone (0.4-0.5 mg/kg body weight). She was lost to follow-up for four years, during which she experienced periodic flares. She discontinued Cyclosporine A in the past 2.5 years; she

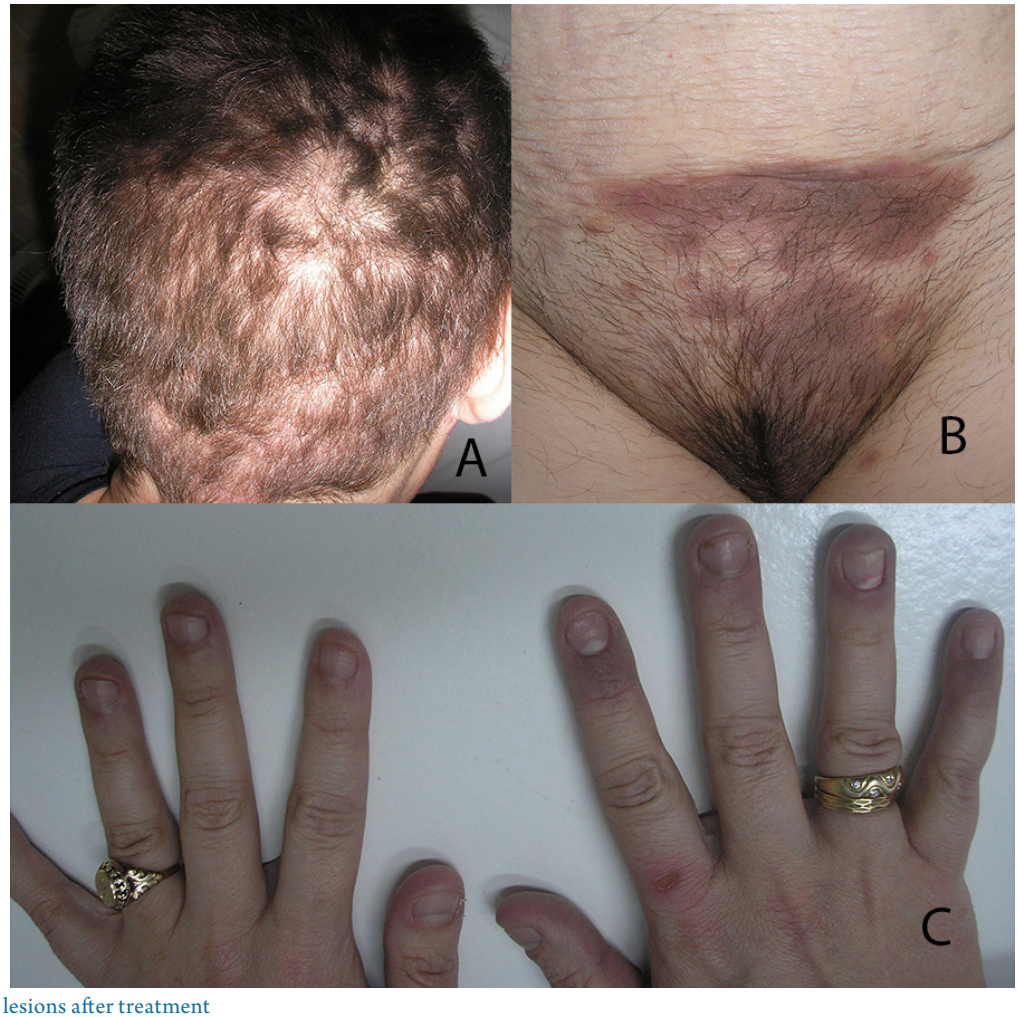

Figure 3. Skin lesions after treatment

occasionally took prednisone (approximately 0.3 mg/kg body weight) for 2-3 months/ per year. On her last visit four months ago, she had no skin or mucosal changes; the indirect immunofluorescence test was negative. She has had no treatment for 16 months.

DISCUSSION

In the PVeg Hallopeau type, pustular lesions with subsequent vegetation are the basic clinical features of the disease (3). Lesions are typically located in the intertriginous areas and oral mucosa (3,5,6). The occurrence of PVeg in non-intertriginous areas is extremely rare. Our patient had no mucosal (oral or genital) or intertriginous involvement (except for two pustulous lesions in her left groin). Initially, the disease may be confined to a single site, but generally, it becomes multifocal (5,7). In rare cases, the disease may manifest as solitary lesions limited to one affected site (6).

The nail apparatus is rarely involved in PVeg, mainly presenting as verrucous paronychia and pachyonychia (5). Jindal et al. described a patient with PVeg presenting as acrodermatitis continua suppurativa (2). Our patient also had uncommon nail changes manifested as pustules with inflammatory edema on her fingernails, which resolved under treatment without evolving into chronic/ verrucous paronychia.

Previous publications reported autoantibodies against Dsg3, while autoantibodies against Dsg1 and desmocollin 1-3 are occasionally detected (5,8,9). Although we had shown autoantibodies against Dsg3 in our patient, no testing against desmocollin was performed.

Histopathologic findings distinctive of PVeg Hallopeau – suprabasal acantholysis with epithelial hyperplasia, intraepidermal eosinophil and neutrophil microabscesses, papillomatosis and acanthosis (5,9) were the characteristics in our case.

The etiopathogenesis of PVeg remains unclear. Development of vegetation on the intertriginous area may be attributed to relative occlusion and maceration with subsequent bacterial infection suggesting a response to superinfection (1). Cytokines play a role in epithelial proliferation and eosinophilic chemotaxis. High counts of blood eosinophils are observed, such as in our patient (10).

Apart from genetic (predisposing) factors, various environmental and hormonal (precipitating) factors are incriminated in the pemphigus group. Published data showed more pregnancies in patients with autoimmune pemphigus (11). Also, most published cases of pemphigus associated with pregnancy are patients with the disease worsening during pregnancy or after delivery. Indeed, our patient did not develop the disease as expected at the beginning or after pregnancy. Still, as she had multiple pregnancies, we could suppose that, in her case, hormonal influence could contribute to the development of PVeg. Bonifazi et al. reported a child with neonatal pemphigus in which the mother had shown no symptoms of pemphigus before the delivery. Still, the symptoms appeared six months after birth (12). None of our patient's children had any clinical signs of pemphigus during the follow-up period. Further studies need to establish the role of sex hormones in the pathogenesis of pemphigus.

Systemic steroids are treatment of choice for PVeg Hallopeau. Relapses, if any, usually correspond to lower doses of corticosteroids. The addition of immunosuppressive agents may improve remission rates and allow a steroid-sparing effect (1). Our patient responded well to systemic steroids and Cyclosporine A, which was introduced as she expressed the desire for another pregnancy despite her doctor's advice (during the follow-up period, she did not get pregnant).

CONCLUSION

This case is interesting due to the disease's rarity, atypic clinical presentation, and the association with multiple pregnancies as a possible precipitating factor. Viral infections have been noted as triggering factors for pemphigus activation or exacerbation. Although bacterial infections were not reported as an inducing/precipitating factor, we have to note that a bacterial infection or infestation preceded several flares in our patient. Flares in the scalp area following infestation could be explained by a possible staphylococcal superinfection.

Conflict of interest: None declared.

Author contribution: All listed authors contributed equally to the conception of the work, the interpretation of data, preparing the draft of the manuscript, and the interpretation of the revised version.

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PEMPHIGUS VEGENTANS HALLOPEAU TIP SA PROMENAMA NA NOKTIMA I BEZ ZAHVAĆENOSTI ORALNE MUKOZE

Marija Tomanović[1], Jovan Lalošević[1, 2], Dušan Škiljević[1, 2], Dubravka Živanović[1, 2]

Sažetak

Uvod: Pemphigus vegetans (PVeg) je najređa forma autoimunskog pemfigusa. Lezije su najčešće lokalizovane na mukozama i u pregibima, mada se mogu javiti i na drugim delovima. Zahvatanje oralne mukoze je često. Postoje dva tipa, Neumann i Hallopeau tip, koji je znatno ređi.

Prikaz slučaja: Prikazujemo pacijentkinju sa Hallpeau tipom PVeg, bez promena u usnoj duplji i sa atipičnim promenama na nokatnim pločama. Tokom deset godina koliko je pacijentkinja praćena, imala je nekoliko recidiva, ali nikada nije razvila promene u usnoj duplji.

Zaključak: Ovaj slučaj je jako zanimljiv, kako zbog atipične kliničke prezentacije ovog tako retkog kliničkog entiteta, tako i zbog višestrukih trudnoća kao potencijalnog etiološkog faktora.

Ključne reči: pemfigus, Hallopeau, vegetacije, pustule

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