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PREOPERATIVE AND POSTOPERATIVE LIVER FUNCTION ANALYSIS AFTER LIVER RESECTION

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Preoperative diagnostics and preparation of patients undergoing liver resection procedures are crucial for the outcome of surgical treatment.

The study included 30 patients who underwent hepatectomy due to primary or secondary tumor changes. Preoperative and postoperative liver parenchyma status was monitored based on the determination of biochemical liver function parameters (alkaline phosphatase, AST, ALT, γGT, bilirubin−T.Bil and D.Bil, LDH, albumin) and metabolic syndrome parameters (glucose, urea, creatinine, blood pressure).

This research provided valuable insights into the characteristics of liver tissue damage following resection, based on liver function monitoring. By applying modern data processing techniques and relevant literature, these findings can contribute to the refinement of therapeutic protocols and postoperative care strategies, offering useful guidance for improving treatment outcomes.

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Key words: *liver surgery, liver resections, liver function*

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Introduction

Liver surgeries represent one of the most challenging aspects of abdominal surgery due to the complex anatomy and vital functions of the liver (1–3). Preoperative diagnostics and preparation of patients undergoing liver resection procedures are crucial for the outcome of surgical treatment. Medical history, clinical examination, and imaging diagnostic procedures form the basis for decision-making regarding indications for liver surgery (1, 2). Moreover, one of the most important steps in liver surgery is a precise preoperative assessment of the patient's current functional capabilities and reserves. In this way, patients with a high surgical risk can be identified, particularly those with liver cirrhosis, jaundice, and those undergoing prolonged chemotherapy (3). Liver resections represent a significant surgical stress for the body, leading to complex physiological and biochemical changes that can compromise liver function. Pathophysiological mechanisms contributing to postoperative liver dysfunction include surgical stress, hemodynamic changes induced by anesthesia, intraoperative blood loss, ischemia reperfusion syndrome, oxidative stress, and hepatocyte apoptosis (2, 4). A vital characteristic of the liver is its ability to regenerate, meaning it can recover from injuries, toxic and ischemic damage, and, most importantly for surgeons, after resection procedures (4). Preoperative preparation and postoperative monitoring of patients after liver resection procedures, in addition to the surgical and anesthesiological parameters monitored during any surgical intervention, also include specific quantitative assessments of the volume of liver tissue remaining after resection, and qualitative evaluation of the functional quality of this residual parenchyma, or its ability to take over further postoperative liver function (5, 6).

Our research aimed to identify liver tissue damage based on laboratory parameters (hepatocyte damage markers, liver excretory function markers—biliary obstruction, liver synthetic function markers, and inflammatory syndrome markers). After reviewing the literature, we formulated the following scientific hypothesis: To determine the degree of liver tissue damage during the surgical procedure based on functional biochemical tests pre- and postoperatively. From this hypothesis, the objectives of the study were set:

To determine the preoperative and postoperative functional status of the liver parenchyma based on the measurement of biochemical liver function parameters (alkaline phosphatase activity, AST, ALT, γGT, bilirubin—TBIL and DBIL, LDH, albumin) and metabolic syndrome parameters (glucose, urea, creatinine).

Materials and Methods

A prospective analysis was conducted on 30 patients who underwent liver resection due to a primary neoplastic process or metastases from colorectal cancer. The patients were hospitalized at the Department of Digestive Surgery, University Clinical Center Niš. The following were analyzed for all patients:

Preoperative parameters:

Standard preoperative tests evaluating liver function in the patients included in the study (alkaline phosphatase, AST, ALT, γGT, bilirubin, LDH, albumin), as well as the presence of metabolic syndrome (glucose ≥ 6.1 mmol/L; urea ≥ 6.1; creatinine ≥ 6.1; blood pressure ≥ 130/85 mmHg).

Postoperative parameters:

Postoperative liver function and the presence of metabolic stress were monitored by analyzing blood samples from patients at the Central Laboratory of the University Clinical Center Niš on the first, third, and fifth postoperative days.

Statistical Data Analysis:

Data are presented as means and standard deviations. Comparison of values between the four measurements (preoperative, days I, III, V) was performed using repeated measures analysis of variance (ANOVA). The Bonferroni test was used as a *post hoc* analysis. Statistical processing was performed using the SPSS 20.0 software package. The null hypothesis was tested with a significance level of $p < 0.05$.

Results

Demographic and Clinical Characteristics of the Study Population

The study included 30 patients (16 male and 14 female) (Figure 1). The average age of the study population was 60.03 years (Min 37, Max 77 years).

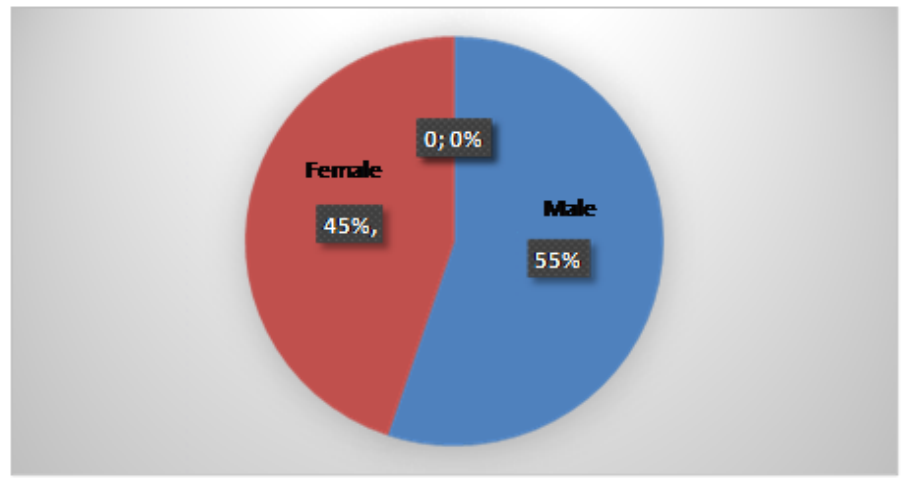

Figure 1. Distribution of the study population by gender

By type of liver resection, a total of 30 surgeries were performed in this study. Of these, four were hepatectomies, four bisegmentectomies, 11 metastasectomies, and 11 segmentectomies (Figure 2). According to the classification of resections into major and minor types, hepatectomies and bisegmentectomies are categorized as major resections, while segmentectomies and metastasectomies belong to minor resections.

Accordingly, in the analyzed group of patients, a total of 8 major resections (26.7%)

and 22 minor resections (73.3%) were performed, with a predominance of less invasive surgical procedures. This distribution indicates a tendency to preserve functional liver parenchyma while performing oncologically adequate surgeries.

The duration of the surgical intervention ranged from 87 to 180 minutes. Seven patients underwent surgery due to a primary liver process, while the remaining 21 patients were operated on because of colorectal cancer metastases. All patients underwent intermittent clamping of the hepatoduodenal ligament (Pringle maneuver) for 15–25 minutes for resection and bleeding control. During the surgical procedure, no blood loss greater than 300 ml was recorded. Five patients received a postoperative transfusion in the form of whole blood (2 x 350 ml) and fresh frozen plasma (2 x 220 ml).

The values of AST and ALT differed statistically significantly between preoperative and postoperative measurements ($p < 0.001$ for both genders). DBIL values were statistically significantly higher after surgery compared to the preoperative period ($p = 0.017$). LDH values were statistically significantly higher after surgery compared to the preoperative period ($p < 0.001$). Albumin values were statistically significantly lower after surgery compared to the preoperative period ($p < 0.001$) (Figure 3).

ANOVA for repeated measurements showed statistically significant changes in the values of the following laboratory parameters during the follow-up period: alkaline phosphatase ($p = 0.001$), AST ($p = < 0.001$), ALT ($p = < 0.001$), γGT ($p = 0.023$), TBIL ($p = 0.033$), DBIL ($p = 0.130$), LDH ($p = < 0.001$), albumin ($p = < 0.001$), glucose ($p = 0.016$), urea ($p = 0.001$), creatinine ($p = 0.004$), STA ($p = 0.324$), DTA ($p = 0.388$). The values of alkaline phosphatase, AST, ALT, LDH, and albumin significantly differ between preoperative measurements and measurements on the 1st, 3rd, and 5th days ($p = < 0.001$, $p = 0.001$, and $p = 1.000$), AST ($p = 0.005$, and $p = < 0.001$), ALT ($p = 0.021$, $p = < 0.001$, and $p = < 0.001$), LDH ($p = < 0.001$, $p = < 0.001$, and $p = < 0.001$), albumin ($p = < 0.001$, $p = < 0.001$, and $p = < 0.001$). The values of the following parameters change between the preoperative period and the first day: γGT ($p = < 0.001$), TBIL ($p = 0.011$), glucose ($p = 0.043$), urea ($p =< 0.001$), creatinine ($p = 0.050$) (Table 1).

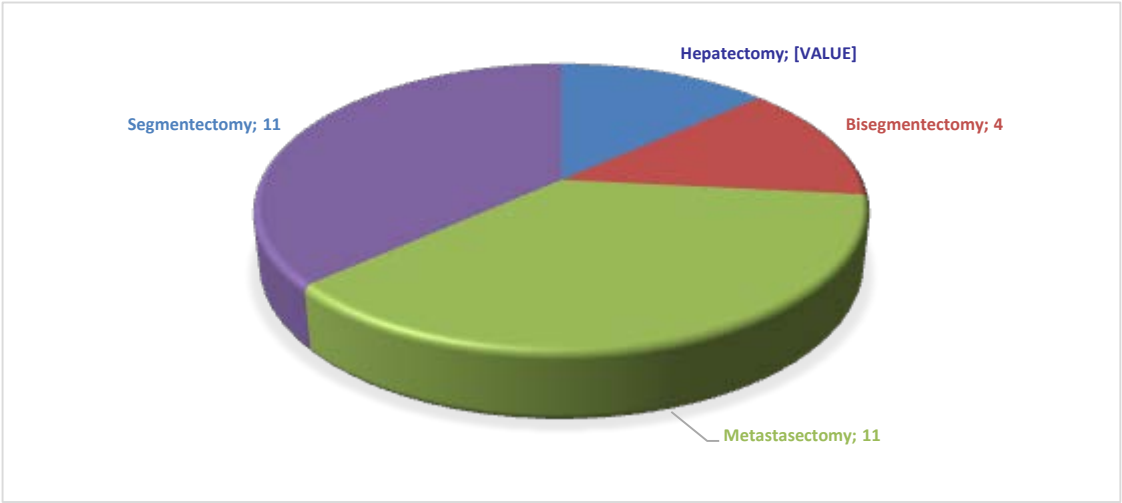

Figure 2. Distribution of surgical procedures by type of resection

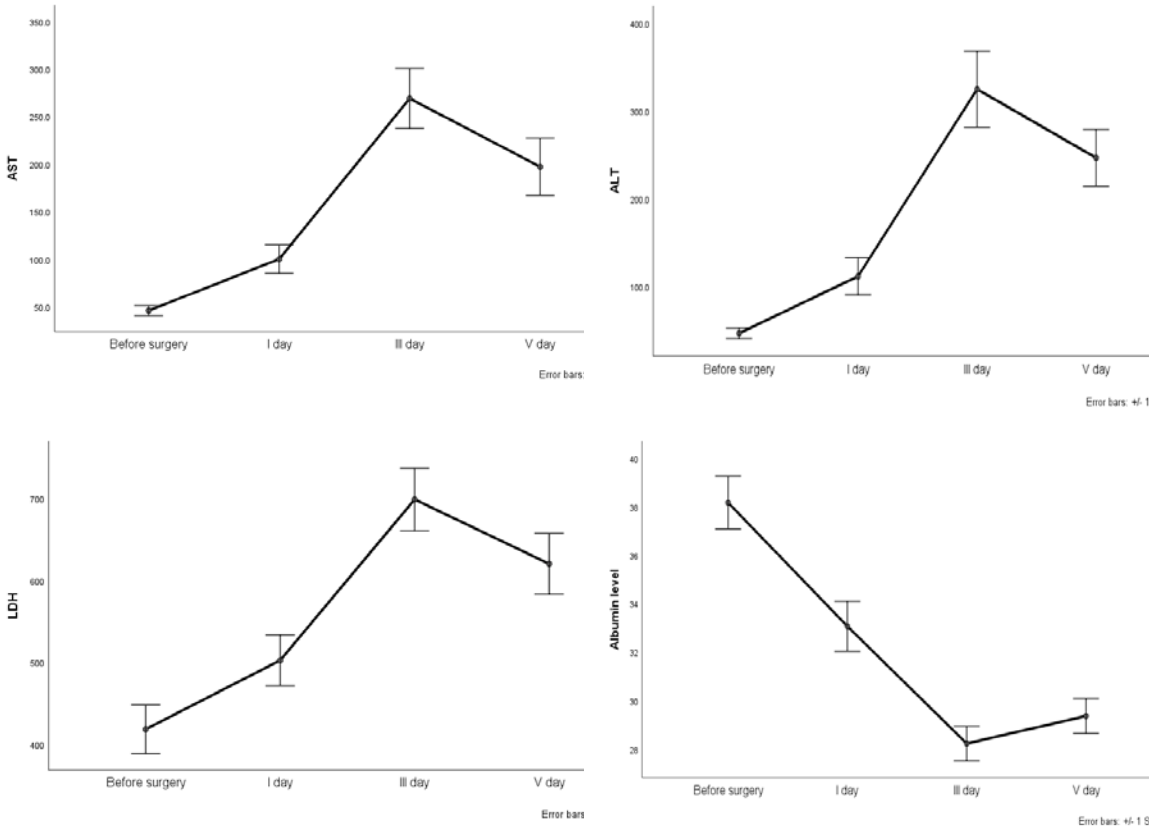

Figure 3. AST, ALT, LDH and albumin levels during the follow-up period

Table 1. Laboratory parameters during the follow-up period in the studied population

Parameter	Preoperative	Day I	Day III	Day V	p^1	$p^{Pre\ vs\ I}$	$p^{Pre\ vs\ III}$	$p^{Pre\ vs\ V}$
Alkaline Phosphatase	119.9 ± 66.15	170.23 ± 89.64	173.4 ± 57.44	123.87 ± 57.69	0.001	< 0.001	0.001	1.000
AST	45.96 ± 28.75	100.1 ± 81.87	269.07 ± 172.72	197.23 ± 164.32	< 0.001	0.005	< 0.001	< 0.001
ALT	47.08 ± 33.32	111.93 ± 116.29	324.73 ± 236.74	246.67 ± 177.64	< 0.001	0.021	< 0.001	< 0.001
γGT	44.53 ± 31.835	85.53 ± 56.201	73.6 ± 80.264	70.13 ± 71.345	0.023	< 0.001	0.174	0.196
TBIL	13.53 ± 9.79	18.59 ± 12.43	17.41 ± 8.15	14.82 ± 7.28	0.033	0.011	0.131	1.000
DBIL	3.93 ± 5.02	6.27 ± 5.63	5.68 ± 4.33	4.68 ± 3.44	0.130			
LDH	418.47 ± 163	502.57 ± 170	699.03 ± 208.51	620.43 ± 204.31	< 0.001	< 0.001	< 0.001	< 0.001
Albumin	38.17 ± 5.97	33.07 ± 5.65	28.23 ± 3.92	29.37 ± 3.93	< 0.001	< 0.001	< 0.001	< 0.001
Glucose	6.76 ± 3.18	7.65 ± 2.7	6.62 ± 2.87	6.52 ± 2.99	0.016	0.043	1.000	1.000
Urea	6.2 ± 2.21	8.18 ± 2.2	6.41 ± 3.31	6.21 ± 3.07	0.001	< 0.001	1.000	1.000

Creatinine	90.02 ± 13.77	105.72 ± 23.37	85.31 ± 18.49	82.97 ± 17.36	0.004	0.050	1.000	0.302
STA	131.07 ± 10.76	174.17 ± 241.17	130.8 ± 12.12	130.67 ± 12.21	0.324			
DTA	80.97 ± 5.288	80.63 ± 5.654	79.53 ± 5.244	79.87 ± 4.925	0.388			

1 ANOVA for repeated measures, *post hoc* p-values for pre vs. day 1, vs. day 3, vs. day 5

Discussion

Liver surgery has a relatively short history compared to other areas of surgery, which were developed and widely accepted many decades earlier around the world. Modern liver surgery dates back to the early 1950s, when, thanks to the joint efforts of surgeons and anatomists, the intrahepatic segmental anatomy of the liver was discovered. The full development of this surgical field became possible only in the last 40 years, thanks to the introduction of modern diagnostic methods, especially ultrasound and computerized tomography (2, 7, 8).

Along with the development of diagnostics, surgical techniques, anesthesia, preoperative preparation, and postoperative intensive care and nursing of these patients also evolved. Today, liver surgery is a safe area of surgery, with a relatively low overall mortality rate (below 5%). However, it is still associated with a relatively high risk of complications, which can reach up to 20%. The most dangerous complications are related to the development of postoperative liver failure (up to 5%) (2, 3). Many patients with hepatobiliary malignancies require large resectional procedures that leave a smaller portion of healthy tissue (8). Therefore, adequate preoperative qualitative analysis of liver function, along with quantitative volumetric tests, is crucial for the success of resection procedures.

Precise assessment of liver function and capacity involves the analysis of the following groups of parameters:

1. **General health of the patient:** The first and fundamental assessment is the general health status of the patient. This includes factors such as age, presence of other chronic diseases or comorbidities, and the ability of the patient to tolerate the surgery, which can be classified according to the ASA (American Society of Anesthesiologists physical status classification system) and Apache II (Acute Physiology and Chronic Health Evaluation II) scores (3, 9).

2. **Type of planned surgical procedure:** The size and type of liver resection also affect patient preparation. Larger resections may require additional preparation.

3. **Liver volumetry (CT/MRI):** Volumetry helps in the quantitative assessment of liver parts that will be removed and those that will remain after the surgery. The necessary minimal remaining liver volume after resection (Future Liver Remnant, FLR) should not be less than 30% for a healthy liver, and 40–50% for a liver affected by cirrhosis, fibrosis, severe steatosis, or damage caused by cytotoxic therapy (6).

4. **Assessment of liver functional capacity:** To assess liver function, biochemical parameters are used (alkaline phosphatase enzyme activity, AST, ALT, γGT, bilirubin—TBIL and DBIL, LDH, albumin) and parameters of metabolic syndrome (glucose, urea, creatinine), which, in combination with clinical findings, can be categorized into more precise scores, such as the Child-Pugh, MELD (Model for End-Stage Liver Disease), ALBI score, APRI score, FIB-4 score, and the LIMON score (a non-invasive monitoring system measuring the elimination of indocyanine green ICG) (10).

The Child–Pugh classification is the easiest and most common method for quantitatively determining the degree of liver insufficiency based on basic clinical and biochemical parameters. By combining indicators of liver excretory and synthetic function and the presence of portal hypertension, this scoring system provides an accurate picture of the overall functional state of the liver. It includes five elements (serum levels of bilirubin and albumin, presence of ascites and encephalopathy, and prothrombin index), based on which patients are categorized into one of three stages: A, B, and C. Patients in stage A have less than 6 points, in stage B from 6 to 9 points, and in stage C more than 9 points (Tabele 2) (6, 8).

Patients with Child A liver insufficiency are considered suitable candidates for resection procedures (postoperative mortality risk of only 1–2%), while patients with Child B are only suitable for limited resections (postoperative mortality risk of 10%). In patients with Child C stage, resection is contraindicated (postoperative mortality risk over 50%) (8).

Table 2. Child–Pugh Classification—Modification by J. M. Henderson (1994)

Points	Bilirubin (µmol/L)	Albumin (g/L)	Prothrombin Index - Quick	Encephalopathy	Ascites
1	< 20	> 35	> 70%	0	0
2	20–30	28–35	40–70%	I–II degree	Small
3	> 30	< 28	< 40%	III–IV degree	Large

One of the functional tests is the retention of Indocyanine green (ICG). Based on clinical experience during the 1980s and early 1990s, an ICG-R value below 10% was considered a safety limit for performing larger hepatic resections in cirrhotic patients. However, with advancements in surgical techniques and perioperative care, this upper safety limit was first extended to 14%, and later to 20% for major hepatectomies. The MELD score, based on values of bilirubin, INR, and creatinine, is commonly used to predict three-month mortality in patients with bleeding esophageal varices. Its values range from 6 (for healthy individuals) to 40 (for terminal liver insufficiency). Today, this score is predominantly used to determine priority for liver transplantation, which is carried out within the MELD score range of 10–20 (8).

The surgery itself causes varying degrees of liver damage. The pathophysiological mechanisms contributing to postoperative liver dysfunction include surgical stress, hemodynamic changes induced by anesthesia, intraoperative blood loss, ischemia-reperfusion syndrome, oxidative stress, and apoptosis of hepatocytes (7). Surgical stress triggers the activation of the hypothalamic-pituitary-adrenal axis, resulting in increased secretion of cortisol and catecholamines. This response contributes to a systemic inflammatory response with enhanced release of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP). These changes can further worsen hepatocellular dysfunction and contribute to postoperative complications. The pharmacodynamic effects of anesthetics significantly affect hepatic perfusion. Volatile anesthetics, such as isoflurane and sevoflurane, can reduce blood flow through the liver, while intravenous anesthetics, such as propofol and remifentanil, can lead to systemic hypotension, further compromising hepatocyte oxygenation (7–11). Intraoperative bleeding can result in hypovolemia and compromised liver perfusion, increasing the risk of ischemia-reperfusion injury. Clamping of the vascular inflow vessels—the Pringle maneuver—can further worsen liver function. Additionally, massive blood transfusions can lead to the development of disseminated intravascular coagulation (DIC) and microthrombosis in intrahepatic sinusoids, further impairing liver function (12–14). The ischemia-reperfusion syndrome arises from the temporary interruption of blood flow through the liver during vascular manipulation. The ischemic phase results in reduced aerobic metabolism and the accumulation of lactate in hepatocytes, while reperfusion induces a sudden increase in reactive oxygen species (ROS), leading to oxidative damage to cell membranes, proteins, and mitochondrial DNA (15, 16). Oxidative stress caused by increased ROS production, including superoxide and hydroxyl radicals, triggers lipid peroxidation and hepatocyte damage. The depletion of antioxidant mechanisms, including superoxide dismutase (SOD), catalase, and glutathione, further contributes to cytotoxicity and liver dysfunction. Apoptotic processes in hepatocytes are initiated by the activation of the mitochondrial (intrinsic) and receptor-mediated (extrinsic) pathways of cell death, thereby increasing the risk of postoperative insufficiency (2, 9, 10).

Postoperative recovery of liver function begins on the third postoperative day and typically normalizes by the tenth day following surgery. Balzan and colleagues demonstrated the significance of the "50-50 criteria," which are based on a combination of elevated serum bilirubin levels (greater than 50 µmol/l or 2.9 mg/dl) and a reduced prothrombin index (less than 50 percent) on the fifth postoperative day (12, 17). This study included 30 patients, 16 men and 14 women, with an average age of 60 years. Data analysis did not reveal a significant correlation between postoperative liver function parameters and demographic characteristics such as gender and age.

The biochemical parameter analysis in our study showed significant changes in liver function following resection. Specifically, AST and ALT values increased significantly after surgery compared to the preoperative period ($p < 0.001$), indicating hepatocyte damage due to the surgical intervention. Additionally, there was a significant increase in DBIL ($p = 0.017$) and LDH ($p < 0.001$), which may suggest liver dysfunction and potential hemolysis. In contrast, albumin levels were significantly lower postoperatively ($p < 0.001$), which may reflect decreased synthetic liver function or increased protein catabolism during the postoperative period. The increase in DBIL ($p = 0.017$) may indicate temporary bile duct obstruction or hepatocellular dysfunction.

Increased plasma levels of AST and ALT following liver resection represent significant clinical indicators used to assess liver function and postoperative recovery. These changes in enzyme

levels may result from various factors, including the type of resection, the amount of blood loss, the duration of surgery, as well as individual patient characteristics. Different types of liver resections have different effects on AST and ALT levels. Research shows that larger resections, such as hemihepatectomy or total hepatectomy, lead to a more significant increase in these enzymes due to greater trauma and hepatocyte damage. The authors noted that transaminase levels peaked within the first 24 hours after surgery and gradually returned to normal over the next five days. Elevated AST and ALT levels were associated with longer surgery duration and larger resections, suggesting that greater surgical trauma leads to more extensive liver tissue damage (18).

These findings are consistent with previous research. Studies show that liver resection often leads to a transient increase in aminotransferases and bilirubin, while decreased albumin levels may be a result of surgical stress and reduced liver reserve function. The increase in LDH further confirms postoperative stress and possible liver cell damage. Monitoring these parameters is crucial for the early detection of complications and optimal management of postoperative recovery in patients (12).

Conclusion

Standard liver function tests remain essential for assessing its functional state and regenerative capacity. In our study, significant changes were identified in biochemical parameters before and after surgery. Surgical stress, anesthetics, bleeding, and ischemia-reperfusion injuries through subtle mechanisms of oxidative stress and apoptosis lead to transient liver damage, while regeneration, starting on the fifth postoperative day, shows a positive trend. The combination of standard biochemical tests with dynamic liver function tests and volumetric studies can be highly useful in distinguishing patients at high risk of complications in liver surgery. In any case, this area of research requires further clinical investigations.

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PREOPERATIVNA I POSTOPERATIVNA ANALIZA FUNKCIJE JETRE NAKON RESEKCIJE

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Lekari koji se bave hirurgijom jetre ističu važnost temeljne preoperativne procene funkcionalnosti jetre i njenih rezervi, koja se sprovodi da bi se minimalizovao rizik od nastanka komplikacija u toku operacije, naročito kod bolesnika sa cirozom jetre, žuticom ili kod bolesnika koji su dugo na hemoterapiji.

U istraživanju je učestvovalo trideset bolesnika kojima je urađena hepatektomija zbog postojanja tumora, uključujući metastaze kolorektalnog karcinoma. Parametri su analizirani u dvema fazama: pre operacije i posle operacije. Praćeno je stanje parenhima jetre na osnovu određenih biohemijskih parametara funkcije jetre (aktivnost enzima alkalne fosfataze, aspartat aminotransaminaze (AST), alanin aminotransferaze (ALT), gama-glutamil transferaze (γGT), kao i bilirubina – ukupnog bilirubina (engl. *total bilirubin* – TBIL) i direktnog bilirubina (engl. *direct bilirubin* – DBIL) – laktat dehidrogenaze (LDH) i albumina) i parametara metaboličkog sindroma (glukoza, urea, kreatinin, krvni pritisak) preoperativno i postoperativno.

Ovo istraživanje je pružilo uvid u karakteristike oštećenja tkiva jetre nakon resekcije jetre, do kojeg se došlo na osnovu parametara za praćenje funkcije jetre. Budući da su zasnovani na primeni savremenih metoda i istraživanju odgovarajuće literature, ovi rezultati mogu pomoći u daljem unapređenju terapijskih postupaka i strategija za postoperativnu negu, s obzirom na to da pružaju korisne smernice za poboljšanje ishoda lečenja.

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***Ključne reči**: hirurgija jetre, resekcija jetre, funkcija jetre*

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CHANGING TRENDS IN THE DIAGNOSIS AND TREATMENT OF LIVER HYDATIDOSIS OVER A 60-YEAR PERIOD: EXPERIENCE OF A TERTIARY REFERRAL CENTER IN AN EUROPEAN ENDEMIC REGION

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Serbia is a well-known endemic region for hydatid liver (LH) disease. Although surgery remains the primary treatment modality, significant changes have occurred in the diagnosis and treatment of this disease in recent years. The aim of this study was to retrospectively analyze the demographic and clinical characteristics of patients who had undergone surgical treatment for LH at a tertiary referral center over the past 60 years. The authors conducted a comparative analysis across three 20-year periods: period I (1960–1980), period II (1980–2000), and period III (2000–2020). The ratio of surgeries performed due to LH in the last period (1.23 ‰) was significantly lower than in the first two periods (5.15 ‰ and 4.86 ‰, respectively). A higher incidence in females (1:2.2), cyst localization, and rate of complications have remained consistent over time. The latest standard diagnostic procedures include Ultrasonography (US), Computed tomography (CT), Enzyme-linked immunosorbent assay (ELISA) and Indirect hemagglutination assay (IHA) test. While the management of LH shifts towards less invasive procedures, open surgery remains the gold standard. The tissue-sparing operations were performed in most cases (61.91%). However, there has been a slight increase in the radical surgeries, rising from 25.4% in the first period to 43.15% in the second and 46% in the third period. The surgical approach by Papadimitriou—partial cystopericystectomy plus omentoplasty (PCPCO)—may be the preferred method as it balances the need for radical treatment with tissue preservation in LH surgery. Minimally invasive techniques such as punation aspiration irrigation and respiration (PAIR) and laparoscopy have gradually been introduced over the last two periods, in a small number of carefully selected cases (increasing from 3.4% to 8.1%, respectively).

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Key words: *echinococcosis, hydatid disease, liver, diagnosis, therapy*

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Introduction

Hydatid disease is a worldwide zoonosis produced by the larval stage of the *Echinococcus* tapeworm, with endemic prevalence in the great grazing regions. Serbia has been a very well-known endemic region for decades, with an average annual incidence of 0.32/100,000 inhabitants (1). Over 75% of hydatid diseases are situated in the liver and are almost exclusively treated with surgery (2). Although there has been an increase in early disease detection due to new diagnostic methods, the incidence of liver hydatid disease has slightly decreased, reflecting changing trends both globally and in our region (1). Although surgery still remains the primary treatment modality, in recent times, there have been notable changes in the diagnosis and treatment of this disease (3–5).

Aim

This study aimed to retrospectively analyze the main demographic and clinical characteristics of the patients surgically treated for liver

hydatidosis (LH) in a tertiary referral institution (University Clinical Center Niš) in the period of 60 years between 1960 and 2020. The authors used comparative analysis for the three 20–year periods (I period from 1960 to 1980, II period from 1980 to 2000, and III period from 2000 to 2020).

Materials and Methods

Over the past 60 years, 323 adult patients have been surgically treated for liver hydatidosis. One hundred and three patients underwent surgery in the first 20 years (1960–1980); 146 within the second period (1980–2000); and 74 patients were invasively treated in the third period (2000–2020)

Results

The number of patients who underwent surgery for LH at the University Clinical Center in Niš dramatically decreased in relation to the total operations performed, from 5.15‰ to 4.86‰ and 1.23‰ in observed periods, respectively. The male-to-female ratio was 1:2.2, with the patient's age being 44.2 years, similar in all observed periods. Cysts were solitary in ¾ of cases and located mainly in the right lobe (66.6%). Bilobar localization was registered in 12.4%. So-called "complex" cysts, hylar, and caval hepatic venous encasement or infiltration, were registered in 20 patients (6.2%), but the incidence was significantly higher in the last observed period (9.5% versus 5.2%). The complication rate was almost similar in both groups (17.4% in total), with a slight increase in preoperatively discovered cysto-biliary fistulas in the last period (10.8% versus 6.4% in the former periods) (Table 1).

Table 1. Demographic and clinical characteristics of patients treated for LH

	I period 1960–1980	II period 1980–2000	III period 2000–2020	Total
Number	103	146	74	323
% of total N° of OP performed in 20 yrs.	103/20.000 (5.15‰)	146/30.000 (4.86‰)	74/60.000 (1.23‰)	
Age	44.1		44.3	44.2
Male/female (1:x)	72/177 (1/2.45)		28/46 (1/1.65)	100/223 (1:2.23)
Number				
Solitary	190/249 (76.3%)		60/74 (81.1%)	250/323 (77.39%)
Multiple	59/249 (23.7%)		14/74 (18.9%)	73/323 (22.6%)
Localisation				
Right lobe	167/249 (67.1%)		48/74 (64.9%)	215/323 (66.6%)
Left lobe	52/249 (20.1%)		16/74 (21.6%)	68/323 (21%)
Bilobar	30/249 (12.8%)		10/74 (13.5%)	40/323 (12.4%)
Complex (problematic)	13/249 (5.2%)		7/74 (9.5%)	20/323 (6.2%)
Complicated	42 (16.9%)		14/74 (18.9%)	56/323 (17.4%)
Cysto-biliary communication (CBC)	16 (6.4%)		8 (10.8%)	24/323 (7.4%)
Abscesses	6 (2.4%)		2 (2.5%)	8/323 (2.5%)
Perforation	20 (8%)		4 (5.4%)	24/323 (7.4%)

Table 2. Therapeutic approach in LH

	I period	II period	III period	TOTAL
	1960–1980	1980–2000	2000–2020	
Percutaneous	0	3 (2%)	8 (10.8%)	11 (3.4%)
Laparoscopic	0	5 (3.4%)	6 (8.1%)	11 (3.4%)
Open	103	138 (94.5%)	60 (81%)	301 (93.2%)
TOTAL	103	146	74	323 (100%)

Table 3. Tissue sparing (conservative) and radical operations in LH

	I period	II period	III period	TOTAL
	1960–1980	1980–2000	2000–2020	
TISSUE-SPARING				
Papadimitriou (PCPCO)	39 (37.86%)	68 (46.57%)	26 (35.13%)	133/323 (41.17%)
Drainage only	24 (23.3%)	10(6.84%)	14 (18.91%)	48 (14.86%)
Capitonnage	8 (7.76%)	-	-	8 (2.47)
Marsupialization	6 (5.82)	5 (3.42%)	-	11 (3.4%)
TOTAL	77 /103 (74.75%)	83/146 (56.84%)	40/74 (59.45%)	200/323 (61.91%)
RADICAL				
Nonanatomical LR (total pericystectomy)	17 (16.50%)	48 (32.87%)	16 (21.62%)	81 (25.07%)
Anatomical LR	9 (8.73%)	15 (10.27%)	18 (24.32%)	42 (13%)
TOTAL	26/103 (25.24%)	63/146 (43.15%)	34/74 (46%)	123/323 (38.08%)

The main diagnostic procedures in the first 20-year period were clinical data, plain radiography, scintigraphy, and basic laboratory (eosinophillia and Casoni–Botteri reaction). The most common clinical symptoms and signs of the disease were pain in the right hypochondrium and hepatomegaly. In the first period imaging method of choice was scintigraphy. However, it is replaced by more simple and precise methods like ultrasound (US), computerized tomography (CT) and magnetic resonance (MR) during the early 1980s of the 20th century. MR and MRCP were selectively used in 18.57% (60 cases). The Indirect hemagglutination test (IH) and the Enzyme-linked immunosorbent assay (ELISA) were the initial screening tests of choice. Standard preoperative antihelmintic regimen was done with one or more cycles of Albendazole (10 mg/kg BM for 4 weeks), after its discovery in 1975, and Mebendazole during the first observed period. All patients underwent some form of invasive procedure. In the first and second observed periods, open surgery was the only way to operate on liver hydatidosis. Percutaneous and laparoscopic approaches were gradually introduced in the last two 20-year periods, in a small number of strongly selected cases, with increasing rate from 2% to 10.8% and 3.4% to 8.1%, respectively (Table 2). Tissue-sparing or conservative surgical operations were performed in most cases (61.91%). However, we noticed a slight increase in radical surgery (from 25.4% in the first to 43.15% in the second and 46% in the third period). Operations according to Papadimitriou (partial cystopericystectomy plus omentoplasty—PCPCO) were most frequent (133 cases or 41.17% in total). Capitonnage and marsupialisation were abandoned in the last

period of time. Increasing tendency of anatomical liver resection from 8.73% to 10.27% and 24.32% during the three observed time periods was registered (Table 3).

The rate of true relapses is very rare, and in our material it amounted to below 5%. The cumulative complication rate at the University Clinical Center in Niš was 17.31%, showing a decreasing trend from 19.42% in the first period to 17.80% in the second, and finally to 13.51% in the third observed period. The average length of hospitalization was the shortest in the third period (4.6 days) compared to the first (20 days) and second (12.4 days) periods. The mortality rate in the first and second periods was 3.21% (equivalent to eight patients) (Table 4).

Table 4. Results of surgical treatment of LH by the observed period

	I period 1960–1980	II period 1980–2000	III period 2000–2020	Total
Biliary fistula	8/103 (7.76%)	7/146 (4.79%)	3/74 (4.05%)	18/323 (5.57%)
Abscesses	7/103 (6.82%)	15/146 (10.27%)	3/74 (4.05%)	25/323 (7.73%)
Pulmonary	5/103 (4.85%)	4/146 (2.73%)	4/74 (5.4%)	13/323 (4.02%)
Total	20 (19.42%)	26 (17.80)	10 (13.51%)	56 (17.3%)
Hospitalization (days)	20	12.4	4.6	
Mortality	8 (3.21%)		-	8/323 (2.47%)
Recurrence	4 (1.60%)		4 (5.40%)	8/323 (2.47%)

Discussion

Hydatid disease is a worldwide zoonosis produced by the larval stage of the *Echinococcus* tapeworm. The two main types of hydatid disease are caused mostly by *E. granulosus* and less often by *E. multilocularis* (2). According to World Health Organization (WHO), the incidence of hydatid disease has an almost unbelievable range from 1 to 200 cases per 100,000 population (2). *E. granulosus* is an endemic disease in great grazing regions like the Mediterranean region, Africa, South America, the Middle East, Australia, and New Zealand. Previously rare, today it is increasingly common in the countries of Western Europe and North America due to the large influx of emigrants, who bring the disease with them. Hydatid disease has been practically eradicated in some countries, thanks to programs to combat this disease, which include systematic anthelmintic vaccination of dogs, pigs and sheep. Based on that program, new cases of hydatid disease have not been registered in Norway since 1982 (2), and incidence has also significantly dropped in some island countries (as epidemiologically closed systems) like Iceland, South Cyprus, part of Argentina and Chile, Tasmania and New Zealand (6). In Serbia, the most frequent intermediate hosts for *E. granulosus* are pigs, with a percentage of infected animals ranging between 4.6% and 57.6% (7). Exact information about the real incidence of human infection in Serbia is uncertain and underestimated due to incomplete and inadequate reporting by clinicians. In 2023, 21 new cases of echinococcosis were reported in the Republic of Serbia, with an annual incidence rate of 0.32 per 100,000 inhabitants. There is a large difference in reporting by gender and region. The highest cumulative incidence rates of echinococcosis were registered in the territory of Zlatibor, Toplica, and Rasina districts (Figure 1). According to the statistical data of the Institute of Public Health in Niš, the average level of cumulative incidence of echinococcosis in the Southern and Eastern Serbia for the period 1988–2001 was 5.83, and 4.028 for the period 2002–2006 (8).

Over the last ten-year period, echinococcosis in Serbia has shown a downward trend in the number of cases, with the highest incidence rate registered in 2017, and the lowest rates recorded in 2020 and 2021, during the period of the COVID-19 pandemic (1) (Figure 2).

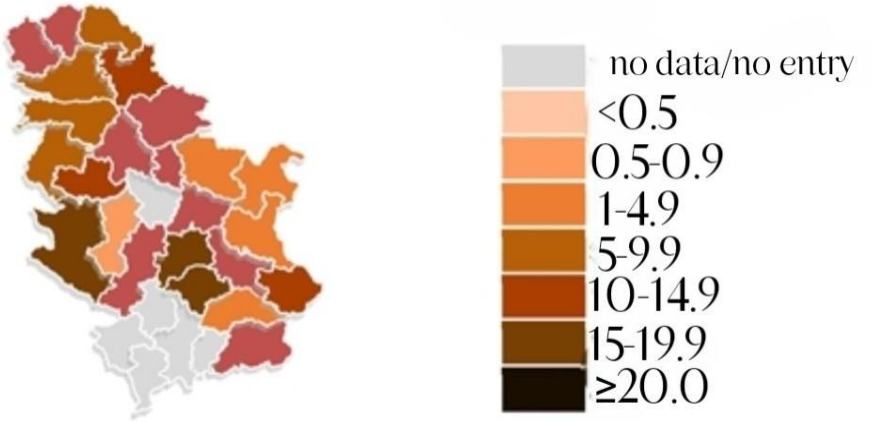

Figure 1. Cumulative incidence of echinococcosis/100,000 inhabitants in Serbia in 2014–2023 period

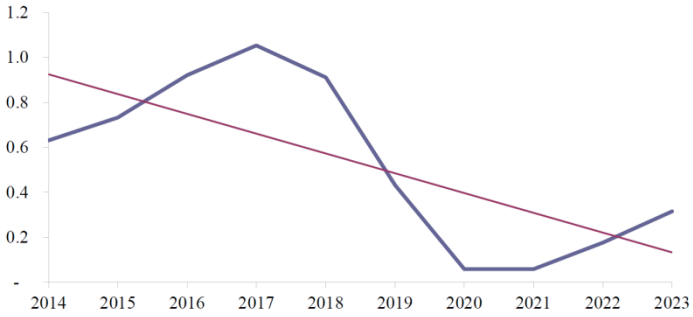

Figure 2. Incidence trend of echinococcosis/100,000 inhabitants in Serbia in 2014–2023 period

University clinical Center in Niš is a tertiary referral health institution covering an area of 2.5 million inhabitants in the middle of an endemic region. Although the total number of surgeries performed in the last period was significantly higher than in the first two periods, the ratio of surgeries performed due to LH (1.23 ‰) was significantly lower than in the first two periods (5.15 ‰ and 4.86 ‰, respectively). The male-to-female ratio was 1:2.2, with the patient's age 44.2 years, similar in all observed periods. Over 75% of hydatid diseases are situated in the liver—liver hydatidosis. The most common localisation in the liver is the right lobe, probably due to its larger size and portal flow (2). Our results are similar, with exactly 2/3 of cysts located in the right lobe. Other common localisation are the lungs with about 20%. Hematogenous dissemination after passing through the hepatic and pulmonary filters is very rare, with a relatively small number of cases reported in the brain, heart, bones, joints, pericardium, and pelvis (9).

Hydatid cysts can often be asymptomatic for many years and are sometimes discovered incidentally during imaging studies (10, 11). In most cases, symptoms are associated with the results of LH complications, which occurred in 17.4% in our study. The most frequently registered complication was inflammation in 7.4% and rupture into the bile ducts with cysto-biliary communication (CBC or fistula) formation 7.4%, abdominal or chest cavity (7.4%). Some complications can even lead to fatal outcomes, for example, anaphylaxis after a cyst's perforation, which we did not note. During the first twenty-year period (1960–1979), the diagnosis of LH was determined by numerous complicated procedures with low sensitivity and specificity (native radiography: angiography, splenoportography, intravenous cholangiography, scintigraphy, Casoni–Botteri test, etc.). Ultrasound and computerized tomography were introduced in clinical practice at our institution during the early 1980s of the 20th century, and were used in the diagnosis of LH in almost the patients. CT examination with high sensitivity rate of 95%, represents the method of choice used in preoperative planning. It enables a precise anatomical picture of the liver, position of the cyst(s), and relation to the great vessels and bile ducts (2, 11) (Figure 3). Magnetic resonance and magnetic resonance cholangio-pancreatography at our institution were selectively used in suspected cystobiliary communication (18.57% of the

patients) (11, 12). Routine blood tests may show non-specific changes. Eosinophilia could be noted in only 25–40% of cases (2, 11). Serological tests include enzyme-linked immunosorbent assay, indirect hemagglutination assay (IHA) and Western blotting (WB). ELISA is the method of choice with a sensitivity of 93.5% and specificity of 89.7%. IHA testing has a sensitivity of 90%; however, if the result is positive, it may remain positive for several years after that (2, 3). WB serology for liver CE has a high sensitivity of 80–100% and a specificity of 88–96% (13, 14).

There are various treatment modalities for this disease. Medical therapy with antihelmintic agents by itself is indicated in cases where surgical intervention is not possible for any reason, after multiple relapses and in the alveolar form of the disease. The most used drug is Albendazole (10 mg/kg daily for 4 weeks) as a neoadjuvant or adjuvant therapy in combination with interventional and surgical procedures (2, 12). However, long-term and non-critical use of Albendazole can lead to liver fibrosis and cirrhosis, as seen in our patients after two years of non-critical continuous use.

Despite advancements in effective medications for treating parasites, surgery remains the preferred method for addressing liver hydatid disease. The main goal of surgical intervention is to remove the cyst and its contents while preventing contamination of the peritoneal cavity (2, 5). Although the concept of management of liver hydatidosis is changing and going to less invasive procedures, open surgery is still the gold standard for complete cure in the complicated cases (15, 16). Surgical techniques can vary, ranging from liver-sparing methods like endocystectomy to more aggressive approaches, including partial or total pericystectomy and various types of hepatectomy (17) (Figure 4).

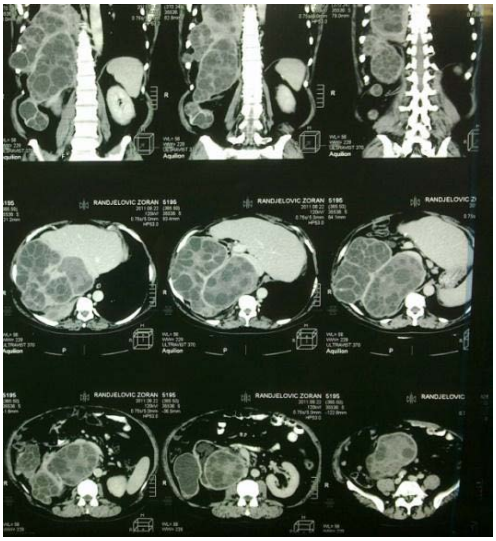

Figure 3. MSCT of giant LH.

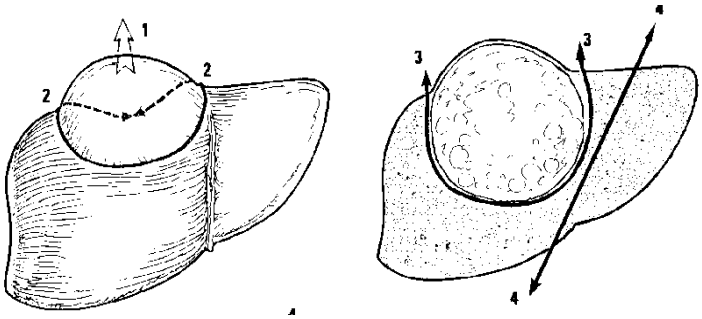

Figure 4. Different types of surgical interventions:
1. Drainage, 2. Partial pericystectomy, 3. Total pericystectomy, 4. Liver resection

When deciding on the most appropriate surgical procedure, several factors must be considered: the location and size of the cyst, its proximity to vascular and biliary structures, and the characteristics of the pericyst (including thickness and infiltration) (2). Since liver echinococcosis is classified as a benign condition, it is vital to minimize the surgical risks to patients. Because of that primary idea, tissue-sparing or conservative surgical operations were suggested for decades (17). We performed these in most cases (61.91%). However, we noticed a slight increase in the number of radical surgeries (from 25.4% in the first to 43.15% in the second and 46% in the third period). Capitonnage and marsupialization were abandoned in the last period. The introduction of a new concept in liver surgery over the past 25 years has led to an increase in anatomical liver resections, rising from 8.73% to 10.27% and then to 24.32% across three observed time periods. Immediate and late results of open surgery are very good. Free of recurrence rate after 1, 5 and 10 years was 100%, 90.9% and 87.9%, respectively (10, 12, 18). The rate of true relapses is very rare and in our material, it amounts to below 5%. However, open surgery is associated with significant morbidity (15–25%) and mortality rate (up to 6.5%) and long hospital stay (10). The relationship between surgical techniques and outcomes is notable: the more radical the surgery, the higher the operative risk, but the lower the risk of recurrence, and vice versa. Radical surgery is considered superior to conservative surgery due to lower morbidity (3–24% compared to 11–25%), lower mortality (1–1.8% compared to 2–5%), and lower recurrence rates (2–6.4% compared to 10.4–40%) as reported by specialized liver centers (19). The cumulative complication rate at our institution was 17.31%, showing a decreasing trend from 19.42% in the first period to 17.80% in the second, and finally to 13.51% in the third observed period. The introduction of new concepts in liver surgery and minimally invasive methods has reduced the average length of hospitalization (11). It was the shortest in the third period (4.6 days) compared to the first (20 days) and second periods (12.4 days). The mortality rate in the first and second period was 3.21% (eight patients). The causes of death were as follows: uncontrolled abdominal sepsis in five patients, cardiopulmonary insufficiency in one patient, hepato-renal insufficiency in another patient. Additionally, one patient died on the operating table due to bleeding from a retrohepatic lesion of the inferior vena cava. No deaths were registered in the third observed period.

Every day, surgeries are performed by general surgeons in underdeveloped countries, where the philosophy is that “benign disease needs benign therapy” (17, 19). Operation according to Papadimitriou (partial cystopericystectomy plus omentoplasty—PCPCO) could be the operation of choice that encompasses both concepts of radicality and sparing in LH surgery. It was the most frequent operation on our material as well (41.17%).

Newer minimally invasive methods of treatment, such as laparoscopic and robotic surgery, have the advantage of less morbidity, lower cost, and shorter hospital stay (20). However, laparoscopy for liver hydatidosis could be a very complex and challenging procedure in the cases of centrally located disease and suspected biliary complications (20–22). Laparoscopic surgery of the LH was performed in the last two periods in 11 cases of peripherally located and solitary, non-complex cysts (3.4%). There were no conversions to open surgery nor any complications. Nonoperative, percutaneous treatment of liver hydatidosis consists of punction, aspiration, irrigation and reaspiration (PAIR) (Figure 5). The most commonly used scolicidal reagents are hypertonic (20–30%) NaCl solution, povidone-iodine and 95% ethanol. It was introduced in the mid-1980s. In this treatment modality, the aim is to destroy the germinal layer using scolicidal agents or to evacuate the entire endocyst.

According to the current guidelines, the best results with PAIR achieved in > 5 cm CE1 (unilocular) and CE3a cysts (with detached membrane) (Table 5). Early pregnancy, lung cysts, superficially localised cysts, and cysts that communicate with the biliary tree are also contraindicated for PAIR. Percutaneous cyst drainage is an effective and safe procedure, with a low complication rate (13, 23, 24). At the University Clinical Center in Niš, PAIR has been gradually introduced in the last two 20-year periods, in a small number of strongly selected cases (from 3.4% to 8.1%, respectively). There was only one severe complication—obstructive jaundice caused by necrotic debris one week after PAIR. It was successfully treated by open surgery.

Recurrence after invasive therapy for liver hydatid disease ranges from 4.6% to 22.0% (25, 26). In present study, the rate of true relapses is very rare, accounting for less than 5%, regardless of the type of intervention used. The main reasons for recurrence appear to be the microscopic spillage of live parasites, failure to remove all viable cysts from inaccessible or difficult locations, and leaving behind a residual cyst wall after the initial operation.

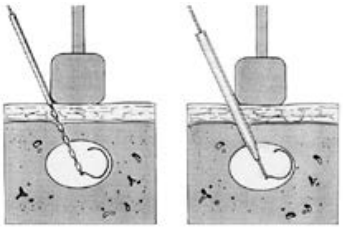

Figure 5. PAIR procedure

Table 5. Comparative description of the WHO-IWGE and Gharbi ultrasound classifications of echinococcal cysts

WHO-IWGE	Gharbi	Description	Stage	PAIR
CE1	Type I	Unilocular unechoic cystic lesion with double line sign.	Active	Indicated
CE2	Type III	Multiseptated "rosette-like" "honeycomb" cyst	Active	Contraindicated
CE3 A	Type II	Cyst with detached membranes [water-lily-sign]	Transitional	Indicated
CE3 B	Type III	Cyst with daughter cysts in a solid matrix	Transitional	Contraindicated
CE4	Type IV	Cyst with heterogenous contents No daughter cysts	Inactive	Contraindicated
CE5	Type V	Solid cyst with calcified wall	Inactive	Contraindicated

Conclusion

Based on the present research, it can be concluded that the incidence of liver echinococcosis is decreasing in the area covered by the University Clinical Center in Niš. In the third period, the ratio of surgeries performed for LH (1.23 ‰) was significantly lower than in the first two periods (5.15 ‰ and 4.86 ‰ respectively). Higher incidence in females (1:2.2), predominant cyst localisation in the right lobe and the rate of complications have been consistent over time. Standard diagnostic procedures employed during the last two periods include US, CT, ELISA, and IH. Although the management of liver hydatidosis is shifting towards less invasive procedures, open surgery remains the gold standard for achieving a complete cure in complicated cases. Laparoscopic techniques and PAIR treatment should be utilized more frequently for treating LH, but only in carefully selected cases. The surgical approach described by Papadimitriou, which involves partial cystopericystectomy plus omentoplasty, could be the preferred method as it balances both radicality and tissue preservation in LH surgery.

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PROMENA STAVOVA U DIJAGNOSTICI I LEČENJU EHINOKOKUSA JETRE U ŠEZDESETOGODIŠNJEM PERIODU: ISKUSTVO TERCIJARNE INSTITUCIJE U ENDEMSKOM PODRUČJU U EVROPI

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Srbija je dobro poznat endemski region kada je reč o hidatidnoj bolesti jetre. Premda hirurgija ostaje primarni modalitet lečenja, došlo je do značajnih promena u dijagnostici i lečenju ove bolesti u poslednje vreme. Cilj ovog istraživanja bio je da retrospektivno analizira demografske i kliničke karakteristike pacijenata koji su podvrgnuti hirurškom lečenju hidatidne bolesti jetre u tercijarnoj referentnoj ustanovi (Univerzitetskom kliničkom centru u Nišu) u poslednjih šezdeset godina (1960–2020). Izvršena je komparativna analiza triju perioda od dvadeset godina: perioda I (1960–1980), perioda II (1980–2000) i perioda III (2000–2020). U poređenju s ukupnim brojem operacija, procenat operacija urađenih zbog hidatidne bolesti jetre bio je značajno manji u poslednjem periodu (1,23‰) nego u prvom i drugom periodu (5,15‰, odnosno 4,86‰). Pokazalo se i da je incidencija veća kod žena (1 : 2,2). Lokalizacija cisti i procenat uočenih komplikacija nisu se menjali s vremenom. Standardne dijagnostičke procedure u novije vreme obuhvataju ultrazvuk (engl. *ultrasound* – US), kompjuterizovanu tomografiju (engl. *computed tomography* – CT), enzimski imunosorbentni test (engl. *enzyme-linked immunorbent assay* – ELISA) i imunohemaglutinaciju (engl. *indirect hemagglutination* – IHA). Iako se u lečenju hidatidne bolesti jetre sve više primenjuju minimalno invazivne procedure, otvorena operacija ostaje zlatni standard za postizanje potpunog izlečenja, posebno u komplikovanim slučajevima. Ispostavilo se da su u celokupnom posmatranom periodu u najvećem broju slučajeva (61,91%) rađene poštedne hirurške intervencije. Međutim, registrovano je blago povećanje broja radikalnih operacija – sa 25,4% u prvom periodu na 43,15% u drugom i 46% u trećem periodu. Prema mišljenju autora, metodu izbora predstavlja operacija po Papadimitriouu – parcijalna pericistektomija sa omentoplastikom – budući da se njome postiže balans između potrebe za radikalnošću i principa poštednosti tkiva jetre. Minimalno invazivne procedure poput perkutanih drenaža i laparoskopije postepeno su se uvodile u pažljivo odabranim slučajevima u poslednjim dvama analiziranim periodima; primećeno je da se njihov broj povećao sa 3,4% na 8,1%.

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***Ključne reči**: ehinokokus, hidatidna bolest, jetra, dijagnoza, terapija*

Original article

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IMPACT OF *ABCG2 421 C>A AND SLCO1B1 521T>C* GENE POLYMORPHISM ON THE CONTROL OF LIPID STATUS IN PATIENTS ON ATORVASTATIN AND ROSUVASTATIN TREATMENT

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Single nucleotide polymorphisms (SNPs) of SLCO1B1 gene (521T>C), encoding OATP1B1 transporter, and ABCG2 gene (421C>A), encoding BCRP transporter, may have impact on statin metabolism, consequently affecting their pharmacodynamic effects. This study aimed to examine the association between transporter gene polymorphisms and lipid status control in relation to the doses of statin administered. In addition, the serum activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were compared among carriers of different transporter genotypes. This cross-sectional pharmacogenetic study enrolled 102 patients with dyslipidemia who had been on atorvastatin or rosuvastatin treatment for more than 4 weeks. The values of lipid status parameters were collected from routine patient check-ups, and the transporter SNP was determined using the real-time PCR method. The frequencies of the mutant 521C and 421A alleles were 32.35% and 19.61%, respectively. Patients carrying the mutant A allele of ABCG2 421C>A, who were taking higher doses of atorvastatin, had significantly lower LDL-c than patients with the wild-type genotype. In addition, the presence of the variant 521C allele of the SLCO1B1 polymorphism resulted in better control of HDL-c in patients receiving higher doses of rosuvastatin. The obtained results did not show an association between AST and ALT activity and the examined SNPs. Our study demonstrates that the presence of the examined SNPs may be linked to the regulation of specific lipid parameters. Further research with a larger cohort and blood drug concentration measurements of statins is needed to better understand the polymorphism-dose-effect relationship.

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Key words: *atorvastatin, rosuvastatin, ABCG2, SLCO1B1, gene polymorphism, lipid status*

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Introduction

Hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, commonly known as statins, demonstrate high efficiency in the management of dyslipidemia (1). Enzyme HMG-CoA reductase catalyzes the rate-limiting step in cholesterol biosynthesis; therefore, its inhibition reduces intracellular cholesterol levels, lowering low-density lipoprotein cholesterol (LDL-c) levels, while potentially increasing high-density lipoprotein cholesterol (HDL-c) levels (2). The beneficial effects of statins on reducing cardiovascular morbidity and mortality are not solely due to their impact on lipid regulation; pleiotropic effects also play a role. These pleiotropic effects contribute to their widespread use in both primary and secondary prevention of cardiovascular events related to atherosclerotic changes in blood vessels (3).

The most commonly prescribed statins in clinical practice are atorvastatin and rosuvastatin. While both drugs are administered in their active acid form, they differ in structure and pharmacokinetic properties. Atorvastatin is a lipophilic drug that undergoes a significant first-pass effect. It is metabolized in the liver by cytochrome P450 (CYP) enzymes, primarily

CYP3A4, and its metabolites are subsequently excreted in the bile (4). In contrast to atorvastatin, rosuvastatin is more hydrophilic and is only partially metabolized by cytochrome P450 enzymes, primarily CYP2C9 and CYP2C19 (5). Apart from CYP isoenzymes, transporters also play a key role in the distribution and elimination of both statins. These include the organic anion-transporting polypeptide (OATP), an influx transporter primarily located on the basolateral membrane of hepatocytes, and the breast cancer resistance protein (BCRP), an efflux transporter found in the liver, intestine, kidney, and other tissues. Their expression is influenced by single nucleotide polymorphisms (SNP), Solute Carrier Organic Anion Transporter Family Member 1B—SLCO1B1 (OATP1B1 transporter) 521T>C (rs41490565) and Adenosine triphosphate (ATP) Binding Cassette G2—ABCG2 (BCRP transporter) 421C>A (rs2231142). In accordance with previous studies, these SNPs may have an impact on statin bioavailability, uptake in the liver and excretion into the bile, consequently affecting the concentration of these drugs in the blood (6–8).

It has been shown that the SLCO1B1 521T>C can lead to increased plasma concentrations of both atorvastatin (a 2.4-fold increase) and rosuvastatin (a 1.7-fold increase) (4). A potential explanation for the influence of this gene polymorphism on statin pharmacokinetics is provided by *in vitro* studies followed by *in vivo* studies (9). It has been shown that the amino acid change from valine to alanine at codon 174, characteristic of the SLCO1B1 521T>C polymorphism, results in decreased function of the OATP1B1 transporter, reduced uptake of statins into liver cells, and consequently, higher drug concentrations in the blood (10). Given the significant frequency of the 521C allele in various populations (9, 11), it is important to examine the frequency of this polymorphism in our study participants and its impact on statin kinetics in order to determine the optimal dosing regimen for effective lipid control. The ABCG2 transporter is a multidrug transporter that handles a wide range of substrates and belongs to a superfamily of 48 human ATP-dependent transporters. It has been found that the ABCG2 rs2231142 variant, located in the nucleotide-binding domain, plays an important role in protein stability (12). The 421C>A results in an amino acid change from glutamine to lysine at codon 141 (Q141K), which is associated with reduced ABCG2 transport activity, either by lowering transporter expression on the plasma membrane or by decreasing its ATPase activity (13). Additionally, researchers found that subjects carrying the variant allele had plasma rosuvastatin levels more than 100% higher than those with the wild-type genotype (1). Given this, it is expected that SNPs in transporter genes may influence the lipid-lowering effects of statins. The aim of this study was to examine the association between transporter gene polymorphisms and lipid status control in relation to the administered doses of atorvastatin or rosuvastatin. Additionally, we compared the serum activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) between carriers of different transporter genotypes.

Materials and Methods

The cross-sectional pharmacogenetic study was conducted from September to November 2024 at the Clinic of Nephrology and the Clinic of Cardiology, University Clinical Centre Niš, and the Research Centre for Biomedicine, Faculty of Medicine, University of Niš, Serbia. All procedures involving human participants were conducted in accordance with the ethical standards of the institutional and/or national research committees, as well as the 1964 Helsinki Declaration and its subsequent amendments, or comparable ethical standards. The protocols were approved by the Ethics Committee of the Faculty of Medicine, University of Niš (No 12-8310-1/2-8 from July 10, 2024) and Ethical Committee of the University Clinical Centre Niš (No 17321/2 from June 19, 2024). This study included 102 patients with dyslipidemia who had been on atorvastatin or rosuvastatin treatment for more than 4 weeks. All patients were enrolled in this study during regular controls at the mentioned clinics. Of all recruited patients, data for 93 patients were complete regarding statin dosing regimen and were included in the further study. Inclusion criteria were applied: age (> 18 years), use of a statin (atorvastatin or rosuvastatin), availability of a dosage regimen, and time since the last dose was taken, as well as recent control of lipid status. The values of lipid status parameters, along with other important biochemical markers such as alanine aminotransferase (ALT), aspartate amino-transferase (AST), serum creatinine, urea, and glucose levels, were collected from routine patient check-ups at one of the mentioned clinics. The pharmacodynamic effect of statins was measured in terms of lipid status control. Desired levels of lipid parameters for which it is considered that the control of the lipid status is optimal, according to the reference laboratory values, were as follows: total cholesterol (TC) ≤ 5.2 mmol/l, low-density lipoprotein cholesterol (LDL-c) ≤ 3.4 mmol/l, high-density lipoprotein cholesterol (HDL-c) ≥ 1 mmol/l, and triglycerides (TG) ≤ 1.7 mmo/l.

Genotyping ABCG2 421C>A and SLCO1B1 521T>C Gene Polymorphism

A fasting blood sample was collected from each patient during the routine control at the Clinic of Nephrology and the Clinic of Cardiology, University Clinical Centre Niš. DNA was extracted from the whole blood (200 µL) with EDTA as an anticoagulant using Genomic DNA Purification Kit (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's instructions. The ABCG2 421C>A (rs2231142) and SLCO1B1 521T>C

(rs4149056) genotyping was performed using TaqMan® Drug Metabolism Genotyping Assays, C_15854163_70 and C__30633906_10, respectively (Applied Biosystems, Carlsbad, CA, USA) on the 7500 Fast Real-Time PCR System (Applied Biosystems), according to the manufacturer's instructions.

Statistical Analysis

The distribution of genotypes for each polymorphism was assessed for deviation from Hardy–Weinberg equilibrium (HWE), given in Figure 1 and Figure 2. Continuous data were expressed as mean with standard deviation and median with interquartile range, and categorical variables were expressed as counts and percentages. Student's t-test (normally distributed data) and Mann–Whitney U test (not normally distributed data) were employed for the comparison of continuous variables between groups. Chi-square ($\chi 2$) test was used to compare data between groups, when data were defined as categorical. All analyses were performed using SPSS statistical analysis software, version 20.0 (SPSS, IBM Corp, Armonk, NY, USA) at the significance level set at $p < 0.05$.

Results

The study included 102 patients with dyslipidemia who were taking atorvastatin (50 of 102 patients) or rosuvastatin (52 of 102 patients). Also, the research population consisted of 41 women (40.2%) and 61 men (59.8%). Baseline characteristics of the patients, along with the genotypic frequencies of the ABCG2 421C>A and SLCO1B1 521T>C gene polymorphisms, are presented in Table 1.

Most common comorbidities among the studied population were ischemic heart disease 59.80% (n = 61), arterial hypertension 55.88% (n = 57), insulin-independent diabetes mellitus 36.27% (n = 37) and renal insufficiency 34.31% (n = 35). It was observed that the frequency of wild-type genotypes was 70.59% (n = 72) and 81.37% (n = 83) for SLCO1B1 521T>C and ABCG2 421C>A gene polymorphism, respectively (Figures 1 and 2).

Table 1. Baseline characteristics and laboratory findings of the examined population

Parameter	Mean ± standard deviation or median (Q1–Q3) or N (%)		
	All patients	Atorvastatin	Rosuvastatin
Male/female	61/41 (59.8/40.2%)	30/20 (60/40%)	31/21 (59.6/40.4%)
Number of patients	102 (100%)	50 (49.02%)	52 (50.98%)
Age	62.4 ± 12.6	63.5 ± 13.56	60.74 ± 11.94
Obesity	20 (19.79%)	11 (22%)	9 (17.31%)
Total Cholesterol (mmol/L)	5.15 ± 1.73 5 (3.81–6.175)	5.00 ± 1.80 5.7 (3.75–5.97)	5.28 ± 1.67 5.15 (3.88–6.34)
High-density lipoprotein cholesterol (mmol/L)	1.25 ± 0.34 1.24 (1–1.47)	1.26 ± 0.36 1.3 (1–1.5)	1.25 ± 0.33 1.25 (1.04–1.44)
Low-density lipoprotein cholesterol (mmol/L)	2.96 ± 1.38 2.66 (1.86–3.79)	2.9 ± 1.49 2.6 (1.84–3.65)	3.02 ± 1.18 2.77 (2.02–3.87)
Triglycerides (mmol/L)	2.09 ± 1.68 1.61 (1.11–2.33)	1.82 ± 1.06 1.53 (1.17–2.25)	2.34 ± 2.1 1.7 (1.09–2.79)
Aspartate aminotransferase (U/L)	46.9 ± 74.08 25 (20–36)	32.91 ± 26.68 25 (20.25–34.5)	61.2 ± 100.41 26 (20–28)
Alanine aminotransferase (U/L)	44.78 ± 77.99 25.5 (19.75–39.25)	34.57 ± 35.00 26 (20–37.5)	54.57 ± 103.26 26 (20–48)
Urea (mmol/L)	10.79 ± 9.21 7.2 (5.35–12.88)	14.36 ± 11.23* 10.85 (6.4–18.7)	7.35 ± 4.71 6.15 (4.83–8.27)
Creatinine (mmol/L)	190.12 ± 222.87 101.3 (81.43–151.25)	276.35 ± 283.01* 141.75 (89.52–360.25)	107.21 ± 84.34 90.65 (75.67–104.17)
Glucose (mmol/L)	7.23 ± 2.81 6.2 (5.3–8.5)	6.77 ± 2.35 5.8 (5.1–8.5)	7.67 ± 3.15 6.5 (5.47–8.5)

SNP— single nucleotide polymorphism; Serum levels of examined parameters are expressed as mean ± standard deviation or median (interquartile range Q1–Q3)
* atorvastatin vs. rosuvastatin, $p < 0.001$

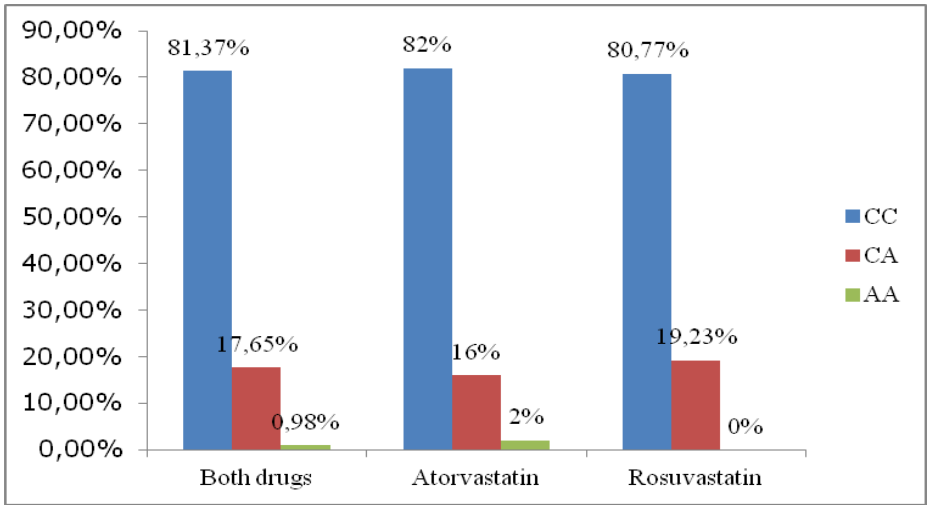

Figure 1. Frequency of ABCG2 421C>A genotypes among studied population (n = 102)
HWE: χ^2 = 0.0005, p = 0.98 (p > 0.05)

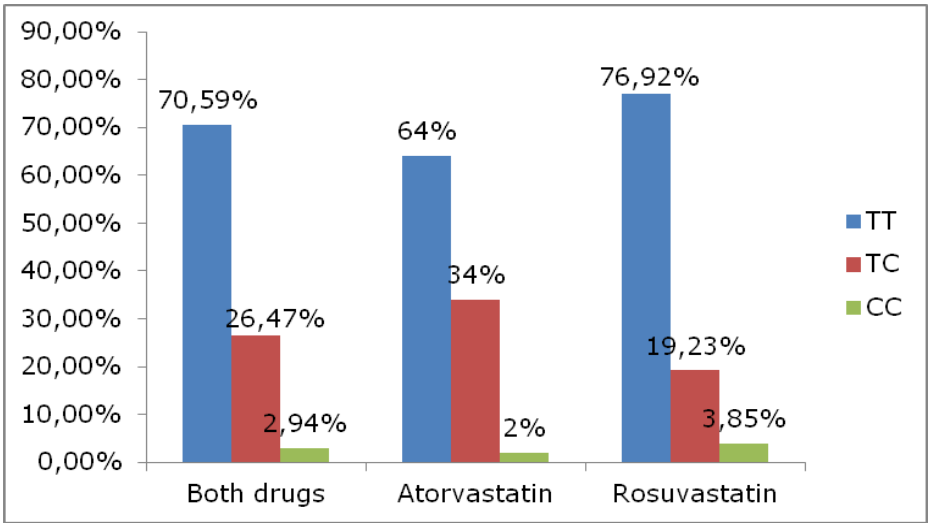

Figure 2. Frequency of SLCO1B1 521T>C genotypes among studied population (n = 102)
HWE: χ^2 = 0.0584, p = 0.81 (p > 0.05)

The frequency of mutant 521C and 421A alleles was 32.35% and 19.61%, respectively. Mean values with standard deviation, as well as median and interquartile range of lipid parameters, are given in Table 1. Serum values of examined biochemical parameters: ALT, AST, creatinine and urea, in some patients showed a drastic deviation, which affected the mean value and standard deviation. For this reason, the median and interquartile difference are more accurate and precise indicators of the value of these parameters in the studied population. Serum creatinine and urea levels were significantly elevated in patients undergoing atorvastatin therapy. This finding can be attributed to the fact that patients receiving atorvastatin were predominantly recruited from the Nephrology Clinic, where the majority of individuals have pre-existing or existing kidney conditions. Consequently, the observed differences in these parameters were anticipated. Atorvastatin was more commonly used in patients with impaired kidney function because the drug is primarily eliminated by metabolism. In addition, differences in serum levels of AST and ALT were examined among the different genotypes of the studied polymorphisms (Table 2). Carriers of the mutant 421A allele exhibited nearly significant (p = 0.082) higher serum AST levels compared to individuals with the wild-type genotype.

Patients were divided into two groups based on the median dose taken which was 20 mg for both atorvastatin and rosuvastatin. Patients who were taking lower doses—5, 10 or 20 mg of

atorvastatin or rosuvastatin, were selected in group I. The II group included patients with 40 or 80 mg atorvastatin or 40 mg rosuvastatin prescribed. Lipid parameters of each patient were considered according to the reference values of each of the investigated parameters individually and expressed as controlled and uncontrolled lipid parameter status (Table 3). Table 3 shows the number of patients with controlled and uncontrolled parameters of lipid status, divided according to dosage regimen (group I and II as previously explained) and according to ABCG2 C>A and SLCO1B1 521T>C genotype.

It was noticed that patients who were carriers of the mutant A allele of ABCG2 C>A in group II had significantly lower LDL-c than patients with the wild-type genotype. This indicates that the presence of the ABCG2 gene polymorphism affected the pharmacodynamic effects of atorvastatin on LDL-c lowering in patients who were taking higher doses. None of the patients carrying the A allele had an uncontrolled lipid status, which indicates greater efficacy of atorvastatin in these patients. There were no significant differences in other parameter values between the other groups of examined gene polymorphisms in patients taking atorvastatin.

Table 4 is structured in the same way as Table 3, with data for rosuvastatin provided. Patients with higher doses of rosuvastatin and the TC/CC SLCO1B1 521T>C genotypes had better-controlled HDL-c (values > 1 mmol/L) compared to patients in the same dosage group with the TT genotype. This suggests increased efficacy of rosuvastatin in patients carrying the C allele, particularly those on higher doses, in terms of elevating HDL-cholesterol plasma levels. No significant differences were observed between the other groups of examined gene polymorphisms in patients taking rosuvastatin.

Table 2. A comparison of groups stratified based on genetic polymorphisms regarding the values of AST and ALT

Gene polymorphism	Genotype	AST	ALT
ABCG2 421C>A	CC	24 (20–36)	25 (19–34)
	CA/AA	30.5 (22.75–86.25)	27.5 (20.25–61.75)
Statistics		Z = -1.737, p = 0.082	Z = -0.550, p = 0.583
SLCO1B1 521T>C	TT	24.5 (20–37.75)	24.5 (18.75–36)
	TC/CC	26 921.5–34)	26 (21–42)
Statistics		Z= -0.678, p = 0.498	Z = 0.583, p = 0.560

AST— aspartate aminotransferase (U/L); ALT— alanine aminotransferase (U/L)
Values are expressed as median with interquartile range (Q1– Q3)

Table 3. Patients taking **atorvastatin**— comparison of groups according to the control of lipid status (controlled/uncontrolled parameter) in relation to the dose of atorvastatin and the investigated gene polymorphism

		ABCG			SLCO1B1		
		CC	CA/AA	Statistics	TT	TC/CC	Statistics
TC	I group	13/8	3/1	χ^2 = 0.250, p = 0.617	12/7	4/2	χ^2 = 0.024, p = 0.876
	II group	6/14	3/2	χ^2 = 1.563, p = 0.211	8/12	1/4	χ^2 = 0.694, p = 0.405
LDL-c	I group	15/6	3/1	χ^2 = 0.021, p = 0.884	13/6	5/1	χ^2 = 0.503, p = 0.478
	II group	7/13	5/0	**χ^2 = 6.771, p = 0.009**	10/10	2/3	χ^2 = 0.160, p = 0.689
HDL-c	I group	18/3	3/1	χ^2 = 0.287, p = 0.592	17/2	4/2	χ^2 = 1.765, p = 0.184
	II group	16/4	3/2	χ^2 = 0.877, p = 0.349	15/5	4/1	χ^2 = 0.055, p = 0.815
TG	I group	11/10	2/2	χ^2 = 0.008, p = 0.930	10/9	3/3	χ^2 = 0.013, p = 0.910
	II group	10/10	2/3	χ^2 = 0.160, p = 0.689	10/10	2/3	χ^2 = 0.160, p = 0.689

I group—patients taking lower doses of atorvastatin (5 mg, 10 mg, 20 mg); II group—patients taking higher doses of atorvastatin (40 mg, 80 mg) TC: total cholesterol, LDL-c: low density lipoprotein cholesterol, HDL-c: high density lipoprotein cholesterol; TG: triglycerides; ABCG2 SNP: Adenosine triphosphate (ATP)-binding cassette transporter single nucleotide polymorphism; SLCO1B1: Solute carrier organic anion transporter family member 1B1 single nucleotide polymorphism. Results are expressed as the total number of patients who had controlled lipid status (TC ≤ 5.2 mmol/l, LDL-c ≤ 3.4 mmol/l, HDL ≥ 1 mmol/l, TG ≤ 1.7 mmol/l) and uncontrolled lipid status (TC > 5.2 mmol/l, LDL-c > 3.4 mmol/l, HDL < 1 mmol/l, TG > 1.7 mmol/l)

Table 4. Patients taking **rosuvastatin**—comparison of groups according to the control of lipid status (controlled/uncontrolled parameter) in relation to the dose of atorvastatin and the investigated gene polymorphism

		ABCG2			SLCO1B1		
		CC	CA / AA	Statistics	TT	TC / CC	Statistics
TC	I group	12/12	3/2	χ^2 = 0.166, p = 0.684	8/9	7/5	χ^2 = 0.358, p = 0.550
	II group	8/4	2/0	χ^2 = 0.933, p = 0.334	8/2	2/2	χ^2 = 1.260, p = 0.262
LDL-c	I group	15/9	3/2	χ^2 = 0.011,p = 0.917	11/6	7/5	χ^2 = 0.121, p = 0.728
	II group	9/3	2/0	χ^2 = 0.636, p = 0.425	9/1	2/2	χ^2 = 2.715,p = 0.099
HDL-c	I group	22/2	3/2	χ^2 = 3.490, p = 0.062	16/1	9/3	χ^2 = 2.162, p = 0.141
	II group	7/5	1/1	χ^2 = 0.049, p = 0.825	4/6	4/0	**χ^2 = 4.200, p = 0.040**
TG	I group	16/8	2/3	χ^2 = 1.250, p = 0.264	10/7	8/4	χ^2 = 0-184, p = 0.668
	II group	4/8	1/1	χ^2 = 0.207, p = 0.649	4/6	1/3	χ^2 = 0.280, p = 0.597

I group—patients taking lower doses of rosuvastatin (5 mg, 10 mg, 20 mg)**;** II group—patients taking a higher dose of rosuvastatin (40 mg) TC: total cholesterol, LDL-c: low density lipoprotein cholesterol, HDL-c: high density lipoprotein cholesterol; TG: triglycerides; ABCG2 SNP: Adenosine triphosphate (ATP)-binding cassette transporter single nucleotide polymorphism; SLCO1B1: Solute carrier organic anion transporter family member 1B1 single nucleotide polymorphism. Results are expressed as the total number of patients who had controlled lipid status (TC ≤ 5.2 mmol/l, LDL-c ≤ 3.4 mmol/l, HDL ≥ 1 mmol/l, TG ≤ 1.7 mmol/l) and uncontrolled lipid status (TC > 5.2 mmol/l, LDL-c > 3.4 mmol/l, HDL < 1 mmol/l, TG > 1.7 mmol/l)

Discussion

Pharmacogenetic testing has expanded in recent years and plays a crucial role in personalized medicine. Personalized medicine was initially intended to be implemented in clinical practice for drugs with a narrow therapeutic index and/or potentially severe side effects (14). However, even for drugs with a wide therapeutic range, pharmacogenetic testing can be important for adjusting doses and avoiding side effects, thereby improving patient compliance and enhancing the effectiveness of therapy. It has already been mentioned that, in addition to CYP enzymes, efflux and influx transporters in the liver may play an important role in exposure to statins. Although various studies have investigated different genes encoding transporters, not all gene polymorphisms have been shown to affect the expression and/or function of transporters, or these polymorphisms may not be present in the population at a frequency sufficient to monitor their effects (15, 16).

Several studies have revealed that the gene polymorphisms ABCG2 421C>A and SLCO1B1 521T>C impact the systemic exposure of atorvastatin and rosuvastatin (17–20). Therefore, it is not surprising that the 2022 updated guidelines of the Clinical Pharmacogenomics Implementation Consortium (CPIC) recommend SLCO1B1 and ABCG2 genotyping, given their strong association with increased systemic exposure to statins (21).

The frequency of minor alleles of examined genes varied among different populations. Tomilson et al. found that the frequency of the 421A variant allele among Asian patients with hypercholesterolemia was 30.5%, which is a higher frequency than in our studied population (19.61%). A slightly higher frequency of the mutant Aallele is also present in the population of Mexicans compared to ours, about 25% (22). On the other hand, the ABCG2 421A variant was found to have the lowest frequency in African populations, ranging from 0% to 5% (23). On the other hand, variant ABCG2 421A had the lowest frequency in African populations, 0–5%. The African population has also been shown to have a low frequency of the SLCO1B1 521C variant allele, ranging from 0% to 7%. A study conducted in the Netherlands had comparable SLCO1B1 521C variant frequency (30.57%) with our results, 32.35% (24).

The impact of the ABCG2 421C>A gene polymorphism has already been mentioned for reducing transporter activity in A allele carriers. Lower efflux transporter activity leads to increased statin absorption in the gastrointestinal tract while reducing drug elimination via the hepatobiliary pathway. Increased absorption and reduced elimination of statins can lead to higher concentrations of the drug in the blood, thereby enhancing its effect. However, it is important to consider that higher drug concentrations in the blood may increase the risk of statin-related side effects. The results of our study indicate a near-significant difference in serum AST levels between the different genotypes of the ABCG2 421C>A polymorphism. It has been suggested that individuals carrying the mutant A allele may have

an increased likelihood of experiencing statin-induced liver toxicity (25). During the first months of statin therapy, a temporary increase in transaminase levels (AST, ALT) can occur. Aminotransferase changes have been observed as early as a few hours after initial statin exposure, extending up to eight months following treatment initiation (26). However, only 3% of patients experience a permanent rise in these enzyme activities (27). Elevated transaminase levels are more common in patients taking higher doses of statins (28). According to current guidelines, statin use does not need to be discontinued if aminotransferase levels increase by ≤ 3 UNL (upper normal limit), but it is recommended to stop statin therapy if levels exceed 3 UNL (29). The temporary increase in liver transaminases may be due to changes in the lipid membranes of liver cells, which increase permeability and cause enzyme leakage. These changes in enzyme levels are considered adaptive, rather than a sign of liver damage (26). Reduced activity of the ABCG2 transporter causes statins to accumulate in liver cells, potentially affecting cell membranes. This may explain why transaminase levels are higher in patients with the 421AA/421CA genotype.

A large study published in September 2024, which enrolled 139,508 Taiwanese participants, found that individuals with the ABCG2 rs2231142 AA genotype had higher HDL-c and lower triglyceride (TG) levels, but no difference in LDL-c levels was observed (30). This study indicates a better lipid profile in AA genotype carriers, primarily reflected in a decrease in TG and an increase in HDL-c, though these changes were not statistically significant in our study. These results highlight the importance of clearly determining the effect of this gene polymorphism on each lipid parameter.

Although most research has focused on the impact of ABCG2 polymorphism on statin metabolism, it is noteworthy that statin therapy also affects the expression of the ABCG2 transporter. Rodrigues et al. investigated baseline mRNA expression levels of ABC and SLCO transporters in peripheral blood mononuclear cells. They reported that atorvastatin treatment significantly downregulated the gene expression of these influx and efflux transporters (31).

The Rotterdam study investigated the impact of SLCO1B1 521T>C and found that patients taking atorvastatin at a starting dose greater than 20 mg had a higher risk of dose reduction or switch if they carried the C allele, compared to those with the TT genotype (24). Although this was not the primary objective of the trial, these results are comparable to those in our group II, where patients also used atorvastatin doses greater than 20 mg and showed a greater reduction in LDL cholesterol. This suggests that higher doses of atorvastatin are more effective in C allele carriers.

Regarding the influence of this polymorphism on the pharmacodynamic effect of rosuvastatin, some studies (20, 32) have shown that the presence of the 521C allele negatively affects the control of LDL cholesterol reduction. It is important to highlight that carriers of the TT genotype had lower drug concentrations in plasma, which correlated with a reduced incidence of side effects. In contrast, carriers of the CC genotype, despite having higher drug concentrations, exhibited poorer control of lipid levels. Therefore, it is essential to better understand the relationship between gene polymorphisms, plasma drug concentrations, and pharmacodynamic effects.

Our study indicated that higher doses of atorvastatin or rosuvastatin in patients with the variant allele had improved response to statin therapy. This is reflected by a more significant reduction in LDL-c and an increase in HDL-c. However, a statistically significant difference was observed only in the better control of LDL-c in patients with the variant 421A allele of the ABCG2 polymorphism on atorvastatin therapy, and in better control of HDL-c in patients with the variant C521C allele of the SLCO1B1 polymorphism during rosuvastatin treatment. It remains unclear why the polymorphisms affect only some lipid parameters, while no statistically significant differences were observed for others. A potential explanation could be that statins exert cholesterol-lowering effects by inhibiting HMG-CoA reductase in hepatocytes. Therefore, when uptake from the blood into the liver is reduced due to the SLCO1B1 c.521T>C variant, it may be associated with reduced pharmacological efficacy. On the other hand, increased systemic exposure may increase the risk of enhanced side effects, which were not specifically investigated in this study but should be considered in future research. It is also important to note that some of the patients enrolled in the study had chronic kidney disease. The data suggest that lowering LDL cholesterol helps prevent major heart-related events in patients with chronic kidney disease and kidney transplant recipients (33). On the other hand, it must be mentioned that patients with chronic kidney disease require careful attention. Chronic kidney disease is a common risk factor for the development of statin-induced myopathy. The risk of this complication increases when other significant factors, such as advanced age, female gender, liver dysfunction, and diabetes mellitus, are present (34).

Some limitations of the study need to be mentioned, in the first row, the small number of patients enrolled in the study. Further research involving a larger cohort of patients is needed, with a more detailed and precise stratification by dosage. In addition, it is important to have measured drug concentrations in the blood, which would provide a more comprehensive understanding of the polymorphism-dose-effect relationship. This study serves as a foundation for future investigations into the pharmacokinetics and pharmacodynamics of rosuvastatin and atorvastatin.

Conclusion

In conclusion, examining the influence of ABCG2 and SLCO1B1 gene polymorphisms on lipid control in relation to the administered statin dose, statistically significant differences were observed in some lipid parameters among patients with polymorphic alleles. These findings could serve as a basis for adjusting statin doses based on the presence of specific polymorphisms. However, more extensive research is needed to provide a clearer understanding of how transporter gene polymorphisms affect individual lipid parameters.

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UTICAJ *ABCG2 421 C>A I SLCO1B1 521T>C* GENSKOG POLIMORFIZMA NA KONTROLU LIPIDNOG STATUSA BOLESNIKA LEČENIH ATORVASTATINOM I ROSUVASTATINOM

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Polimorfizmi gena za transportere lekova SLCO1B1 (OATP1B1 transporter) 521T>C i ABCG2 421C>A (BCRP transporter) mogu imati uticaja na metabolizam statina i, posledično, na farmakodinamičke efekte ovih lekova. Cilj ove studije bio je da se ispita povezanost između genskih polimorfizama za transportere lekova i kontrole lipidnog statusa kod bolesnika u čijem su lečenju korišćene različite doze statina. Dodatno, poredili smo serumsku aktivnost aspartat aminotransferaze (engl. aspartate aminotransferase – AST) i alanin aminotransferaze (engl. *alanine aminotransferase* – ALT) kod nosilaca različitih genotipova ispitivanih polimorfizama. Farmakogenetička studija preseka obuhvatila je sto dva bolesnika sa dislipidemijom koji su bili na terapiji atorvastatinom ili rosuvastatinom najmanje četiri nedelje. Vrednosti lipidnih parametara prikupljene su prilikom rutinske kontrole bolesnika. Za ispitivanje genskih polimorfizama korišćena je metoda *Real-Time* PCR. Frekvencije mutiranih alela 521C i 421A bile su 32,35% i 19,61%, redom. Pokazalo se da bolesnici koji su nosioci mutiranog A-alela ABCG2 421C>A i koji uzimaju više doze atorvastatina imaju statistički značajno niže vrednosti LDL holesterola od bolesnika koji imaju *wild type* genotip. Osim toga, prisustvo varijantnog 521C alela SLCO1B1 polimorfizma uticalo je na bolju kontrolu serumskih vrednosti HDL holesterola kod bolesnika koji uzimaju veće doze rosuvastatina. Dobijeni rezultati nisu pokazali statistički značajnu povezanost između aktivnosti AST-a i ALT-a i ispitivanih genskih polimorfizama. Studija je pokazala da prisustvo ispitivanih genskih polimorfizama može biti povezano s kontrolom određenih "parametara lipida". Neophodna su dalja istraživanja na većem broju ispitanika. Takođe, poželjno je određivanje koncentracije leka u krvi da bi se dobila potpunija slika o vezi između genskih polimorfizama, doze i efekta statina.

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***Ključne reči**: atorvastatin, rosuvastatin, ABCG2, SLCO1B1, genski polimorfizmi, lipidni status*

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ESTIMATION OF WORKING CAPACITY AMONG WORKERS WITH ALLERGIC CONTACT DERMATITIS IN THE TEXTILE INDUSTRY

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Working capacity is the physical, mental and intellectual capacity of the worker to perform certain work duties under specific conditions, all the while without harming their health. Should the sensitized person have repeated contact with a potential sensitizer during the performance of those duties, occupational allergic contact dermatitis may occur—skin inflammation of the eczema type, which can impact working capacity and even fully incapacitate the individual from performing those work duties. The aim of this paper was the estimate of the working capacity of 98 examined workers in the textile industry, 9 of whom have been diagnosed with allergic contact dermatitis. The medical part of the expertise for the estimate of working capacity encompassed a precise and comprehensive allergologic history, positive patch tests to certain potential allergens and responses to exposure—elimination test. The practical part of the estimate of the working capacity demanded a full job description, as well as a description of the conditions in which the specific job was performed. In estimating the working capacity of a patient with occupational contact dermatitis, we kept in perspective the psycho-social approach of the affected person, including their age, level of professional qualification, and the likelihood that the company would accept the suggestions given by medical and other professionals. Every case where we performed an estimate of the working capacity was done in isolation and with due respect for the individual.

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Key words: *allergens, allergic contact dermatitis, working capacity evaluation, textile dye*

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Introduction

Working capacity is the physical, mental and intellectual capacity of the worker to perform certain work duties under specific conditions without harming their health.

Allergic contact dermatitis is an eczema-type skin inflammation that occurs upon repeated contact of the sensitized person with a sensitizer. If this occurs during the performance of work duties, it is classified as Occupational Allergic Contact Dermatitis (OACD), which is more frequently registered in women (1).

Data shows (2) that out of all occupational diseases, dermatoses make up between 20–90% (in different countries), while the highest percentage belongs to contact dermatoses. Based on extensive resources, Knajter (3) makes the deduction that in the whole occupational pathology, skin impairment represents 20–50%, depending on the work group, industry, region, state and other factors. There are reports (4) stating that from 30% to 40% of all occupational skin diseases are eczema, with eczematous dermatitis (5) being the most frequent reason for occupational skin morbidity in the USA.

The acute stage of allergic contact dermatitis is characterized by erythema, papulae, tiny vesicles and oozing, while the chronic stage is marked by infiltration, lichenification and desquamation.

However, the clinical picture may vary, depending on the type of allergen. This is exactly what happens with allergic contact dermatitis caused by textile—Textile contact dermatitis (6).

It may present in the shape of:

-Erythema multiforme-like lesions as an atypical manifestation of hypersensitivity to disperse dyes (7, 8).

-Purpuric contact dermatitis caused by hypersensitivity to textile dyes and resins (9, 10). This partly depends on climatic factors (heat, humidity), leading to profuse sweating.

-Papular contact dermatitis caused by textiles is a rare condition. Cases have been described after exposure to formaldehyde. The description is similar to papular and purpuric dermatitis (11).

-Pigment contact dermatitis is an atypical manifestation registered in multiple cases. It may be the result of hypersensitivity to disperse dyes (12) and to azo dyes, which contain Naphthol AS (13).

-Phototoxic reaction to textile has been described (14), as well as contact depigmentation to azo dye, Solvent Yellow 3 (15).

-The clinical picture of atopic dermatitis which occurs on flexures is not rare (16).

The estimation of working capacity is one of the most complicated and delicate tasks that qualified institutions with adequate professional teams need to perform.

Aim

The aim of this paper was the estimation of the working capacity of workers with diagnosed allergic contact dermatitis employed in the textile industry.

Materials and Methods

Ninety-eight workers were examined in the textile factory DOO Evro Mak, Negotino in the Republic of North Macedonia. An extensive history was recorded for all of them. The clinical presentation of allergic contact dermatitis on their hands was registered in 9 workers, 8 of whom were women and 1 man.

Epicutaneous tests of the European Standard Series of allergens (ESS) were performed on the workers who exhibited skin changes. Testing was done at the University Clinic of Dermatology in Skopje.

Work ability assessment was performed on 9 out of 98 textile industry workers diagnosed with allergic contact dermatitis. Ninety-four (95.91%) of the workers examined were female, while 4% (4.08%) were male. Eight (88.88%) of the workers diagnosed with allergic contact dermatitis were women, and only one (11.11%) was a man (Table 1).

Three workers tested positive to one allergen, four workers tested positive to two allergens, and two workers tested positive to three allergens.

The most common allergens for our patients were textile dye mix, p-paraphenylenediamine (PPD) and formaldehyde.

Three workers reacted to one allergen, all to the textile dye mix

Four workers reacted to two allergens, all to the textile dye mix, PPD

Two workers reacted to three allergens, all to the textile dye mix, PPD and formaldehyde.

It was determined that all workers with allergic contact dermatitis experienced skin changes caused by harmful substances. The primary skin lesions were located in areas matching the maximum exposure sites, and the duration of exposure was consistent with the nature of the suspected agents and the type of skin disease.

With these workers, exposure outside of the workplace was eliminated, and it was determined that there was solely occupational exposure at the workplace.

Exposure elimination tests were performed. In all patients, they showed that the duration of the elimination was beneficial to the improvement of skin symptomatology.

The assessment of the working capacity was conducted by a Commission for Working Capacity Assessment, following the company's Rulebook on the members and functioning of the assessment commission.

There was 1 female worker aged < 20 years, 3 female workers aged 21-30 years, 2 female workers and 1 male worker aged 31-40 years, 1 female worker aged 41-50 years and 1 male worker aged 51-60 years (Table 2).

The most common allergens for our patients were textile dye mix, PPD and formaldehyde.

Three (33%) of the workers tested were positive for one allergen, all to the textile dye mix. Four (44%) were positive for two allergens, all to the textile dye mix, PPD. And two were positive for three allergens, all to the textile dye mix, PPD and formaldehyde (Table 3).

Table 1. Distribution of examined workers and workers with skin changes by sex

Examined workers						
Sex	Men		Women		Total	
	No.	%	No.	%	No.	%
	4	4.08%	94	95.91%	98	100%

Workers with skin changes—type KD						
Sex	Men		Women		Total	
	No.	%	No.	%	No.	%
	1	11.11%	8	88.88%	9	100%

Table 2. Distribution of workers with skin changes—type KD by age.

Total number of examined workers with changes to their skin by age			
Age	Men	Women	Total
< 20 years	-	1	1
21–30 years	-	3	3
31–40 years	1	2	3
41–50 years	-	1	1
51–60 years	-	1	1
> 60 years	-	-	-
Total	1	8	9

Table 3. Number of workers tested positive to one, two and three allergens.

Allergen number	1 allergen	2 allergens	3 allergens
Number and % of workers	3 (33%)	4 (44%)	2 (22%)

Discussion

According to the latest data, occupational dermatoses make up 1–2% of the total number of all occupational diseases, including occupational injuries (6). Should occupational injuries be excluded from this group, skin diseases represent 35–50% of all occupational diseases. Occupational skin diseases, contact dermatitis in particular, represent a significant problem (7, 8) and are the reason for 75% of sick day leaves.

Occupational skin diseases among workers in the textile industry are a continuous problem that affects quality of life of workers. The joint emergence of occupational irritant contact dermatitis and occupational allergic contact dermatitis and their synergy are of great importance among workers (9). Those workers are in constant contact with textile products of different kinds, and the main culprit for changes in the skin among them is the chemicals used in the process of fabric treatment to give it certain quality and performance. The constant contact leads to the possibility of sensitization of the skin and the occurrence of changes in the same region of contact, most frequently the hands.

Textile fibers are natural wool, flax, cotton and silk and the synthetic derivatives of cellulose and polyamides (17). Other materials such as metals, rubber components, and dyes may be added to give the fibers specific features (18).

It used to be a common belief that wool could cause an allergic reaction. However, the absence of evidence of allergy is now evident. It is obvious that the allergens are linked to the processing of wool (ex., chemical dyes) added to modern clothing made of wool. Wool can irritate only if the fibers are of a larger diameter. Clothing made from Merino wool is better tolerated as the fibers are of a smaller diameter (19).

Allergy to cotton is extremely rare (20). Cotton clothing may cause erythema or itching because of skin irritation.

The same applies to silk, although there is a case of contact urticaria to silk (21).

However, allergic contact dermatitis is not rare, and this is because textile is prepared with biocides (22) which cause contact dermatitis. To name a few: triclosan, zinc pyrithione, MCI/MI, dichloro-octylisothiazolinone, dimethyl fumarate and silver particles (23–25). Substances used after dying (benzanthrone) or textile treatment (sulphites) may cause allergic contact dermatitis (26, 27). Formaldehyde, urea-formaldehyde resin, and melamine-formaldehyde have been used in the textile industry since 1920 to prevent wrinkling. It has been found that they all may cause a reaction. Based on numerous studies in various countries, the release of formaldehyde is documented for various types of fibers. However, it is suggested that wool is most certainly the textile material for this sensitivity (19, 28–34).

Textile dyes are rarely the cause of allergic reactions of type I (32, 33). It is more frequently the case of type IV reactions. The classification of dyes is conducted according to chemical structure or according to method of application. Different dyes are used for synthetic and natural fibers. Disperse dyes (DDs) are used for coloring synthetic textiles, polyester, nylon and mixed fibers (16). Around 60% of all DDs are azo dyes, while about 25% are anthraquinone dyes containing quinophthalone, methine, naphthylamide, naphthoquinone and nitro dyes (34).

Before DDs were included in the baseline series, PPD was considered the screening allergen for textile dye dermatitis. It was later discovered that PPD is not a marker allergen for the detection of sensibilization to all azo dyes found in textiles (34). A total of 26 DDs is used for testing.

The most common allergens are textile dye mix, which is a global allergen dominated by azo and anthraquinone bases; PPD, which is used in textile dyes; and formaldehyde, which is used as an anti-wrinkle finish. One must not ignore the effect of nickel, as most textile workers are in contact with it when performing their jobs (10).

Testing for textile dermatitis is recommended with the use of the European baseline series, which includes TDM, Textile series and own material “as is” as well as with extracts made from it.

The estimation of working capacity is performed by a Work Capacity Committee based on:

-Worker’s personal history (atopic constitution or previous allergic manifestations on the skin or other organs)

-Work history (job position they occupy and where the changes occurred)

-Job description of the position the worker occupies (contact with fabrics, textile dust,

scissors, chalk, and duration of contact—in the course of the full working day or occasionally)

If the contact is continuous, workplace exposure should exist for at least a year, and 2–3 years if the contact is occasional.

Dermatologist's report provides the diagnosis of Allergic Contact Dermatitis with description of the clinical condition and course of the disease—chronic illness with severe relapses, course of the disease at the workplace and home, duration of relapses after exposure and whether rehabilitation occurs with or without treatment.

The trend to create prevention programs to minimize skin contact with allergic substances, improving safety measures, health education and good personal hygiene, should, in turn, have an important impact on lowering the number of workers with occupational dermatoses (11).

Conclusion

When estimating the working capacity of a patient with OACD, one must keep in perspective the psycho-social approach to the diseased person, including their age, level of professional qualification and the likelihood that the company would accept the suggestions given by medical and other professionals. Every case where an estimate of working capacity is done must be viewed in isolation and with due respect to the state of the individual with occupational skin diseases. The estimation of working capacity should include a description of job operations and the conditions in which the job is performed for every specific post.

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PROCENA RADNE SPOSOBNOSTI RADNIKA SA ALERGIJSKIM KONTAKTNIM DERMATITISOM U TEKSTILNOJ INDUSTRIJI

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Radna sposobnost predstavlja fizičku, psihičku i intelektualnu sposobnost radnika da pod određenim uslovima na radnom mestu obavljaju određeni posao, a da pritom ne nanose štetu svom zdravlju. Ako prilikom obavljanja posla dolazi do ponovljenog kontakta senzibilne osobe sa mogućim senzibilizatorom, nastaje profesionalni kontaktni alergijski dermatitis–zapaljenje kože poput ekcema, koje može uticati na smanjenje radne sposobnosti i dovesti do potpune nemogućnosti obavljanja posla. Cilj ovog rada bila je procena radne sposobnosti do koje se došlo obradom podataka dobijenih posle pregleda devedeset osam radnika zaposlenih u tekstilnoj industriji. Kontaktni alergijski dermatitis dijagnostikovan je kod devet radnika. Medicinska ekspertiza za ocenu radne sposobnosti obuhvatila je preciznu i iscrpnu alergološku anamnezu, *patch* testove pozitivne na određene sumnjive alergene i odgovor na test ekspozicije/eliminacije. Praktični deo ocenjivanja radne sposobnosti podrazumevao je zahteve radnih operacija i uslove u kojima su se one odvijale na konkretnom radnom mestu. Pri ocenjivanju radne sposobnosti ispitanika sa profesionalnim kontaktnim alergijskim dermatitisom uzeti su u obzir psihosocijalni pristup oboleloj osobi, godine starosti, stručna sprema, kao i mogućnost preduzeća da usvoji predloge medicinskih i drugih stručnjaka. Svaki ispitanik je prilikom ocenjivanja radne sposobnosti posmatran izolovano i sa dužnim poštovanjem.

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***Ključne reči**: alergeni, alergijski kontaktni dermatitis, procena radne sposobnosti, tekstilne boje*

Original article

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THE EFFECT OF METFORMIN ON BIOCHEMICAL PARAMETERS IN PATIENTS WITH METABOLIC DYSFUNCTION-ASSOCIATED STEATOTIC LIVER DISEASE

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common form of chronic liver disease in the modern world. The importance of this condition is that it can progress to nonalcoholic steatohepatitis, which increases the risk of developing liver cirrhosis and hepatocellular carcinoma. The study aimed to examine the effects of metformin on achieving positive biochemical responses in patients with MASLD. The study included 146 patients, 96 men and 50 women, with MASLD diagnosed by ultrasound. Biochemical analyses were performed as well. The values of all parameters were measured at baseline, after three and after six months of therapy. On each visit, the body weight and body mass index (BMI) were obtained. All patients at baseline received 750 mg of metformin twice a day. There was a reduction in body weight, which was statistically significant after six months. The BMI decrease reached no statistical significance. Liver enzyme values showed a significant decrease in values relative to baseline after three and six months of metformin therapy. Serum cholesterol and triglyceride levels were reduced during treatment with metformin, and changes reached statistical significance at six months relative to baseline. There was a statistically significant decrease in the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) value after three and six months compared to baseline. Results showed that metformin may be an appropriate addition to diet, weight reduction, and physical activity, as it led improvements in metabolic parameters, with minimal adverse events and good tolerance of therapy.

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Key words: *metabolic dysfunction-associated steatotic liver disease metformin, body mass index*

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Introduction

There is an obvious increase in the number of people with metabolic dysfunction-associated steatotic liver disease (MASLD). Today, MASLD is the most common form of chronic liver disease in the modern world. The importance of this condition is that it can progress to nonalcoholic steatohepatitis (NASH), which increases the risk of developing liver cirrhosis and hepatocellular carcinoma. Therefore, MASLD is a growing health problem which affects 20–30% of the population, while in type 2 diabetes mellitus (DM), it may be as high as 75% (1). MASLD is a state of accumulation of fat in the liver with no significant alcohol abuse. There is a wide range of changes in the liver, from the common hepatic steatosis to nonalcoholic steatohepatitis, liver damage which is similar to that of alcoholic liver disease. MASLD is clearly associated with the risk of developing type 2 DM and cardiovascular disease (2). MASLD is a common cause of asymptomatic elevation in liver enzymes, in particular, alanine aminotransferase (ALT) (3).

The pathogenesis of MASLD is not well understood. It is assumed that insulin resistance is a major pathogenetic disorder that leads to liver steatosis. Steatosis itself further increases the sensitivity of the liver to metabolic damage, leading to the progression of hepatic steatosis to steatohepatitis and fibrosis (4). Ultrasound examination of the abdomen is the most common diagnostic tool for MASLD. If the percentage of fat

in the liver is 20–30%, the sensitivity for detection of hepatic steatosis is 85%, a specificity of 94% (5). You mayfind mild to moderate elevations in ALT and AST enzymes with a ratio of AST:ALT less than 1. Hepatic enzymes can be considered normal in 75% of cases, so that the increase in enzyme is not sensitive for the diagnosis of MASLD (6). For now, ideal therapy for MASLD does not exist. Changing lifestyle, diet and weight loss are difficult to achieve and maintain. As insulin resistance is a key pathogenetic mechanism of MASLD use of insulin sensitizers may be an answer to medical therapy. Metformin, as a representative of this group of drugs, may play an important role and exert a positive effect on biochemical parameters and histological changes in patients with MASLD.

The study aimed to examine the effects of metformin on achieving positive biochemical responses in patients with MASLD.

Meterials and Methods

The study included 146 patients, 96 men and 50 women, treated at the Clinic of Gastroenterology and Hepatology, Clinical Center Niš, in the period from 2022 to 2024, with the patient's signed consent. The study has been approved by a local ethics committee. The study included patients with MASLD diagnosed by ultrasound. Ultrasound parameters for diagnosis were the presence of two of the four ultrasound criteria: i) liver echogenicity exceeding that of the renal cortex, ii) loss of definition of the diaphragm, iii) poor delineation of the intrahepatic architecture, vi) attenuation of the ultrasound wave. Ultrasound examinations were performed by one ultrasonographer on the camera.

The inclusion criteria for the study required participants to be either abstinent from alcohol or to consume no more than two drinks a week, with a daily alcohol intake of less than 20 g/day. Participants also needed to show an increase in serum ALT and AST levels greater than 1.5 x ULN, and to have negative viral markers for HBsAg and HCV. Additionally, there should be no data of chronic liver disease, Wilson's disease, or hemochromatosis. The study did not include patients with DM, Cushing's disease, liver cirrhosis, chronic renal failure, heart failure III and IV according to New York Hecrt Associotian Classification. Patients receiving certain drugs such as methotrexate, amiodarone or those who had previously used metformin as well as patients with lactate values exceeding 2.2 mmol/l were not considered for the study. All patients underwent ultrasound examination of the liver upon diagnosis with NAFLD. Biochemical analyses measuring ALT, AST, gamma-glutamyl transferase, alkaline phosphatase enzyme, glucose, cholesterol, and triglycerides were conducted as well.

Further, insulin, C-peptide levels and HOMA-IR were determined. The values of all parameters were measured at baseline, after three and six months of therapy. On each visit, the body weight and body mass index (BMI) were obtained. The biochemical analyses were performed at the Institute of Biochemistry, Clinical Center Niš, and fasting insulin and C-peptide concentrations were determined at the Center of Nuclear Medicine, Clinical Center Niš.

At baseline, all patients received 750 mg of metformin twice daily. Data are presented as mean ± standard deviation (SD). Student's t-test and chi-square test were used to determine differences between the groups, and a p-value < 0.05 was taken as significant.

Results

The basal values of observed parameters, as well as values obtained after three and six months from the inclusion of metformin, are given in Table 1. There was a reduction in body weight, which was statistically significant after six months as compared to baseline, but not in relation to the value after three months. The BMI decrease reached no statistical significance. There were no significant changes in fasting blood glucose value. Serum cholesterol and triglyceride levels were reduced during treatment with metformin, and changes reached statistical significance at six months relative to baseline. The impairment of AST had statistical significance after three and six months compared to baseline, as well as the impairment after six months compared to the value after three months. ALT level showed a statistically significant reduction after three and six months compared to baseline, and no significant differences after six months compared to value after three months. There was a statistically significant decrease in HOMA-IR value after three and after six months compared to baseline, and no significant differences after six months compared to the value after three months.

Table 1. Changes in biochemical parameters and HOMA-IR

	Baseline	Three months	Six months
Body weight kg	88.7 ± 12.4	86.8 ± 10.7	84.6 ± 10.5*
BMI	29.7 ± 3.3	29.3 ± 2.7	28.7 ± 3.0
Fasting glucose mmol/l	5.1 ± 1.3	5.0 ± 1.1	5.0 ± 1.2
Cholesterol mmol/l	6.24 ± 2.73	5.53 ± 2.26	5.19 ± 2.59*
Triglycerides mmol/l	4.01 ± 1.85	3.69 ± 1.45	3.12 ± 1.73*
AST IU/L	79.63 ± 10.48	62.25 ± 14.68*	50.07 ± 9.55* **
ALT IU/L	85.12 ± 11.17	69.73 ± 17.44*	59.36 ± 14.33*
HOMA-IR	7.2 ± 2.4	4.0 ± 1.4	4.1 ± 1.7

*statistical significance after six months compared to baseline, $p < 0.01$
**statistical significance after six months compared to three months, $p < 0.01$

Discussion

Metformin was introduced as a first-line treatment for type 2 DM for more than half a century. The effect of metformin lowering blood glucose is explained by reducing hepatic gluconeogenesis, stimulating glucose taking into muscle and an increase in fatty acid oxidation in the adipose tissue (7). The final result is improving peripheral insulin sensitivity. Activation of AMPK by metformin has beneficial effects on lipid metabolism. The mechanism of loss of body fat is not only through direct inhibition of adipogenesis, but also by changing the synthesis and secretion of adipokines. Under the action of metformin, adiponectin stimulates AMPK and prevents hepatic lipid accumulation by increasing the β-oxidation of free fatty acids as well as decreasing synthesis.

The study included 146 patients, 96 men and 50 women. Maruti et al. suggest that the prevalence of in MASLD men is 31% and in women 16%, which means that the male sex is a risk factor for this disease. In our patients, there is a decrease in body weight and BMI after three and six months of the introduction of metformin therapy. All patients were on a prescribed diet and nutrition before monitoring. No statistically significant changes in body weight and BMI were observed after three and six months of treatment with metformin. Most authors observed similar changes, weight loss, with no statistically significant changes (8).

It was observed that even a smaller reduction in body weight can lead to improvements in markers of MASLD, in particular ALT and imaging markers of liver fat (9). There is also no significant difference in blood glucose value during follow-up. Cholesterol and triglyceride levels are reduced after three and six months, with a statistically significant change after six months compared to baseline values. Metformin significantly reduces the percentage of patients with MASLD in impaired fasting glucose (IFG) compared to patients treated with diet alone. Metformin therapy also significantly lowered the percentage of patients who met the diagnostic criteria for metabolic syndrome (10). There is data on different lipid changes during the treatment of MASLD with metformin, from mild to moderate decrease. Additionally, during the follow-up of 12 months, there were no changes in lipid levels, even with the increase during that period (11). Our results show a reduction in lipid values after three and six months of therapy.

The role of insulin resistance in the development of MASLD is complex, so that both hepatic and peripheral insulin resistance are clearly associated with the onset of MASLD. There is a diminished ability of insulin to suppress lipolysis, which increases the inflow of free fatty acids from adipose tissue to the liver. There is a reduced ability of insulin to inhibit gluconeogenesis, which leads to hyperglycemia and increased insulin resistance. Metformin has a positive effect on all of these processes by improving insulin sensitivity. Patients with MASLD have significantly higher levels of insulin and HOMA-IR index. Our results show a significant reduction in HOMA-IR after three months, with the maintenance of those values without significant changes after 6 months. It has been observed that people with higher levels of insulin and HOMA-IR have a higher risk over five years to develop MASLD. Reducing the levels of insulin using metformin reduces the risk that becomes similar to risk that in people who have had low or normal basal insulin levels. High insulin levels probably result in primary insulin resistance rather than decreased hepatic extraction of insulin in any liver disease (12, 13).

Liver enzyme values showed a significant decrease in values relative to baseline after three and six months of metformin therapy. There is a statistical significance of changes in AST after six months compared to baseline and after three months, while ALT level after six months shows a value similar to those after three months of treatment. Most studies indicate that metformin therapy significantly reduces ALT and AST, with normalization of ALT in as many as 56% of patients (12). This can be important because there is a greater risk of disease progression with higher values of transaminases (3).

Liver biopsy is the gold standard for the diagnosis of MASLD, but because of possible complications, cost and inconvenience for patients is often replaced by ultrasound and CT diagnostics. The percentage of patients with MASLD was higher when assessed by liver biopsy, allowing for accurate data on the presence of MASLD or NASH. Studies investigating the histological changes of liver biopsy showed no significant difference in histological findings during the course of metformin treatment. Despite the good response and the improvement in metabolic parameters, only about 30% of patients showed noticeable improvement in the level of steatosis, and only 20% of patients showed an improvement in the degree of inflammation after one year of metformin use (11, 14). There is a question of treatment duration and the daily dose of metformin. There are various study durations and doses of metformin that have been administered. Duration ranges from 4–12 months, a total daily dose of metformin from 0.85 gr to 3 g, average 1.5 g. Because of this, for now, there is limited number of studies, and the optimal dose of metformin and duration of treatment have not yet been defined (15).

Conclusion

The specific drug therapy of MASLD is not yet defined and available, and no drug can be a substitute for lifestyle modification. Our results indicate that metformin may be an benefitial addition to a regimen that includes diet, weight reduction and physical activity. It improves metabolic parameters, with almost no adverse events and good tolerance of therapy. However, it does not lead to significant histological changes in the liver. Reducing the risk of metabolic syndrome and cardiovascular disease is crucial.

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UTICAJ METFORMINA NA BIOHEMIJSKE PARAMETRE KOD BOLESNIKA SA NEALKOHOLNOM MASNOM JETROM

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Metabolička disfukcija povezana sa masnom bolešću jetre (engl. *metabolic dysfunction-associated steatotic liver disease* – MASLD) predstavlja najčešći oblik hronične bolesti jetre u savremenom svetu. Može napredovati u nealkoholni steatohepatitis, što povećava rizik od razvoja ciroze jetre i hepatocelularnog karcinoma. Cilj ove studije bio je da se ispitaju efekti metformina na postizanje pozitivnih biohemijskih odgovora kod bolesnika sa MASLD-om. Studija je obuhvatila 146 bolesnika (96 muškaraca i 50 žena) sa MASLD-om, koji je dijagnostikovan ultrazvukom. Urađene su biohemijske analize. Vrednosti svih parametara merene su na početku, posle tri meseca terapije i posle šest meseci terapije. Prilikom svake posete lekaru mereni su telesna težina i indeks telesne mase (engl. *body mass index* – BMI). Svi bolesnici su na početku primali 750 mg metformina dva puta dnevno. Nakon šest meseci terapije došlo je do statistički značajnog smanjenja telesne težine. Ispostavilo se da smanjenje BMI-ja nema statistički značaj. Pri poređenju početnih vrednosti enzima jetre sa vrednostima posle tri meseca i posle šest meseci terapije metforminom, zabeleženo je njihovo značajno smanjenje. Nivoi holesterola i triglicerida u serumu opali su u toku lečenja metforminom. Statistički značajna razlika u vrednostima primećena je pri poređenju početnih vrednosti sa vrednostima nakon šest meseci lečenja. Došlo je do statistički značajnog smanjenja vrednosti HOMA-IR (engl. *homeostatic model assessment for insulin resistance*) indeksa posle tri meseca i posle šest meseci lečenja u odnosu na početnu liniju. Rezultati ove studije pokazuju da metformin može biti adekvatan dodatak ishrani, s obzirom na to da doprinosi smanjenju telesne težine i fizičkoj aktivnosti, postiže poboljšanje metaboličkih parametara, skoro bez neželjenih efekata, i da se dobro podnosi.

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***Ključne reči**: metabolička disfukcija povezana sa masnom bolešću jetre metformin, indeks telesne mase*

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RISK FACTORS OF PHANTOM LIMB PAIN IN PATIENTS WITH LOWER LIMB AMPUTATIONS

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Phantom pain is a common and complex complication following limb amputation. Current pharmacological, psychological, and rehabilitative approaches are only partially effective, indicating the need for further research.

This study aimed to examine risk factors associated with the occurrence of phantom pain in patients with lower limb amputations.

A cross-sectional study was conducted at the Clinic of Medical Rehabilitation of the University Clinical Center of Vojvodina in Novi Sad, including 53 patients with acquired lower limb amputation in either the pre-prosthetic or prosthetic phase of rehabilitation. Data were collected from medical records and patient history regarding age, sex, presence of cardiovascular comorbidities, diabetes mellitus, various types of pain, body mass index, level of amputation, and characteristics of the residual limb. Statistical analysis was performed using JASP software, applying descriptive statistics, the Student's t-test, the χ^2 test, and binary logistic regression to examine factors associated with the occurrence of phantom limb pain. Statistical significance was set at $p < 0.05$.

Phantom limb pain was reported in 16 patients (30.18%). A statistically significant association was found between phantom pain and the level of amputation ($p = 0.046$), as well as the phase of rehabilitation ($p = 0.011$).

The level of amputation and the phase of rehabilitation represent significant risk factors for the development of phantom limb pain.

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Key words: *phantom pain, lower limb amputation, diabetes mellitus*

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Introduction

According to data from the World Health Organization from 2023, approximately 1.5 million amputations are performed worldwide each year. Limb amputation can have significant physical consequences, such as impaired gait and reduced limb mobility, as well as psychological consequences such as depression and anxiety, and socio-economic consequences (1). After limb amputation, patients often develop different types of pain, including residual limb pain, phantom limb pain, and musculoskeletal pain, which can be acute but are often chronic in nature (2). Despite innovations in surgical techniques, rehabilitation procedures, and prosthetics, literature data report the prevalence of phantom limb pain among patients after limb amputation ranging from 6.7% to 88.1% (3). The occurrence of phantom limb pain is one of the most common complications following limb amputation. Previous studies have shown that the intensity of pain prior to amputation, the presence of residual limb pain, and phantom sensations are significant predictors of phantom limb pain development in patients with lower limb amputation (4, 5). Additional risk factors include amputation due to diabetic complications, higher (proximal) level of amputation, older age, lack of preoperative counseling, and inadequate pain control before surgery (6, 7). The exact mechanism behind this phenomenon is still not fully clarified; it is considered to result from a complex combination of peripheral, central, and psychological mechanisms. Consequently, the treatment of

phantom limb pain must be multidisciplinary. Since all pharmacological, psychological, and rehabilitation procedures used in the treatment of phantom limb pain are only partially effective, it is necessary to further investigate the mechanism of onset of this disorder as well as the risk factors contributing to its development (8).

Aim

This study aimed to investigate individual risk factors that, according to the literature, are associated with the development of phantom limb pain as a complication following lower limb amputation.

Materials and Methods

A cross-sectional study was conducted at the Clinic of Medical Rehabilitation of the University Clinical Center of Vojvodina (UCCV) in Novi Sad and included patients who had undergone lower limb amputation and who were evaluated as part of the pre-prosthetic phase of rehabilitation treatment or were admitted for the prosthetic phase of rehabilitation in the outpatient clinic service, day hospital, or inpatient ward of the Clinic.

The study included 53 participants with acquired lower limb loss. Based on anamnesis data and medical documentation, information was collected regarding patient age, sex, presence of cardiovascular (CVS) comorbidities (valvular diseases, episodes of heart failure, arrhythmias, cardiomyopathies, hypertension, hypotension, oedema, angina pectoris, heart and stroke history) and diabetes mellitus (DM), as well as the presence of pain (phantom limb pain, residual limb pain, or musculoskeletal pain). Clinical examination included measurement of body weight and height to calculate the body mass index (BMI), determination of amputation level, and assessment of residual limb condition, including formation and length. The study was conducted with prior approval of the Ethics Committee, approval number 00-364, issued on Oct. 15, 2024.

The collected data were entered into a specially created Microsoft Excel 2013 database. During statistical processing, descriptive statistical methods were used: measures of central tendency (arithmetic mean) and measures of variability (standard deviation). For the complete statistical analysis, JASP software was used. Student's t-test or χ^2 test was applied to determine statistically significant associations or differences. Binary logistic regression was used to examine the relationship between different factors (CVS diseases, DM, BMI, level of amputation, rehabilitation phase, residual limb formation and length) and the occurrence of phantom limb pain, which was used as a dependent variable with possible values yes/no. P-values < 0.05 were considered statistically significant.

Results

Of the total 53 participants, 39 (74.13%) were male and 14 (25.86%) were female. The mean age of participants was 64 years, with the oldest participant being 85 and the youngest 35 years old. A total of 22 (41.51%) patients had transtibial amputation, while 31 (58.49%) had transfemoral amputation. Sixteen patients experienced phantom limb pain, three reported musculoskeletal pain, and five had localized residual limb pain. Descriptive statistical measures are shown in tabular form (Table 1).

Table 1. Descriptive statistics are presented for all quantitative variables, including mean value, standard deviation, minimum and maximum values

	AGE	HEIGHT	WEIGHT	BMI	LENGTH OF RESIDUAL LIMB
Number	53	53	53	53	53
Missing	0	0	0	0	0
Mean	64.434	173.396	78.151	25.809	31.472
Standard deviation	9.912	9.400	19.671	5.691	9.758
Minimum	35.000	155.000	47.000	16.700	0.000
Maximum	85.000	188.000	143.000	44.100	49.000

Using the χ^2 test, no statistically significant association was found between phantom limb pain and sex ($p = 0.473$) or residual limb formation ($p = 0.293$), nor with the presence of CVS comorbidities ($p = 0.692$) and DM ($p = 0.632$). On the other hand, statistically significant associations were found between phantom limb pain and amputation level ($p = 0.046$) and rehabilitation phase ($p = 0.011$). Logistic regression results also indicated that these two factors represent statistically significant predictors of phantom limb pain occurrence. For amputation level, the coefficient was -4.44, with $p = 0.019$, where the negative coefficient indicates that patients with transtibial amputation (coded as 1) have a significantly lower probability of developing phantom limb pain compared to those with transfemoral amputation (Figure 1). For the rehabilitation phase, the coefficient was -2.59, with $p = 0.031$, indicating that patients in the prosthetic phase of rehabilitation have a lower probability of developing phantom limb pain compared to those in the pre-prosthetic phase (Figure 2). Binary logistic regression did not show a statistically significant association between CVS comorbidities and DM with phantom limb pain. Stratified analyses were also performed: the group of patients with transfemoral amputation was analyzed for DM, and patients in different rehabilitation phases (prosthetic and pre-prosthetic) were analyzed for CVS comorbidities. In both stratified analyses, no statistically significant associations were found. Using Student's t-test, no statistically significant difference was found between the occurrence of phantom limb pain and age ($p = 0.146$), BMI ($p = 0.072$), or residual limb length ($p = 0.968$). No statistically significant differences were found for any of the examined parameters in relation to residual limb pain or musculoskeletal pain.

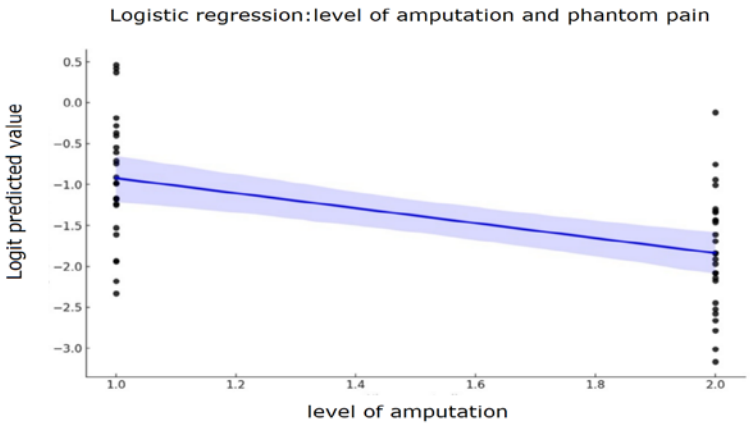

Figure 1. Graphical representation of the applied logistic regression, which in this case was used to examine the association between the level of amputation and the occurrence of phantom limb pain. The blue line represents the logistic regression prediction, while the shaded area corresponds to the 95% confidence interval

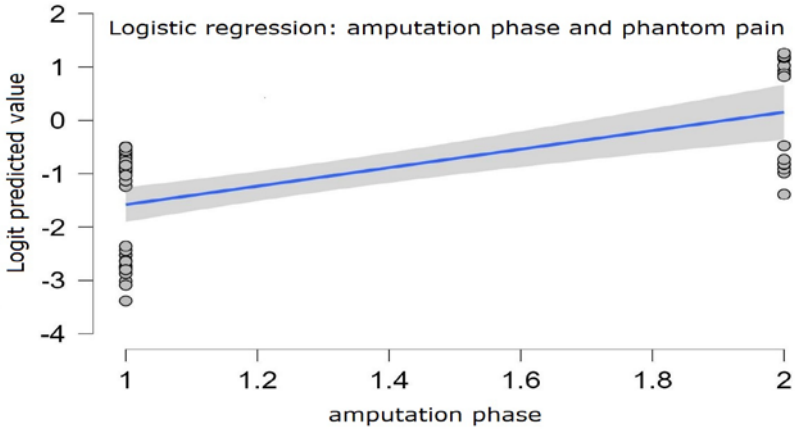

Figure 2. Graphical representation of the applied logistic regression, which in this case was used to examine the association between the amputation phase and the occurrence of phantom limb pain. The blue line represents the logistic regression prediction, while the shaded area corresponds to the 95% confidence interval

Discussion

Our results indicate that there is an association between phantom limb pain and both the level of amputation and the rehabilitation phase, whereas no association was found with sex, CVS comorbidities, or DM, nor were there statistically significant differences related to patient age, residual limb length, or residual limb formation. The exact mechanism of pain persistence in the limb even after its removal is still not fully understood. The mechanisms of phantom limb pain can be divided into four sub-processes, like all pain pathways: transduction, transmission, perception, and modulation. After limb amputation, the motor cortex remains intact, and the brain continues to receive signals as if they are coming from the missing limb, creating a mismatch between motor commands and proprioceptive or somatosensory impulses from the limb (9). There is also a conflict between signals from the missing limb and efferent motor signals, which results in an incomplete somatosensory-motor feedback loop. This interaction between cortical functions and descending pain-inhibitory pathways (thalamus, periaqueductal gray matter, nucleus gigantocellularis, and raphe nucleus) leads to reduced pain inhibition and increased activation of N-methyl-D-aspartate (NMDA) receptors in the spinal cord, causing increased sensitivity (allodynia, hyperalgesia) (10). Factors reported in literature to be associated with the development of phantom limb pain include sex, age, pain before surgery, presence of peripheral nerve damage prior to surgery (diabetic polyneuropathy, etc.), higher level of amputation and shorter residual limb length, prosthetization, inadequate prostheses, etc (11). Regarding the association between sex and risk of developing phantom limb pain, study results vary. Some studies report a higher prevalence in males, with one proposed explanation being more frequent traumatic lower limb amputations among men, often of occupational nature (e.g., military service, high-risk jobs). On the other hand, several studies found no difference between sexes (12). Some studies indicate that younger individuals have an increased risk of developing phantom limb pain due to greater neuroplasticity and nervous system reactivity (13), while older patients more commonly experience chronic phantom limb pain due to more frequent vascular comorbidities and DM (14). In patients with DM, complications such as diabetic neuropathy, vascular complications, and inflammatory processes may increase the risk of developing phantom limb pain (15, 16). Other studies have shown opposite results, where the presence of diabetic neuropathy was associated with a lower incidence of phantom limb pain, explained by reduced nerve sensitivity due to severe nerve damage (17). Literature data suggest that phantom limb pain is more common in patients with transfemoral amputation compared to transtibial amputation, explained by the greater degree of nerve injury during higher-level amputations. This is linked not only to a higher risk of phantom limb pain but also residual limb pain (18, 19). Similarly, some studies have demonstrated an inverse relationship between residual limb length and phantom limb pain occurrence (20). Phantom limb pain generally occurs more frequently in the pre-prosthetic phase compared to the prosthetic phase. A simple explanation for this is that these symptoms are more common immediately after surgery, when nerve damage has just occurred, before the pain has had the opportunity to become chronic. Other explanations include the psychological component of pain and the improvement in overall patient quality of life after receiving a prosthesis, as well as better neurological integration between the residual limb and the central nervous system (21).

Our study had several limitations. Considering that this was a cross-sectional study, it was not possible to determine longitudinal relationships between different parameters or causality. Additionally, the small sample size and heterogeneity of the participants limit the ability to draw definitive conclusions.

Conclusion

The results of this study indicate a statistically significant association between phantom limb pain and amputation level as well as rehabilitation phase. On the other hand, no association was found with sex, prevalence of CVS diseases and DM, nor were statistically significant differences found regarding age, residual limb length, or formation. Further research and a better understanding of these risk factors and the mechanisms behind phantom limb pain, as one of the most common complications following lower limb amputation, are necessary for improved prevention and adequate treatment of this condition.

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FAKTORI RIZIKA ZA NASTANAK FANTOMSKOG BOLA KOD PACIJENATA NAKON AMPUTACIJE DONJIH EKSTREMITETA

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Fantomski bol je česta i kompleksna komplikacijakoja se može javiti nakon amputacije ekstremiteta. S obzirom na to da su postojeće farmakološke, psihološke i rehabilitacione metode samo delimično efikasne u prevazilaženju kod problema, postoji potreba za dodatnim istraživanjima. Cilj ovog rada bilo je ispitivanje faktora rizika povezanih s pojavom fantomskog bola kod pacijenata kojima je obavljena amputacija donjih ekstremiteta. Studija preseka je sprovedena u Centru za fizikalnu medicinu i rehabilitaciju Univerzitetskog kliničkog centra Vojvodine. U studiju su bila uključena 53 pacijenta (39 muškaraca, 14 žena) podvrnuta amputaciji donjih ekstremiteta ispod kolena (22) i iznad kolena (31), u pretprotetičkoj i protetičkoj fazi rehabilitacije. Podaci su sakupljeni uvidom u medicinsku dokumentaciju i kliničkim pregledom pacijenata. Fantomski bol je zabeležen kod 16 (30,18%) pacijenata. Ustanovljena je statistički značajna povezanost između pojave fantomskog bola i nivoa amputacije ($p = 0,046$), kao i između pojave fantomskog bola i faze rehabilitacije ($p = 0,011$). Nivo amputacije i faza rehabilitacije predstavljaju statistički značajne faktore rizika za razvoj fantomskog bola.

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***Ključne reči**: fantomski bol, amputacija donjih ekstremiteta, dijabetes melitus*

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THE OCCURRENCE OF DIFFERENT TYPES OF DEPRESSION IN PATIENTS WITH CERVICAL CANCER

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When a person is diagnosed with a severe and life-threatening disease, experiences sudden hospitalization, undergoes surgical intervention or faces other treatment options threatening their bio-psycho-social integrity, it can create a stressful situation that may result in an acute stress reaction or adjustment disorders.

The global aim of the research was to examine the impact of the type of cervical cancer therapy on the psychological status of patients and the occurrence of depression.

The basic principle of the research was a comparative analysis of the results obtained using instruments to assess the degree of depression in the experimental and control groups. Within the first experimental group, a comparison was made between patients who underwent surgery and postoperative chemoradiation. Within the second experimental group, a comparison was made between patients who did not undergo surgery, but received radical chemoradiation therapy. The following research instruments were used: semi-structured psychiatric interview, gynecological parameters—local findings and stage of the disease, the Hamilton scale for depression.

Univariate logistic regression analysis showed that patients before surgery (OR = 24.17; 95% PI: 4.81–121.57 and $p < 0.001$) and patients before radical chemoradiation (OR = 45.99; 95% PI: 8.70–243.15 and $p < 0.001$) had a significantly higher risk of depression than women from the control group.

Radical hysterectomy is accompanied by an increase in depression in the preoperative period as well as after surgical treatment. Radical chemoradiation leads to an increase in depression to a greater degree compared to radical hysterectomy in the period before and after the treatment.

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Key words: *cervical cancer, depression, therapy*

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Introduction

Globally, cervical cancer is the fourth most common cancer in women, with around 660,000 new cases in 2022. In the same year, in low- and middle-income countries, about 94% of the 350,000 deaths were caused by cervical cancer (1). Cervical cancer has a good prognosis if diagnosed and treated at an early stage of the disease. A multidisciplinary team must examine the patient and determine the type of therapy for the patient, which can be surgery, radiotherapy, and systemic therapy. Also, psychological support is very important (2, 3). Genitals, along with secondary sex characteristics, denote the biological gender of an individual and gender identity is formed during the developmental process. Patients hospitalized for somatic illnesses may develop autonomic and emotional dysregulation as a consequence of numerous factors, including illness uncertainty, its course, therapy and outcome, dependency of care, as well

as accompanying physiological impairments of the somatic disease and its treatment. Just the information that a person is suffering from a severe and life-threatening disease, sudden hospitalization, surgical intervention and other treatment options threatening the bio-psycho-social integrity of an individual may pose a stressful situation that may result in acute stress reaction or adjustment disorders (4–9).

In addition to the described changes, hysterectomy triggers numerous penetrating fears related to injury and destruction, being placed in a dependent position, helplessness, fear of cancer, death, separation fear related to leaving a significant person, fear of losing sphincter control, also specific fears related to the impact of the operation on sexual functioning. Risk factors for the occurrence of depressive disorders in patients with cancer are: earlier mood disorders, alcoholism, advanced stage of cancer, the presence of inadequately controlled intense pain, the existence of a gross somatic disease or complication, and the use of chemotherapy known to cause depression. Hysterectomy leads to real losses (loss of organs, menstruation, and ability to give birth) but also symbolic losses in the field of social, family, sexual and professional functioning. All kinds of loss, and this one as well, should be acknowledged, pain should be shared with the right people around you, one should allow the grief run its course and prevent depression onset after surgery. A significant mood shift, such as depression, usually occurs on postoperative days 4 or 5, which can be understood as a part of the grieving process referred to as anticipatory grief (9–15).

Aim

The global aim of the research was to examine the impact of the type of cervical cancer therapy on the psychological status of patients and the occurrence of depression. Special attention was paid to the impact of surgical treatment on patients who underwent surgery compared to patients who did not undergo surgery for medical and personal reasons. According to data from the literature on possible types of psychological reactions in patients with cervical cancer, the degree of depression related to the type of therapeutic procedure was analyzed.

Special attention was paid to the possible influence of control variables, age, education, socio-economic status, marital status, personality (extroversion, neuroticism, introversion) of constitutive factors related to the respondents: first menstruation, cycle—regular, irregular, prolonged, shortened, number of births, number of abortions, menopause, year of first intercourse, number of partners until diagnosis, infections and whether they were treated, habits—smoking, alcohol, using contraceptives, going to the gynecologist, colposcopic examination, Papanicolaou test.

Methods

The basic principle of the research is a comparative analysis of the results obtained using instruments to assess the degree of depression in the experimental and control groups. Within the first experimental group, a comparison was made between patients who underwent surgery and postoperative chemoradiation. Within the second experimental group, a comparison was made between patients who did not undergo surgery, but received radical chemoradiation.

Experimental group I consisted of 30 patients who underwent surgery and in whom cytostatic monochemotherapy with cisplatin in a dose of 40 mg/m^2 was applied along with postoperative radiation therapy. Experimental group II consisted of 30 patients who did not undergo surgery and in whom cytostatic monochemotherapy with cisplatin, also at a dose of 40 mg/m2, was applied along with radical combined radiation therapy. The control group consisted of 30 healthy subjects.

Criteria for entering the research were: age of 30–60 years, completed minimum secondary education, marital status—married, presence of malignant lesions on the cervix, absence of serious somatic diseases, and absence of serious psychiatric disorders treated in hospital in personal history. The research was conducted in the Gynecology and Obstetrics Clinic and the Oncology Clinic of the University Clinical Center of Niš.

The following research instruments were used: semi-structured psychiatric interview, gynecological parameters—local findings and stage of the disease—and Hamilton scale for depression. The semi-structured psychiatric consisted of: 1. A list of general socio-demographic and medical history data that includes: first and last name, age, marital status, and education. 2. Psychosocial functioning. Gynecological parameters included: age at the time of first menarche, cycle duration, period between cycles, age at first birth, number of births, number of abortions, year of first sexual intercourse, number of sexual partners, infections during life, visits to the gynecologist, type of gynecological procedures performed, and reviews.

The Hamilton scale for depression was constructed due to the need to standardize the phenomenology of the depressive syndrome and to assess the severity of the depressive disorder. The scale is used in clinical and pharmacological studies. It is suitable for quantifying the intensity of depressive symptoms, and it is also a standard for evaluating the validity of other scales in measuring depression. The basic characteristics of the scale are that it is not too long, it covers the most important symptoms for assessing depression, and it is reliable even when used by two examiners. The Hamilton depression assessment scale belongs to the group of individual scales and is completed by the examiner themselves. It is used in two original versions, one

with 21 items and the other with 17, with the following scoring system: 0–7 no depression, 8–15 minor depression, 16 and over, major depression. The total score of the Hamilton scale (21 items) determines the severity of depression as follows—less than 8, suggests no depression, 17–24 indicates moderate depression in patients treated in outpatient conditions, and over 24 signifies severe depression, often associated with hospitalized patients. The absence of depression can be assessed if the total score at the end of treatment or assessment is reduced by 50% from the initial score. The scale is reliable, especially for average scores. In order to properly fill out this instrument, it is necessary to strictly adhere to the instructions that define the way of conducting the interview, the questions from the scale and the scoring of individual answers.

The Excel program from the Microsoft Office program package was used for entering, ranking, grouping, tabular and graphical presentation of data. Calculations were made using the SPSS program and the StatCalc program from the EPI-INFO program package.

Results

In the first group, the average value of the Hamilton scale for depression before therapy was 6.73 ± 3.73, then it significantly increased 3 months after surgery to 9.47 ± 4.34 (ANOVA and Dunnett's test: $p = 0.034$), and 6 months after surgery increased significantly once and reached a value of 12.33 ± 4.31 (ANOVA and Dunnett's test: $p = 0.038$). In the second group of patients, the average value of the Hamilton depression scale also increased significantly, from 9.50 ± 4.10 before the start of therapy to 12.53 ± 4.40 three months later (ANOVA and Dunnett's test: $p = 0.023$) and 16.23 ± 3.48 six months later (ANOVA and Dunnett's test: $p = 0.002$). In the control group, the average value of the Hamilton depression scale was 3.00 ± 2.39. At the first test, the value of the depression scale in patients assigned to radical chemoradiation was statistically significantly higher than in patients assigned to surgery (ANOVA and Dunnett's test: $p = 0.025$) and in the control group (ANOVA and Dunnett's test: $p < 0.001$).

Patients scheduled for surgery had a significantly higher average value of the depression scale than subjects from the control group (ANOVA and Dunnett's test: $p < 0.001$). When retested after three and six months, the value in patients who underwent radical chemoradiation remained significantly higher compared to women who underwent surgery (Mann–Whitney U test: $Z = 3.66$, i.e., $Z = 4.29$ and $p < 0.001$) (Table 1).

The average values of the Hamilton scale for depression in patients who underwent radical chemoradiation increased to a greater degree than in patients who underwent surgery, but the differences between the compared groups in this sample were not statistically significant (Table 2).

Table 1. Average values of the Hamilton scale for depression (Xsr ± SD), by groups and time of testing

Group	Testing time			Comparison by test time (Dunnett's test)
	Before therapy	After 3 months	After 3 months	
Patients who underwent surgery	6.73 ± 3.73	9.47 ± 4.34	12.33 ± 4.31	I vs. II: p = 0.034 I vs. III: p < 0.001 II vs. III: p = 0.038
Patients who underwent radical chemoradiation	9.50 ± 4.10	12.53 ± 4.40	16.23 ± 3.48	I vs. II: p = 0.023 I vs. III: p < 0.001 II vs. III: p = 0.002
Control group	3.00 ± 2.39	...	...	-
Group comparison	Dunnett's test I vs. II: p = 0.025 I vs. III: p < 0.001 II vs. III: p < 0.001	Mann–Whit ney U test Z = 3.66, p < 0.001	Mann–Whitney U test Z = 4.29, p < 0.001	

Table 2. Comparison of the resulting differences in the values of the Hamilton scale for depression within groups (Xsr ± SD), by the time of testing

Group	Differences between average scale values		
	Before therapy vs. after 3 months	After 3 months vs. after 6 months	Before therapy vs. after 6 months
Patients who underwent surgery	2.73 ± 2.86	2.87 ± 2.78	5.60 ± 3.52
Patients who underwent radical chemoradiation	3.03 ± 2.57	3.70 ± 3.24	6.73 ± 3.62
Mann–Whitney U test	n.s.	n.s.	n.s.

Moderate depression according to the Hamilton scale was present before the start of therapy in 60.00% of patients who underwent surgery, and severe depression in 3.33% of cases. Three months after the operation, the prevalence of moderate depression in this group rose to 63.33%, and severe depression to 13.30%. After six months, the frequency of moderate depression remained at the same level, and the frequency of severe depression rose to 20.00%. The Friedman test showed a significant difference in the representation of different degrees of depression between repeated tests (χ^2 = 15.93 and p < 0.001).

In the group of patients who underwent radical chemoradiation, moderate depression was present in a slightly higher percentage (66.70%) before the start of therapy, as well as pronounced (10.00%). After three months, the prevalence of moderate depression decreased to 63.30%, while the prevalence of severe depression increased to 20.00%. After six months, the share of women with moderate depression decreased to 53.30%, while the share of those with severe depression increased to 43.30%. In this group as well, Friedman's test showed a significant difference in the representation of different degrees of depression between repeated tests (χ^2 = 22.69 and p < 0.001).

There were no significant differences between the prevalence of certain degrees of depression in the group of patients who underwent surgery and patients who underwent radical chemoradiation before the start of therapy and after three months. After 6 months, the proportion of women with severe depression in the second group was more than twice as high as in the first group (43.30%:20.00%). The Mantel–Haenszel Chi-square test confirmed that this difference in the prevalence of severe depression was at the limit of statistical significance among the examined groups (χ^2 = 3.71 and p = 0.054).

In the control group, moderate depression was present in 6.70% of the subjects, and severe depression was not registered. The percentage of women without depression in this group was significantly higher than in the first group (χ^2 = 20.8 and p < 0.001) and second group (χ^2 = 29.7 and p < 0.001), while the percentage of women with moderate depression in the control group was significantly lower than in patients who underwent surgery (χ^2 = 18.9 and p < 0.001) and radical chemoradiation (χ^2 = 22.9 and p < 0.001) (Table 3).

Table 3. Representation of certain degrees of depression by groups and time of testing

Group	Degree of depression	After 3 months			Comparison by test time (Friedman's test)
		Before therapy	After 3 months	After 6 months	
Patients who underwent surgery	Without depression	11 (36.70%)	7 (23.30%)	5 (16.70%)	χ^2 = 15.93 p < 0.001
	Moderate	18 (60.00%)	19 (63.30%)	19 (63.30%)	
	Severe	1 (3.30%)	4 (13.30%)	6 (20.00%)	
Patients who underwent radical chemoradiation	Without depression	7 (23.30%)	5 (16.70%)	1 (3.30%)	χ^2 = 22.69 p < 0.001
	Moderate	20 (66.70%)	19 (63.30%)	16 (53.30%)	
	Severe	3 (10.00%)	6 (20.00%)	13 (43.30%)	
Control group	Without depression	28 (93.30%)	...	...	-
	Moderate	2 (6.70%)	...	...	-
	Severe	-	...	...	-

Comparison by groups (Mantel–Haenszel or Fisher 's test)	Without depression	I vs. II: n.s. I vs. III: χ^2 = 20.8 and p < 0.001 II vs. III: χ^2 = 29.7 and p < 0.001	n.s.	n.s.	
	Moderate	I vs. II: n.s. I vs. III: χ^2 = 18.9 and p < 0.001 II vs. III: χ^2 = 22.9 and p < 0.001	n.s.	n.s.	
	Severe	I vs. II: n.s. I vs. III: n.s. II vs. III: n.s.	n.s.	χ^2 = 3.71 p = 0.054	

Table 4. Correlation between the values of the Hamilton scale for depression and individual control and constellation factors at the first test, results of univariate logistic regression analysis

Factor		OR	95% IP		P
			lower	upper	
Group	Control	1.00	Reference		
	Before surgery	24.17	4.81	121.57	p < 0.001
	Before HRT	45.99	8.70	243.15	p < 0.001
Age		1.00	0.92	1.08	0.96
School education	Secondary	3.32	0.83	13.21	0.09
	High school	1.00	Reference		
Menarche (year)		1.34	0.98	1.83	0.07
Cycle duration (day)		0.87	0.61	1.23	0.43
Period between cycles (day)		0.98	0.83	1.16	0.84
Age at first birth (year)		0.96	0.86	1.09	0.61
Number of births		1.63	0.89	2.98	0.11
Number of miscarriages		1.05	0.86	1.28	0.58
First sexual intercourse (year)		0.97	0.77	1.24	0.86
Number of sexual partners		0.60	0.34	1.08	0.09
Smoking status	Smoker	0.81	0.35	1.89	0.64
	Non smoker	1.00	Reference		
Infections	Yes	0.36	0.15	0.86	0.02
	No	1.00	Reference		
Contraceptions	Preservative	0.15	0.01	1.33	0.09
	Pills	1.71	0.66	4.43	0.26
	No contraceptions	1.00	Reference		
Visits to gynecologist	Twice a year	1.00	Reference		
	Once a year	2.00	0.17	22.94	0.58
	Once in 2 years	3.63	0.35	37.44	0.28
	More than 5 years	7.74	0.84	71.29	0.07
Medical examinations	Never	1.00	Reference		
	Once	0.96	0.33	2.81	0.95
	Once a year	0.11	0.01	1.14	0.07
	Twice a year	0.13	0.02	0.71	0.02
	Periodically	1.14	0.28	4.54	0.85

Table 5. Correlation between the values of the Hamilton scale for depression and individual control and constellation factors at the first test, results of multivariate logistic regression analysis

Factor		OR	95% IP		P
			Lower	Upper	
Group	Control	1.00	Reference		
	Before surgery	31.07	5.64	170.96	p < 0.001
	Before HRT	52.19	9.17	296.84	p < 0.001
Infections	Yes	0.26	0.08	0.84	0.02
	No	1.00	Reference		

Univariate logistic regression analysis showed that patients before surgery (OR = 24.17; 95% PI: 4.81–121.57 and p < 0.001) and patients before radical chemoradiation (OR = 45.99; 95% PI: 8.70–243.15 and p < 0.001) had significantly higher risk of depression than women from the control group (Table 4).

Subjects who underwent examinations once every two years had a significantly lower risk of developing depression than women who never had an examination (OR = 0.13; 95% CI: 0.02–0.71 and p = 0.02).

The multivariate regression model included all factors that the univariate logistic regression analysis showed to influence the onset of depression with an estimation error probability of less than 0.1 (10%), i.e., group, schooling, age of first menstruation, number of sexual partners, infections, use of contraception, visits to gynecologist and examinations (Table 4).

By step-by-step backwards (Backwards Wald) from the multivariate model, all those factors that did not show a significant influence on the occurrence of depression were excluded, so in the last step, the following were defined as statistically significant: group and previous infections. In patients before surgery, the risk of developing depression was 31 times higher than in the control group (OR = 31.07; 95% CI: 5.64–170.96 and p < 0.001), while in patients before radical chemoradiation, the risk was even 52 times higher than in women in the control group (OR = 52.19; 95% CI: 9.17–296.84 and p < 0.001) (Table 5). In subjects who had infections, the risk of depression was 4 times lower than in those without infections (OR = 0.26; 95% CI: 0.08–0.84 and p = 0.02) (Table 5).

Bleeding during intercourse significantly increases the risk of depression (OR = 3.42; 95% CI: 1.33–8.76), as well as pain (OR = 9.75; 95% CI: 3.54–26.82) and irregular menstrual bleeding (OR = 3.42; 95 % IP: 1.33–8.76). The time to see a doctor, the attitude of the subjects towards the disease and the stage of the disease have not been proven as factors that significantly influence the occurrence of depression before the start of therapy.

During the second and third testing, none of the control, constellation and clinical factors, nor group affiliation, significantly impacted changes in the level of depression (no depression/moderate or severe depression). In the second test, only pain caused an increased risk for the occurrence of moderate or severe depression, but at the level of the probability of an error of the statement less than 0.1 or 10% (OR = 3.65; 95% IP: 0.96–13.90 and p = 0.058) (Table 6).

Table 6. Correlation between Hamilton depression scale values and clinical factors at the first test, results of univariate logistic regression analysis

Factor		OR	95% IP		P
			Lower	Upper	
First symptom	Bleeding during sexual intercourse	3.42	1.33	8.76	0.01
	Acyclic bleeding	2.71	0.65	11.23	0.17
	Increased secretion	0.48	0.20	1.11	0.09
	Painful	9.75	3.54	26.82	p < 0.001
	Irregular menstrual bleeding	4.32	1.72	10.82	0.002
Time to see a doctor longer than 6 months		0.76	0.22	2.59	0.67
Talk openly about the disease		1.36	0.42	4.32	0.60
Stage of disease	Ib	1.00	Reference		
	IIb	1.73	0.44	6.72	0.42
	IIIb	3.18	0.59	17.08	0.18

Discussion

Cancer is related to depression, which is a pathological affective response to the loss of normality as a result of cancer diagnosis, treatment or forthcoming complications. Depression presents itself with symptoms of sadness, feelings of fear and panic and longing for the lost object (organ); it is accompanied by a dysfunction increase, feelings of worthlessness and low self-esteem, suicidal preoccupations or inability to accept anything with pleasure (16–19). In our research, a higher percentage of patients treated with radical chemoradiation openly talk about their illness, but the difference compared to patients who underwent surgery in the examined sample is not statistically significant. In patients who were surgically treated, the average value of the Hamilton depression scale before therapy was 6.73 ± 3.73, then it increased significantly three and six months after surgery. The average value of the Hamilton depression scale also increased significantly in patients who underwent radical chemoradiation, from 9.50 ± 4.10 before the start of the therapy, after three and six months after the completion of the therapy. At the first test, the value of the depression scale in patients who were assigned to radical chemoradiation was statistically significantly higher than in patients who were assigned to surgery, as well as in the control group, which agrees with data from the literature (20). When retested, the value in patients who underwent radical chemoradiation

remained significantly higher than in patients who underwent surgical treatment.

In a study by Aziza et al., the HADS measurement showed that patients with cervical cancer had mild and severe levels of anxiety and depression. The results of this study also confirmed that emotional functioning, fatigue and insomnia were the main predictors of anxiety and depressive disorders in women with cervical cancer (4). Zhao et al. showed in their study that the prevalence of anxiety and depression was 44.9% and 36.1% in cervical cancer patients who underwent surgery. Compared to healthy individuals, patients with cervical cancer had a higher prevalence and worse severity of anxiety and depression. They also stated that the presence of cervical cancer and the fear of recurrence of cervical cancer may cause a severe psychological burden and worsening anxiety/depression in patients with cervical cancer who have undergone surgery. Impairment of physical and social functions, financial burden of treatment, and psychological stress of surgery may also lead to the prevalence and severity of anxiety/depression in cervical cancer patients undergoing surgery. Their study found that diabetes, FIGO stage II were independent predictors for a higher risk of anxiety, and diabetes and lymph node metastases were independent predictors for an increased risk of depression in cervical cancer patients undergoing surgery. It has been shown that a higher stage of the disease can cause a higher level of anxiety and depression (21). In accordance with the study by Zhao and our study, Tosic Golubovic et al. showed that depression and anxiety scores were relatively high among a group of patients with cervical cancer. The levels of anxiety and depression severity were different between the studied groups and FIGO stages. In the group with a more advanced FIGO stage of the disease, depression scores were higher (22). Environmental factors play a role in determining whether a vulnerable woman may develop depression major or generalized anxiety disorder, meaning that there is an environmental factor (not related to genetic factors) that is only depressogenic or only anxiogenic. Anxiety and depression are common comorbidities in cancer patients and may affect patients' survival. Providing appropriate information and social support may play a role in patients' psychological well-being, but different patients may favour different types of information and lifestyles. In assessing clinical features of depression in patients with somatic diseases, it is very important to take the full medical history that indicates the presence of depression symptoms, excludes the presence of some other psychiatric disorder, as well as the presence of psychosocial factors associated with depression (23–28).

The result of the Dhakal et al. (29) review showed that various methods (such as exercise, telephone counseling, educational brochure, family education, consultation sessions, lecture presentations, self-study package, face-to-face interviews, medication, psychotherapy, nurse support) can contribute to the effectiveness in solving the psychological support care needs "anxiety and depression" of cervical cancer patients. Very often, due to the lack of understanding of the situation and condition female patients face, there are wrong interpretations that trigger unnecessary fear, suspicion and premonition. Patients with anxious personality traits were greatly correlated with preoperative anxiety onset, but also with ongoing anxiety after hospital discharge, along with increased depression, fatigue, low frustration tolerance threshold, resulting in frequent visits to the doctor. Assessment measures conducted in studies (23, 30–32) determined the range of emotional disturbances. The results of these studies show that simply talking with patients may be useful in revealing women at risk of anxiety, depression, or both.

Conclusion

Radical hysterectomy is accompanied by an increase in depression in the preoperative period as well as after the surgical treatment. Somatic disorder and hospitalization are crisis events that trigger the feeling of helplessness, risk of loss and passive position, as well as adjustment disorder with dominant depressed mood as a clinical manifestation. Radical chemoradiation leads to a greater increase in depression compared to radical hysterectomy in the period before and after the treatment.

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POJAVA RAZLIČITIH VRSTA DEPRESIJE KOD PACIJENTKINJA SA KARCINOMOM GRLIĆA MATERICE

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Informacija da osoba boluje od teške bolesti koja je opasna po život, iznenadna hospitalizacija, hirurška intervencija i druge mogućnosti lečenja koje mogu ugroziti biopsihosocijalni integritet pojedinca predstavljaju stresnu situaciju koja može dovesti do akutne reakcije na stres ili poremećaja prilagođavanja.

Globalni cilj ovog istraživanja bio je da se ispita uticaj vrste terapije karcinoma grlića materice na psihički status pacijentkinja i pojavu depresije.

Osnovni princip istraživanja bila je komparativna analiza rezultata dobijenih korišćenjem instrumenata za procenu stepena depresije u eksperimentalnoj i kontrolnoj grupi. U okviru prve eksperimentalne grupe izvršeno je poređenje pacijentkinja koje su podvrgnute operaciji i postoperativnoj hemoradijaciji. U okviru druge eksperimentalne grupe urađeno je poređenje pacijentkinja koje nisu bile operisane, ali su bile podvrgnute radikalnoj hemoradijaciji. Korišćeni su sledeći instrumenti istraživanja: polustrukturirani psihijatrijski intervju, ginekološki parametri (lokalni nalazi i stadijum bolesti) i Hamiltonova skala za depresiju.

Univarijantna logistička regresiona analiza pokazala je da je kod pacijentkinja pre operacije (OR = 24,17; 95% PI: 4,81–121,57 i p < 0,001) i kod pacijentkinja pre radikalne hemoradijacije (OR = 45,99; 95% PI: 8,70–243,15 i p < 0,001) postojao značajno veći rizik od razvoja depresije nego kod pacijentkinja iz kontrolne grupe.

Radikalna histerektomija je praćena porastom depresivnosti i u preoperativnom periodu i nakon operativnog lečenja. Radikalna hemoradijacija dovodi do povećanja depresivnosti u većem stepenu nego radikalna histerektomija i pre i posle tretmana.

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***Ključne reči**: karcinom grlića materice, depresivnost, terapija*

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DELIBERATE SELF-POISONING IN ADOLESCENTS: A 3-YEAR SINGLE CENTRE STUDY

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Adolescence represents a period during which a child, in a transition period to adulthood, undergoes significant changes, often accompanied by risky behaviors such as intentional self-poisoning. This study aimed to identify the sociodemographic characteristics of adolescents, substance distribution and the impact of school success and completeness of the nuclear family on self-poisoning incidents. A retrospective study examined the records of 412 patients treated at the Institute for Child and Youth Health Care of Vojvodina over a three-year period. The average age of the patients was 15.7 ± 1.8 years. The most commonly used substances were alcohol (67%), benzodiazepines (23.6%), and cannabis (5.8%). One-fifth of the subjects took at least two substances simultaneously, with a higher frequency among female adolescents who experienced more frequent episodes of repeated self-poisoning. Half of the subjects (51%) lived in complete nuclear families, and a significant difference was found regarding alcohol consumption compared to other subjects. There is a significant difference in the consumption of benzodiazepines and alcohol concerning academic success. Alcohol and benzodiazepine medications were most commonly used for self-poisoning, with a significant gender difference. Due to the widespread availability of benzodiazepine medications, they were the most frequently used drugs. Girls were at a higher risk of repeated self-poisoning. Adolescents living in complete nuclear families with excellent academic success more often consumed alcohol, while those from incomplete families with poor success more frequently consumed benzodiazepines.

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Key words: *deliberate self-poisoning, adolescence, puberty, alcohol, benzodiazepines*

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Introduction

The World Health Organization (WHO) defines adolescents as individuals aged 10 to 19 years old (1). Adolescence represents the transition from childhood to adulthood, during which a child is expected to achieve maturity and functionality as an adult (2). During this period, there are changes in school relationships and social integration, resulting in less time spent with parents and more time with friends, increasing their autonomy (3). Adolescents undergo significant physical, mental and emotional changes during this period, which make them susceptible to risky behaviors, including deliberate self-poisoning. According to the WHO, deliberate self-poisoning is defined as an act with a non-fatal outcome in which an individual deliberately ingests a substance in doses exceeding therapeutic ones, resulting in harm to the body (4–8). The most common substances involved in poisoning are medications, alcohol and drugs. These incidents often stem from conflicts in school and family (9). Adolescent self-poisoning has a low mortality rate but is often a predictive factor for mental health disorders. Acute poisonings are a significant cause of morbidity and mortality among adolescents, representing one of the most common urgent conditions (10–14). According to WHO data, acute poisonings result in over 45,000 deaths annually in individuals under 20 years old (15). Poisonings, along with injuries, are ranked third in the most common causes of hospitalization among adolescents in Vojvodina, after respiratory and digestive diseases. Acute poisoning accounts for 2.35% of hospital morbidity in children in Vojvodina, with a mortality rate of 0.19% (16). The objectives of this study were to determine the demographic characteristics of adolescents who committed intentional self-poisoning, to identify

the most commonly consumed substances and reasons for self-poisoning, to determine the incidence of repeated self-poisoning, as well as the role of school success in relation to the type of substance ingested and the role of family nuclear completeness.

Materials and Methods

A retrospective study was conducted analyzing data from the medical records of 412 patients treated at the Institute for Child and Youth Health Care of Vojvodina from January 1st 2016, to January 1st 2019, aged 10 to 18 years and diagnosed with acute poisonings and self-poisonings. Out of 412 patients diagnosed with acute poisoning, 364 were identified as intentional self-poisoning cases. Data from patients' medical records, including physician reports, psychologist reports, medical history, laboratory results and other tests were used. Data on the number of patients admitted to the institute, demographic data, laboratory and toxicological analysis results, anamnestic data on reasons for self-poisoning, suicidal intent, family nuclear completeness and school success were analyzed. Statistical analysis was performed using Microsoft Office Excel 2010, presenting data as means and percentages. The chi-square test and Student's t-test were used to determine statistical significance. The study was approved by the Ethics Committee of the Institute for Child and Youth Health Care of Vojvodina.

Results

Reviewing the medical records of 412 patients aged 10 to 18 years treated at the Institute for Child and Youth Health Care of Vojvodina from January 1st 2016, to January 1st 2019, who were diagnosed with acute poisoning and self-poisoning, 364 patients were found to have actively and intentionally committed self-poisoning. There were 184 male patients (50.55%) and 180 female patients (49.45%) with an average age of 15.75 years. The distribution of self-poisoning by gender and age is shown in Figure 1.

According to the chi-square test results, there was a statistically significant difference between boys' and girls' self-poisoning regarding the poisoning agent ($p < 0.01$). Boys/men more frequently used alcohol for self-poisoning, while girls/women used benzodiazepine, antipyretic and analgesic medications for poisoning more often. Figure 2 shows the most commonly used substances for intentional self-poisoning and their distribution by gender.

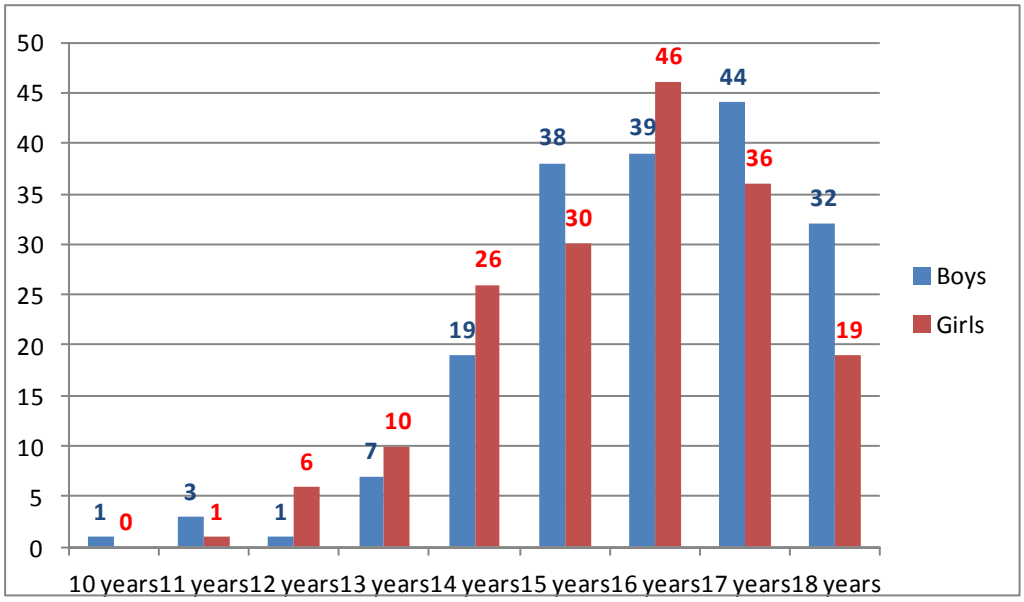

Figure 1. Distribution of self-poisoning by gender and age

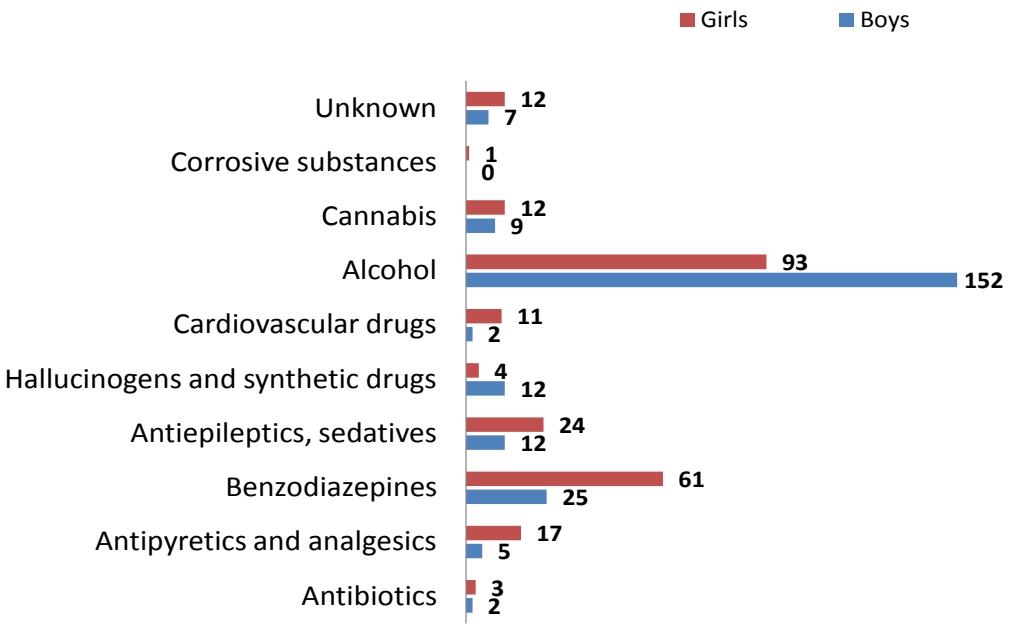

Figure 2. Gender distribution of self-poisoning by causes

Out of the total number of subjects (364), 79 subjects consumed more than one substance (21.4%), including 29 boys/men (36.71%) and 50 girls/women (63.29%). Adolescents most commonly combined benzodiazepines with other substances, 48 subjects in total (60.76%). The most common among those substances were antiepileptic, sedative and antiparkinsonian drugs, 15 subjects in total (31.25%) and alcohol, 13 subjects in total (27.08%). The second most frequent combination was alcohol with other substances, 30 subjects in total (37.97%), most usually in combination with benzodiazepines, 13 subjects in total (43.33%). Student's t-test results showed a statistically significant difference between the number of self-poisoning boys and girls regarding the number of ingested substances ($p < 0.05$). Girls consumed multiple substances simultaneously more often. Anamnestic data on previous self-poisoning attempts were obtained for 340 subjects (93.4%). Out of these, 295 subjects had data on their first self-poisoning attempt (86.76%), with 151 male subjects (51.19%) and 144 female subjects (48.8%). Student's t-test results showed no statistically significant difference in the frequency of first self-poisoning attempts between boys/men and girls/women. Using the chi-square test, a statistically significant difference was found by gender regarding repeated self-poisoning attempts, with girls/women having more repeated self-poisoning attempts than boys/men ($p < 0.05$). Data on the reasons for self-poisoning were obtained for 304 subjects and are presented in Figure 3.

Anamnestic data on family structure were obtained for 122 subjects (33.52%). Out of these, 63 subjects confirmed living in a complete nuclear family (51.64%), while 59 subjects lived in an incomplete nuclear family (48.36%). According to the T-test results, there is a statistically significant difference between adolescents living in complete nuclear families and those living in incomplete nuclear families in terms of alcohol consumption ($p < 0.05$) and benzodiazepine consumption ($p < 0.05$), with patients from complete families more frequently having alcohol poisoning and those from incomplete families more frequently having benzodiazepine poisoning. Data on school success were found for 173 subjects (47.53%). Out of these, 45 were excellent students (26.01%), 58 were very good (33.53%), 25 were good (14.45%), 5 were sufficient (2.89%), 17 were insufficient (9.83%), and 23 adolescents dropped out of school (13.29%). The relationship between consumed substances and school success is shown in Figure 4.

Chi-square test results showed a statistically significant difference ($p < 0.05$) in benzodiazepine and alcohol consumption according to school success. Students achieving excellent and very good grades more frequently consumed alcohol for self-poisoning purposes, while students with insufficient grades or those who dropped out of school more frequently consumed benzo-diazepines.

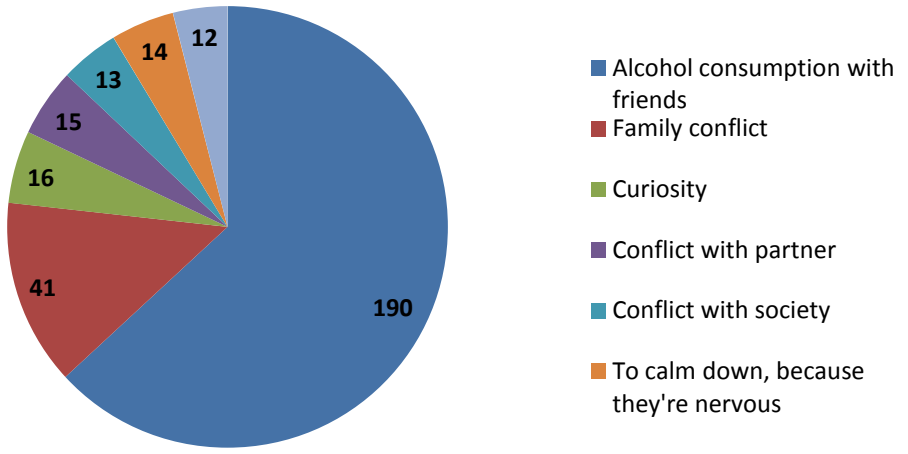

Figure 3. Distribution of self-poisoning according to the intent of self-poisoning

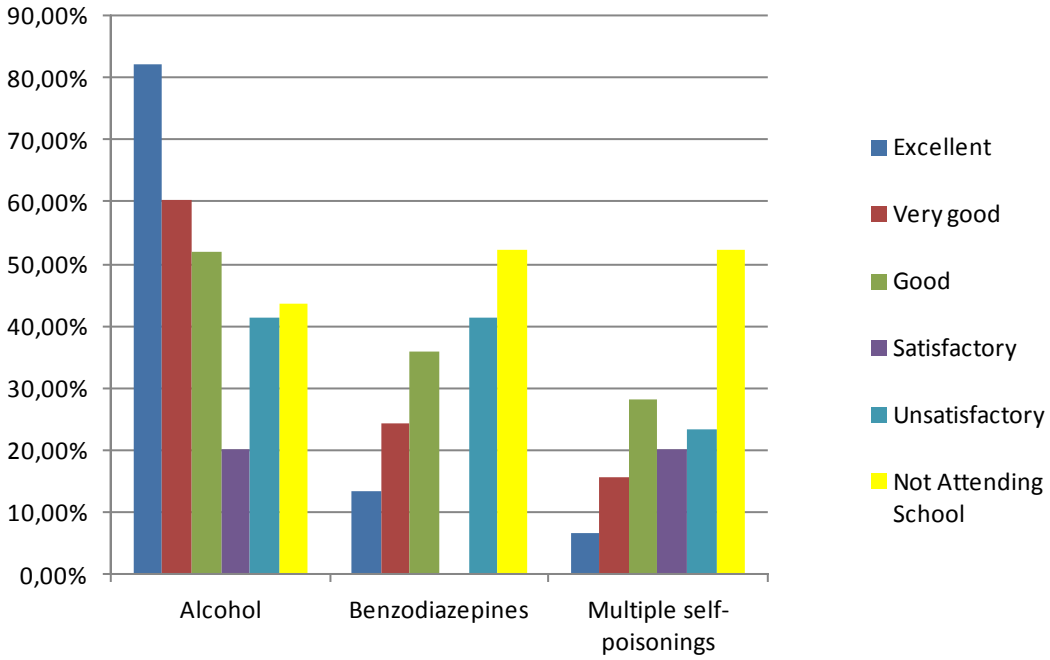

Figure 4. Distribution of substance use leading to self-poisoning in relation to school success

Discussion

There was no statistically significant difference between boys and girls who committed self-poisoning, although according to the literature (17–20), girls more frequently attempt self-poisoning. In this study, the majority of adolescents were 16 and 17 years old. Le Valliant (20) found that the children diagnosed with intentional self-poisoning were most often aged 10 to 14 years. The results of this study may differ from Le Valliant's results because his study included individuals under 10 years of age. The results of this study are consistent with the results of Nistor, Zakharov, and Navratil (9, 21, 22). The most commonly used substances leading to self-poisoning were alcohol, benzodiazepines, antipyretics, analgesics and drugs from the antiepileptic, sedative and antiparkinsonian groups. Similar results were obtained by other authors (9, 14). The most common cause of self-poisoning in adolescence was alcohol, with boys more frequently being consumers, consistent with other authors' results (9, 14). The most common

drugs used for self-poisoning were benzodiazepines. This differs from other authors' results, where acetaminophen was most commonly used for poisoning. However, in this study, analgesics and antipyretics were third in frequency (9, 20). This could be explained by the fact that benzodiazepines are much more commonly prescribed and abused in Serbia than in Western countries, making them more accessible to adolescents (21). Zakharov and Navratil (22) found that 30% of subjects consumed more than one substance, with more girls doing so than boys. Results are consistent with the results of this study, in which a statistically significant difference in favor of girls was observed. The majority of subjects (86%) had their first self-poisoning attempt as other authors concluded (22, 23). This study found a statistically significant difference between boys and girls who self-poisoned multiple times, with results indicating that girls more frequently had repeated self-poisoning attempts. In a Canadian study, the percentage of adolescents re-exposed to self-poisoning over a five-year observation period was around 16% (11). Boys more frequently consume alcohol during adolescence, and intoxication most often results from alcohol consumption (9, 14, 24). Conflicts within the family as well as partners, and conflicts within society were more often triggers for self-poisoning in girls (67.5%) than in boys (32.5%), consistent with Nistor's research (9). This could be explained by the fact that girls are more empathetic during adolescence than boys (25). Family structure and relationships within it are significant factors in developing self-harm ideation among children (26). Divorce, poor economic and social relationships within the family, as well as living with only one parent, also represent risk factors for developing self-harm ideation (27). The results show a statistically significant difference between adolescents living in complete nuclear families and those living in incomplete nuclear families in terms of alcohol and benzodiazepine consumption. Adolescents from complete nuclear families more frequently had alcohol poisoning as a cause of self-poisoning, while those from incomplete families more frequently consumed benzodiazepines for self-poisoning. There is a statistically significant difference in benzodiazepine and alcohol consumption according to school success. Students achieving excellent and very good grades more frequently poisoned themselves with alcohol, while students with insufficient grades or those who dropped out of school more frequently consumed benzodiazepines. In his study, Blair (27) demonstrated that students who achieve very good and excellent results in school have a lower tendency towards risky and delinquent behavior compared to poor-performing students, but alcohol use is equal among them. However, the use of psychoactive substances is slightly more common among adolescents with poorer school performance.

Conclusion

Based on the study results and objectives, the following conclusions can be drawn. The average age of adolescents who committed self-poisoning was 16 years. Alcohol was the most common substance consumed for self-poisoning, more often among boys/men than girls/women, while benzodiazepines were the most commonly consumed pharmacologically active substances, more often among girls/women than boys/men. Girls/women statistically significantly more frequently used multiple substances simultaneously for self-poisoning. The most common reason for self-poisoning was "enjoyment" of alcohol in social settings, more often among boys/men than girls/women, while the most common reasons for self-poisoning among girls/women were "family conflict" and "social conflict." The results showed that girls/women more frequently had repeated episodes of self-poisoning compared to boys/men. Adolescents from complete nuclear families more frequently consumed alcohol for self-poisoning, while those from incomplete nuclear families more frequently consumed benzodiazepines for self-poisoning. There is a statistically significant difference in benzodiazepine and alcohol consumption according to school success, whereas students achieving excellent and very good grades more frequently poisoned themselves with alcohol, while students with insufficient grades or those who dropped out of school more frequently consumed benzodiazepines.

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NAMERNA SAMOTROVANJA ADOLESCENATA: TROGODIŠNJE ISKUSTVO JEDNOG ZDRAVSTVENOG CENTRA

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Adolescencija je period u kojem dete doživljava važne promene i u kojem je podložno rizičnom ponašanju. Jedan oblik takvog ponašanja čine namerna samotrovanja, koja nisu retka pojava. Cilj ovog rada bio je da se utvrde sociodemografske karakteristike adolescenata, distribucija korišćenih supstanci, kao i uticaj koji uspeh u školi i kompletnost nuklearne porodice imaju na čin samotrovanja.

Sprovedena je retrospektivna studija uvidom u dokumentaciju 412 pacijenata koji su lečeni u Institutu za zdravstvenu zaštitu dece i omladine Vojvodine u trogodišnjem periodu.

Prosečna starost ispitanika bila je 15,7 godina. Najčešće korišćene supstance bile su alkohol (67%), lekovi iz grupe benzodijazepina (23,6%) i kanabis (5,8%). Petina ispitanika je uzimala najmanje dve supstance istovremeno; to je bilo češće kod adolescentkinja, kod kojih su zabeležene i češće epizode ponovljenih samotrovanja. Polovina ispitanika (51%) živela je u kompletnoj nuklearnoj porodici. Kada je reč o konzumiranju alkohola, utvrđena je značajna razlika između ispitanika iz pomenute grupe i ostalih ispitanika. Postoji značajna razlika u konzumiranju benzodijazepina i alkohola i kada se u obzir uzme uspeh u školi.

Za samotrovanje su najčešće korišćeni alkohol i lekovi iz grupe benzodijazepina, s tim što postoji značajna razlika među polovima. Zbog činjenice da se lekovi iz grupe benzodijazepina lako mogu nabaviti, oni se u najvećoj meri koriste za samotrovanje. Kod devojaka postoji veći rizik od ponovne epizode samotrovanja. Adolescenti koji žive u kompletnim nuklearnim porodicama i imaju odličan uspeh u školi češće su konzumirali alkohol, dok su se adolescenti iz porodica koje nisu kompletne nuklearne porodice i koji imaju loš uspeh u školi uglavnom odlučivali za benzodijazepine.

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***Ključne reči**: namerno samotrovanje, adolesencija, pubertet, alkohol, benzodijazepini*

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THE SYNERGY OF FORENSIC MEDICINE AND BLOODSTAIN PATTERN ANALYSIS IN DETERMINING THE MANNER OF DEATH

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A forensic pathologist and a bloodstain pattern analyst are involved in determining the manner and the mechanism of death, but based on different scientific approaches. Our work presents the benefits of a holistic approach to forensic expertise. We have analyzed and described three cases. In all cases, the initial police investigation had concluded the wrong manner of violent death (accident, suicide, homicide), but the subsequent synergy of forensic autopsy and bloodstain pattern analysis (BPA) determined the true nature of death. BPA in Serbia is a relatively new forensic discipline. Police authorities (Crime scene evidence technicians) in Serbia are familiar with BPA, but according to our legislation, they are not allowed to provide expertise in the courtroom. The benefits of synergistic expertise in forensic medicine and BPA are more than evident in our cases. Therefore, the education of specialists in forensic medicine in BPA will be useful for solving suspicious and indistinct cases.

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Key words: *forensic medicine, autopsy, bloodstain, bloodstain pattern analysis*

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Introduction

A forensic pathologist and a bloodstain pattern analyst are differently educated experts. They are both involved in determining the manner and the mechanism of death, but based on different scientific approaches.

The forensic pathologist performs autopsies to determine the cause and manner of death (homicide, suicide, accidental, natural, undetermined). Also, the forensic autopsy reveals different pathological processes, injuries, and diseases that directly or indirectly initiate a series of events that lead to a person's death (the mechanism of death). The forensic pathologist examines a corpse and documents all injuries, along with the possible cause of injuries. Based on those findings, along with police crime scene reports, the forensic pathologist can determine the manner of death and the mechanism of injuries. Sometimes, those findings are insufficient for determining the manner of death and the mechanism of injuries, so another field of forensic expertise must be included (1, 2).

The bloodstain pattern analysis (BPA) is based on scientific examination of the size, shape, distribution, and location of the bloodstains to recreate the actions that caused the bloodshed and to form opinions about what did or did not happen. BPA uses principles of biology, medicine, physics, chemistry, and mathematics, so this is a multidisciplinary branch of forensic science. BPA analysts can determine types of bloodstains by gathering information from spatter patterns, transfers, voids, and others, to assist investigators in recreating the sequence of events that occurred during and after bloodshed. This important information assists the investigators in reconstructing the crime, including or excluding potential perpetrators from the investigation, and scientifically testing statements from witnesses. Based on facts and information gathered from BPA expertise, it is possible to determine the manner of death (3–7).

Currently, it is not often that a forensic expert is simultaneously educated in forensic medicine and BPA. In Serbia, there are only two forensic experts with those qualifications. Our work presents the benefits of a holistic approach to forensic expertise of violent death (4, 8).

Aim

The aim was to evaluate the benefits of the holistic approach in determining the manner of

death with the synergy of forensic medicine and bloodstain pattern analysis.

Material and Methods

Material was obtained from expert reports of the Institute of Forensic Medicine in Niš, Serbia. We have simultaneously analyzed reports of three cases, where the autopsy and the BPA expertise were performed, but individually they couldn't explain all important circumstances of the death.

Results

We have analyzed and described three cases. In all cases, the initial police investigation had concluded the wrong manner of violent death (accident, suicide, homicide), but the subsequent synergy of forensic autopsy and BPA determined the true nature of death.

Case 1: The deceased, a 73-year-old man, was found in an auxiliary house on his farm (Figure 1). From the house to the barn, many bloodstains were found on the ground, forming a drip trail pattern. Crime scene investigators found some bloodstains in front of the barn on the white bag, but also in the barn, bloodstains were found in one of the buckets with water. Police assumed that the man was injured in the barn by one of the cows, where the bloodstains began, and that he reached the place where the corpse was found. So, investigating authorities had believed at first it was an accidental death. The autopsy revealed that the death was due to a head injury, but it was unlikely that it was an accidental death.

Figure 1. A. Sitting position of the victim with bloodstains; B. Drip bloodstains forming a drip trail pattern from the victim to the barn; C. Bloodstains in the barn; D. Spatter bloodstains on the white plastic bag in front of the barn with a pool of blood (red circle) and established area of convergence of spatter pattern (red-yellow stars)

On-site BPA analysis revealed that at least three spatter patterns of bloodstains were found on the white plastic bags in front of the barn. Further analysis revealed that the point of origin of those spatter patterns was consistent with the victim's head position at the time of injury (point of origin marked by red-yellow stars). In front of the barn, a larger pool of blood was found on the ground, indicating that the victim had been motionless on the ground for a long time after suffering head injuries. Comparative analysis of blood traces in the barn with traces of diluted bloodstains on the shirt of the victim indicates that the victim was in the barn when there was already liquid blood on him, and then the victim came in contact with water. Furthermore, traces of blood flow on the head and the front of the body indicate that the injured person was in a standing position for some time after suffering a head injury. The injuries to the head were otherwise of such a nature that he could move independently for a certain period. Based on the results of BPA

expertise, the established sequence of events was: 1) victim getting injured in front of the barn; 2) entering the barn and contacting the water; 3) then probably moving independently to the auxiliary house, where the body of the deceased was found. It was then absolutely confirmed that it was a homicide.

Although the autopsy unequivocally revealed violent death, the manner of death was inconclusive. Subsequent BPA at the crime scene revealed the position of the deceased at the time of sustaining injuries, which was inconsistent with the previous assumption of accidental death. The combination of facts obtained from both reports was terminated by the arrest of two offenders.

Case 2: A man reported that he and his wife had been attacked by unknown assailants. The man stated to police that he had been attacked in front of the house, that he had sustained a head injury and lost consciousness, but immediately after his awareness, he called a neighbor to contact the police. A clinical forensic examination revealed that he was indeed injured in the occipital region, so his story was not in doubt.

The deceased 48-year-old woman was found in the bedroom, and many bloodstains were found on the wall, bed, and around the corpse (Figure 2). The on-site BPA analysis revealed that at least four spatter patterns had been located on the wall above the headboard of the bed, and placed one over the other. This practically means that each spatter pattern corresponded to at least one blow to the victim`shead. Interesting was the area with no bloodstains, and that area was the victim's pelvic region. This void area of bloodstains corresponded to some object, which was moved after inflicting injuries on the victim, and that object was the assailant.

Figure 2. A. Position of the victim with the general aspect of the crime scene; B. Bloodstains around the victim with a void area of spatter bloodstains in the pelvic area (between green lines); C. Spatter pattern on the wall above the headboard of the bed with established four areas of convergence of the spatter pattern (red-yellow stars); D. Spatter stains on the back of the T-shirt

A corpse examination of the victim revealed that there were head injuries inflicted with at least 12 blows. Most of the wounds were large linear lacerations with multifragmenting skull fractures. Those injuries were inflicted by an elongated cylindrical object similar to a pole or stick. The number and manner of inflicting injuries indicated a strong emotional discharge of the assailant, which could also mean a close emotional connection between the victim and the assailant, and at that time, the suspicion that the husband might be the perpetrator was raised. The entire house was inspected, and a bloodstained man's T-shirt was found in the laundry basket, with bloodstains on the front and back of the T-shirt. The dilemma about the identity of the person who was wearing this shirt was easily solved by later DNA analysis, and it was the husband of the victim.

Based on the corpse examination and the bloodstain pattern analysis, we concluded:

1. The victim sustained head injuries in approximately the same position of her head in the space as her corpse was found, by repeated blunt force strikes to her head.

2. The weapon used in this homicide was an elongated cylindrical object similar to a pole or stick. Later, a bloodstained metal bar was found in a cesspit.

3. The assailant was positioned above the victim, face to face, probably sitting across her pelvic region or by some other part of his body covering her pelvic region. From this position, the assailant, who wore a gray T-shirt during a critical event, swung a blood-stained weapon over his head and his right shoulder, and he probably held the weapon in his right hand when blood spatters were projected on the back of this T-shirt, at the feet of the victim and on the bedding along her feet.

Case 3: This case was a homicide of an 82-year-old woman with shotgun pellets. An autopsy of the victim revealed two gunshot wounds, one massive gunshot wound on the left forearm, and a lot of single wounds on the front side of the chest and abdomen (Figure 3). Based on autopsy findings and the degree of pellet scattering, a wound on the left forearm was inflicted from a discharge range of about 2 meters, while gunshot wounds on the torso were inflicted from a discharge range of about 18–20 meters. At some point during the autopsy, it was assumed that the left forearm was raised in front of the body, that the attacker did indeed shoot from a distance of about 2 meters, and that the gunshot wounds to the torso were caused by secondary scattering of projectiles on the left forearm bones. Analysis of gunshot residues couldn't provide a scientific answer, but BPA could clear this situation. To prove this, we conducted a detailed laboratory examination of the victim's shirt by a BPA expert.

The victim's shirt was colorful and extremely difficult to analyze, so in those situations, BPA uses the benefits of the infrared camera. An infrared camera helps BPA experts to clear out patterns of textiles and turn dark colors into bright ones, while bloodstains remain dark. Using this method, it has been shown that there are spatter patterns of bloodstains around most entrance wounds on the torso. Those spatters confirmed our theory that the pellets came to the torso together with the cloud of small blood drops. The source of blood for those blood drops was the gunshot wound on the left forearm.

After completing both autopsy and BPA expertise, it was concluded that there was a single shot from a close range, with a secondary scattering of pellets at the broken forearm bone fragments. At first, it seemed to be two shots, one from close range in the forearm and the other from a distance in the chest and abdomen, by the synergy of forensic autopsy and BPA revealed the true mechanism of injury.

Figure 3. A. General aspect of the victim with clothing; B. Massive gunshot wound of the left forearm and scattered pellet wounds on the front side of the torso; C. Close aspect of the shirt; D. Close-up aspect of the rupture on the front side of the shirt in infrared red camera live-view with dark blood spatters (note the bright clear color of the shirt)

Discussion

The organization scheme is very diverse across forensic laboratories worldwide, so it means there is still no perfect one. Authorities in the forensic science community have different views on writing reports by experts educated in different fields of forensic science. Also, some of those authorities have different opinions on the importance and proportion of generalist and specialist experts. Bigger laboratories have the privilege of having a wide range of different experts. Moderately sized laboratories have a limited number of employees, so it would be more appropriate to educate one forensic scientist in a few diverse fields of forensic science, but also in the basic principles of BPA (4, 5, 8–11).

A forensic pathologist and a BPA analyst are both interested in the wounds of a victim and bloodstains, and they are both involved in determining the manner and the mechanism of death, but based on different facts. They use different methodologies for different objects of interest, resulting in a similar final goal. By combining knowledge of those two forensic disciplines, we can gain an overall evaluation and more reliable facts about crime events. There are only a few experts in the world simultaneously educated in those disciplines. Our work presents the benefits of the holistic approach to violent death-related forensic expertise. BPA is grounded on principles of physics, biology, chemistry, and medicine. Rising out of those ground principles, it is clear that a forensic pathologist is the most suitable forensic scientist to be additionally educated in BPA (1–3, 6).

BPA is relatively new in Serbia in the form that it exists in leading world countries. For most judges and prosecutors this is something new. Most people are untrustworthy to new things, especially in new scientific areas. Recent scientific research revealed that conclusions in BPA reports were often erroneous (11.2%) and often contradicted other analysts (7.8%). Those results suggest a need for improved standards in BPA, but those results could be a consequence of insufficient knowledge of experts. Contradictory interpretations contributed to errors and disagreements, which could have serious implications if they occurred in casework. Confidence in BPA expertise could be obtained through strict standards, defined terminology, accredited education, and clear expertise by a BPA analyst (3, 5, 8).

Conclusion

BPA in Serbia is a relatively new forensic discipline. Police authorities (crime scene evidence technicians) in Serbia are familiar with BPA, but according to our legislation, they are not allowed to provide expertise in the courtroom. The benefits of synergistic expertise in forensic medicine and BPA are more than evident in our cases. Therefore, our work showed that educating forensic medicine specialists in the BPA is useful for solving suspicious and indistinct cases.

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SINERGIJA SUDSKE MEDICINE I ANALIZE OBRAZACA KRVNIH MRLJA PRILIKOM ODREĐIVANJA PRIRODE SMRTI

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Specijalista sudske medicine i analitičar obrazaca krvnih mrlja uključeni su u utvrđivanje prirode i mehanizma smrti, s tim što se njihov naučni pristup razlikuje. U ovom radu predstavljaju se prednosti holističkog pristupa forenzičkoj ekspertizi. Analizirana su i opisana tri slučaja. Početna policijska istraga je u svim slučajevima ustanovila prirodu nasilne smrti koja je bila pogrešna (nesrećni slučaj, samoubistvo, ubistvo). Međutim, naknadnom sinergijom sudske medicine i analize obrazaca krvnih mrlja (engl. *bloodstain pattern analysis* – BPA) utvrđena je prava priroda smrti. BPA je u Srbiji relativno nova forenzička disciplina. Mada su policijski istražni organi (kriminalistički tehničari na mestu zločina) u Srbiji upoznati sa osnovnim principima BPA, naše zakonodavstvo im ne dozvoljava da obavljaju veštačenja u sudnici. Prednosti zajedničkog sudskomedicinog i BPA veštačenja više su nego očigledne u prikazanim slučajevima. Stoga, može se zaključiti da bi edukacija specijalista sudske medicine u oblasti BPA bila korisna u rešavanju sumnjivih i nejasnih slučajeva.

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***Ključne reči**: sudska medicina, obdukcija, krvna mrlja, analiza obrazaca krvnih mrlja*

Review article

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PREOPERATIVE ASSESSMENT FOR NON-SMALL-CELL LUNG CANCER SURGERY

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Lung cancer is the most common malignant tumor and globally the leading cause of death from malignant diseases in the general population, accounting for approximately 25% of all cancer deaths. According to current data, approximately 7,000 people are diagnosed with lung cancer in Serbia annually, and about 5,000 die from the disease. Treatment for lung cancer is complex and multidisciplinary, with surgery playing a central role in stages I to III-A. Despite numerous studies confirming that surgical resection offers the best chance of recovery, only 20–30% of patients are eligible for surgery at the time of diagnosis. There are many reasons for this, including advanced disease, comorbidities, weakened respiratory function, and poor performance status. Considering that surgical lung resections are often accompanied by peri- and postoperative complications, a detailed preoperative risk assessment is crucial for determining the outcome of treatment. The remainder of this text will outline the currently valid guidelines and protocols for preoperative risk assessment, with a particular focus on high-risk patients (elderly, smokers, chronic obstructive pulmonary disease—COPD—patients, and obese individuals). It will also delve into the role of spirometric-diffusion parameters and stress tests in this assessment.

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Key words: *lung cancer, surgery, spirometry, diffusion, stress tests*

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Introduction

Lung cancer is the most common malignant tumor and the leading cause of death from malignant diseases in men. In women, it is the third most common malignant tumor and the second most common cause of death (1). In Serbia, according to the data of the "Dr. Milan Jovanović Batut" Institute of Public Health, 6,863 people were diagnosed and 5,242 people died from lung cancer in 2019 (2).

Treatment for lung cancer can be surgical or conservative (chemotherapy, radiation therapy, targeted molecular therapy, and immunotherapy). Depending on their overall health status, non-small-cell lung cancer (NSCLC) patients with stage I, II, and III-A may be candidates for surgical treatment (3). Radical surgical intervention offers the best chance of cure. This is supported by the fact that the median survival of NSCLC patients who were not surgically treated and were diagnosed with stage I disease was only 13 months or 25 months (if detected by screening versus symptomatic disease) (4). Unfortunately, at the time of diagnosis, as much as 70–80% of patients are not eligible for surgery, mainly due to advanced disease, comorbidities, and, consequently, poor general health. According to some authors, the percentage of patients with anatomically resectable NSCLC who are not suitable for surgical treatment solely due to poor respiratory function is as high as 37% (5–7). Moreover, 50–70% of NSCLC patients have COPD, arterial hypertension, diabetes mellitus, peripheral vascular disease, and/or other significant comorbidities, which can further complicate potential surgical treatment (8, 9).

Considering the above, it is clear that a properly conducted preoperative evaluation is central to preventing complications, which are otherwise relatively frequent following NSCLC resection (5, 10). Therefore, addressing this issue necessitates a team effort (involving thoracic surgeons, cardiologists, pulmonologists, and anesthesiologists) and an extremely responsible approach.

Preoperative Assessment of Cardiorespiratory Function

The most common cardiorespiratory complications following NSCLC resection include: prolonged mechanical ventilation, reintubation, acute respiratory distress syndrome, pneumonia, atelectasis requiring bronchoscopy, pulmonary embolism, unstable angina pectoris, myocardial infarction, heart failure, and arrhythmias (11). According to Motono et al., the main predisposing factors associated with the development of the aforementioned conditions include male gender, age over 65 years, COPD, upper lobectomy, surgery duration > 2.5 h, lymphovascular invasion, and body mass index (BMI) of less than 21.68 (10). Petrella et al. came to similar conclusions, claiming that malnutrition (BMI < 18.5), obesity (BMI > 30), active smoking, obstructive sleep apnea, COPD, and asthma are the primary triggers for the development of postoperative complications (11).

The basic parameters to be considered when determining the functional operability of an NSCLC patient include age, general health condition, performance status, cardiorespiratory function, physical fitness, and extent of lung resection (8). The latter is significantly correlated with the rate of postoperative mortality, which, according to the results of Powell et al., reaches 2.3% after lobectomy and 7% after pneumonectomy (13).

Regarding the patient's age, today, most authors believe it is not a contraindication for surgical treatment of NSCLC (more than 30% of patients are over 70 years old) (6, 14). However, the risk of postoperative mortality increases with age, primarily due to comorbidities and impaired cardiorespiratory function. In patients over 70 years of age, it reaches 7% after lobectomy and 14% after pneumonectomy (14). Despite this, current recommendations are that elderly patients should be evaluated using the same algorithms as younger people (14).

European Respiratory Society (ERS) and European Society of Thoracic Surgeons (ESTS) experts recommend that all candidates for NSCLC resection should undergo spirometry and diffusing capacity for carbon monoxide (DLco) measurement (14, 15). Forced expiratory volume in 1 s (FEV_1), as the most critical spirometric parameter, provides insight into the state of pulmonary ventilation, while DLco indicates the integrity and function of the alveolar-capillary membrane (16). Following tumor resection, and due to the loss of the surrounding healthy lung parenchyma, the values of both parameters decrease, with the decrease being proportional to the extent of the resection itself. Numerous researchers have addressed this issue. Table 1 shows the average (%) postoperative values of FEV_1 and DLco expressed relative to the preoperative values obtained by some of the authors in their studies (17–19).

Table 1. Average postoperative (%) values of FEV_1 and DLco compared to preoperative values (17–19)

	Lobectomy			
	After one month	After 3 months	After 6 months	After 12 months
FEV_1	71.4%	75.6%	84.3%	84.2%
DLco	64.5%	70.1%	91.3%	96.5%
	Pneumonectomy			
	After one month	After 3 months	After 6 months	After 12 months
FEV_1	48.1%	50.7%	65%	75%
DLco	50.6%	55.9%	80%	85%

The table shows that the most significant recovery of respiratory function occurs in the period 3 to 6 months post-surgery, while the recovery is insignificant later.

ERS and ESTS experts believe that patients with FEV_1 and DLco ≥ 80% can safely undergo surgical resection up to pneumonectomy, without the need for additional analyses (15). Candidates with FEV_1 > 1.5 L can undergo lobectomy, while segmentectomy or wedge resection may be considered if FEV_1 is > 0.6 L (15, 20). Otherwise, patients should not be lightly excluded from consideration for surgical treatment, but should instead be further investigated. In such circumstances, it is suggested that stress tests (described below) be performed to determine maximal oxygen consumption (VO_2max). A VO2max > 20 mL/kg/min indicates that resection up to pneumonectomy can be safely performed. In contrast, a VO_2max < 10 mL/kg/min suggests

that surgical treatment should be abandoned due to the high risk of postoperative complications and death (15). Patients with a reduced aerobic capacity (VO_2max between 10 and 20 mL/kg/min) fall into a potentially operable category. In these cases, alternative treatment methods such as wedge resection, stereotaxic radiotherapy, or radiofrequency ablation should be considered in addition to radical surgical resection (21, 22).

If one still opts for radical surgical intervention, it must be kept in mind that reduced aerobic capacity indicates impaired cardiopulmonary function. Accordingly, the scope of potential resection should be adjusted to the predicted postoperative pulmonary reserve, i.e., predicted postoperative value (ppo) FEV1 and ppoDLco. The two formulas most commonly used to calculate these values are as follows (14):

1) ppoFEV_1 = preoperative FEV_1 × (1—fraction of total perfusion for the resected lung)

2) ppoFEV_1 = preoperative FEV_1 × (19 segments—the number of segments to be removed—the number of non-functional segments/19—the number of non-functional segments)

The term non-functional segment refers to those bronchovascular segments with no adequate gas exchange taking place due to broncho-obstruction, atelectasis, emphysema, etc. The expert consensus is that the first formula should be used when planning a pneumonectomy (the perfusion fraction of the right and left lung is normally 55% and 45%, respectively), and the second formula should be applied when planning a lobectomy or segmentectomy (8, 14). If both parameters are > 30%, resection up to lobectomy can be performed. If one of the parameters is < 30%, ppoVO_2max should also be calculated. A ppoVO_2max > 10 mL/kg/min is sufficient for resection up to lobectomy. Otherwise, due to the high risk of peri- and postoperative mortality, surgery is contraindicated, and preference should be given to another option (21, 22). High postoperative mortality (29% and 100%) in patients with ppoVO_2max < 10 mL/kg/min was confirmed by the results of two studies independently conducted by Bechard et al. and Bolliger et al. (23, 24). As far as ppoFEV_1 is concerned, if its value is < 30%, the percentage of postoperative complications reaches as much as 41% (19).

There has been a debate among authors concerning the VO_2max value that would rule out the risk of postoperative complications. Most agree that a VO_2max > 20 mL/kg/min (23, 25, 26) is sufficient for pneumonectomy, and a VO_2max > 15 mL/kg/min for lobectomy (25, 27, 28). A VO_2max of 10 mL/kg/min is often considered the safe lower limit of resection. However, some argue that a VO_2max < 15 mL/kg/min already indicates functional inoperability (23, 29).

Stress Tests

Stress tests are diagnostic procedures used to assess the function of the cardiorespiratory system during exertion. They are designed to assess the body's maximum oxygen intake and consumption capacity during intense exercise. The most important parameters measured in these tests include: heart rate, stroke volume and cardiac output, pulmonary ventilation, VO_2max, and saturation of peripheral oxygen (SpO_2) (30).

The most common stress tests include the bicycle and treadmill stress tests (30). Regarding the latter, in addition to treadmill grade, the speed of ascent also shows a linear correlation with VO_2max. A speed of ascent of 15 m/min approximates VO_2max = 20 mL/kg/min (sufficient for pneumonectomy), and a speed of 12 m/min approximates VO_2max = 15 mL/kg/min (sufficient for lobectomy) (8, 31, 32).

In the absence of demanding and expensive tests, we can perform the 6-minute walk test (6-MWT), stair climbing test, or shuttle walk test. Although ERS and ESTS experts do not recommend the 6-MWT in the routine evaluation of NSCLC operability, Pierce et al. claim it is the best predictor of postoperative respiratory failure (33). At the same time, Holden et al. believe that completing > 1,000 steps indicates a low risk of postoperative complications and mortality (34).

The stair climbing test can be used as a first-line evaluation of candidates for surgical resection of NSCLC, to detect the patients who require more precise evaluation using more sophisticated methods (15, 35). Patients who can climb five floors without pausing (equivalent to FEV_1 > 2 L or VO_2max > 20 mL/kg/min) can undergo pneumonectomy safely, while patients who climb three floors (equivalent to FEV_1 > 1.7 L) can undergo lobectomy (32, 36, 37). Specifically, a 22 m ascent is the limit for safe pneumonectomy, while a 14 m ascent is the limit for safe lobectomy (35). The patient's climbing pace should also be considered. Bernasconi et al. argue that a speed of ascent > 15 m/min indicates safe resection of NSCLC up to pneumonectomy. Otherwise, physicians should not rush to decide to perform surgical treatment but should instead evaluate VO_2max using more precise tests (38).

When performing the shuttle walk test, the subject walks along a ten-meter-long path in two directions, gradually speeding up (usually 12 minutes) (39). Tsubochi et al. report that > 400 m traveling indicates a low risk of postoperative complications and death (5). Although the shuttle walk test is the least used for diagnostic purposes, its application is significant in the period of postoperative rehabilitation of patients.

Gas Analyses and Saturation

The gas analysis involves measuring O_2 and CO_2 concentrations in arterial blood to assess respiratory function, metabolism, and acid-base balance.

SpO_2 measures the percentage of hemoglobin in arterial blood that is bound to O2 molecules. It is measured using the non-invasive pulse oximetry procedure.

According to some authors, hypoxemia (partial pressure of oxygen < 60 mmHg), hypercapnia (partial pressure of carbon dioxide > 45 mmHg), and SpO_2 < 90% (or desaturation > 4% during the stress test) are relative contraindications for NSCLC surgery (20, 40, 41).

American College of Chest Physicians (ACCP) Guidelines

According to the current 2013 ACCP guidelines, the two key assessment parameters for postoperative risk following surgical resection of NSCLC are ppoFEV1 and ppoDLco. If the values of both parameters are > 60%, the postoperative risk is low. If at least one of them is between 30–60%, ACCP experts suggest performing one of the two tests listed above—the stair climbing test or the shuttle walk test—to grasp the risks involved more comprehensively. If the patient achieves a result > 22 m in the first test or > 400 m in the second, they are considered a suitable candidate for lung resection up to pneumonectomy. Cardiorespiratory function and VO_2max should be assessed using more sophisticated tests if either or both are < 30%. In that case, and based on the obtained results, the categories of low-risk and high-risk patients include those with ppoVO_2max > 20 mL/kg/min and ppoVO_2max < 10 mL/kg/min, respectively (42).

Conclusion

Given that surgical resections of NSCLC are among the most complex procedures (technically challenging, accompanied by numerous comorbidities and relatively frequent peri- and postoperative complications), a detailed preoperative risk assessment is crucial for the treatment outcome and disease prognosis. For this reason, spirometry, lung diffusion, and stress tests in high-risk patients are now considered standard and imperative.

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PREOPERATIVNA PROCENA KANDIDATA ZA HIRURŠKO LEČENJE NEMIKROCELULARNOG KARCINOMA PLUĆA

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Karcinom pluća je najčešći maligni tumor i vodeći uzrok smrti od malignih bolesti u opštoj populaciji, sa udelom od otprilike 25%. Prema aktuelnim podacima, od ove bolesti u Srbiji na godišnjem nivou oboli skoro sedam hiljada ljudi, a umre oko pet hiljada. Lečenje je kompleksno i multidisciplinarno. Hirurgija ima centralnu ulogu u lečenju tumora od I do III-A stadijuma. Mada su brojne studije potvrdile da hirurška resekcija tumora pruža najveće šanse za izlečenje bolesnika, u vreme postavljanja dijagnoze može se operisati samo između 20% i 30% bolesnika. Razlozi za to su brojni, a kao najčešći se izdvajaju odmakla bolest, komorbiditeti, oslabljena disajna funkcija i loše opšte funkcionalno stanje. S obzirom na to da su hirurške resekcije pluća relativno često praćene perioperativnim i postoperativnim komplikacijama, treba istaći da je detaljna preoperativna procena rizika izuzetno važna za ishod lečenja. U radu su predstavljene trenutno važeće smernice i protokoli za preoperativnu procenu rizika, sa posebnim osvrtom na bolesnike kod kojih postoji povišen rizik od razvoja ove bolesti (stare osobe, pušači, osobe sa hroničnom opstruktivnom bolesti pluća i gojazne osobe) i na ulogu spirometrijsko-difuzijskih parametara i testova opterećenja.

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***Ključne reči**: karcinom pluća, hirurško lečenje, spirometrija, difuzija, testovi opterećenja*

Review article

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CERTAIN IMMUNE MECHANISMS INVOLVED IN NEONATAL SEPSIS DEVELOPMENT

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Development of neonatal sepsis, especially in preterm neonates, is one of the main factors for high morbidity and mortality in the neonatal period. Preterm neonates, with incompletely matured immune system, have enhanced susceptibility to sepsis development, compared to term infants. Innate immune system activation represents the main protective mechanism, in preterm neonates, against sepsis development. Different components of the innate immune system provide basic protection, as well as they may serve as early biomarkers for neonatal sepsis development. In this review, we analyzed basic mechanisms of innate immune response to pathogen presence and different markers included in the initiation of the inflammatory process. Better understanding the mechanisms involved in sepsis development may provide earlier prediction of sepsis development and results in more potent therapeutic efficiency.

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Key words: *neonatal sepsis, preterm neonates, immune system, innate immunity*

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Introduction

Neonatal sepsis represents a leading cause of morbidity and mortality in preterm infants. With a high rate of morbidity and mortality, preterm neonates are especially vulnerable due to their immune system immaturity and lack of maternal protection (1). The coexistence of other respiratory or cardiovascular disorders may also have an impact on preterm sepsis development (2). On the other hand, based on the time it occurs, preterm sepsis may be formed as early onset sepsis (EOS), which develops in the first 72 h of life and late onset sepsis (LOS), which occurs after the first 72 h of life. The development of EOS is associated with infections, transmitted vertically, from mother to infant, while LOS is usually caused by pathogens collected by delivery or the community environment (3).

Appropriate and efficient treatment of neonatal sepsis may diminish morbidity and mortality rates in neonates. Therefore, recognition of early markers, signs and symptoms of neonatal sepsis represents key factors to overcome harmful effects, especially in preterm neonates. However, since nonspecific symptoms and signs are often presented during neonatal sepsis development, the diagnosis of neonatal sepsis is very difficult, and a consensus definition still lacks (3, 4). Identification of a specific pathogen, by positive blood culture, provides a gold standard for sepsis diagnosis. Nevertheless, antibiotic administration and low bacteriemia may provide false or delayed results (5). Moreover, in recent years, novel techniques (pathogen genome hybridization and polymerase chain reaction) have been used to determine the pathogen presence. The results showed that these procedures provide no information about antibiotic resistance, nor the distinction between viable or nonviable pathogens (6), indicating that positive blood culture still provides better results (3, 7).

Taking into account that inflammation has a key role in sepsis development, different studies have been conducted to evaluate the potential role of some pro- and anti-inflammatory mediators (8, 9). However, the precise mechanism of neonatal response to infection is not clearly identified, resulting in no reliable and rapid marker for neonatal sepsis development (7). Therefore, the current study intended to provide the basic mechanisms of the immune system, as well as its components, involved in neonatal sepsis development and to provide a better understanding of this pathological process with possible implications in therapeutic strategies.

Epidemiology

Usually, preterm birth is termed as birth before 37 weeks of gestation and remains the

main cause of neonatal death (10). It is estimated that preterm birth incidence in the USA is around 13%, in other developed countries is between 4.5–8%, while in the European Union is in the range of 5–10% (11, 12). Also, it is observed that, besides preterm mortality, consequences of preterm birth may persist in the neonatal period as well as in adulthood (12). On the other hand, EOS incidence is around 20 per 1,000 infants (infants born before 29 weeks of gestation), and LOS incidence is in the range of 12–28% (infants born before 26 weeks of gestation), with increasing incidence as gestational age decreases (13, 14). Furthermore, neonatal sepsis (EOS and LOS) induce a neonatal mortality rate between 5–20% in developed countries, while the rate of mortality rises to 70% in middle or low-income countries (2). Accordingly, a rapid and respectable marker for neonatal sepsis prediction is a major challenge in neonatal sepsis treatment.

Certain Immune Mechanisms in Neonatal Sepsis Development

In recent years, most of the research has focused on determining specific inflammatory components which may serve as potential biomarkers for early diagnosis of neonatal sepsis. Initial research proposed a potential role of some acute-phase reactant proteins, including C-reactive protein (CRP) and procalcitonin (PCT). Even though CRP can induce opsonization and to activate the complement system, this protein has a 24–48 h half-life and needs 10–12 h to reach elevated plasma levels (15), indicating that CRP is not able to serve as an early predictor of neonatal sepsis development but, rather, as monitoring factor of sepsis therapy efficiency (3). Additionally, levels of PCT showed physiologically altered values during the neonatal period (16), suggesting that this biomarker may not provide enough diagnostic ability to rule out neonatal sepsis (4).

Innate Immunity

Following the birth, the immune system in neonates is not fully developed, especially in preterm neonates (17). An incompletely developed innate and adaptive immune system, together with a lack of communication between these both immune system parts, often leads to sepsis development in preterm neonates (4). Transplacental antibodies transmission from mother to fetus represents the main defense mechanism from different pathogens. Taking into account that this process is enhanced after 32 weeks of gestation, preterm neonates usually lack this way of protection (18). Consequently, immune system protection is mainly based on the innate immunity, which is not very potent due to its immaturity. In line with this, previous findings demonstrate that various soluble proteins and peptides in blood plasma, with antimicrobial properties and opsonization ability, have been significantly reduced in preterm neonates (19). These antimicrobial proteins and peptides (APPs) are mainly cationic molecules released by neutrophils, eosinophils, monocytes and epithelial cells of the gastrointestinal or respiratory system, including defensins, caprotectin, protegrins, lactoferrins and lysosomes (20). All APPs have the ability to bind to various pathogens and provide elimination of pathogens through different mechanisms. This was supported by previous findings indicating that application of lactoferrin reduces the incidence of LOS in preterm neonates (21).

As part of innate immunity, complement system activity (classical, alternative and lectin pathway of activation) is also reduced in preterm neonates (4). Namely, in preterm neonates, there is decreased production of C1 and C4 components (involved in classic pathway activation), factor B (included in alternative pathway activation) and mannose-binding lectin (necessary for lectin pathway activation) compared to the term infants (22). The inability of complement activation leads to a reduction of phagocytosis activity and eradication of different pathogens, enabling preterm neonates to be especially susceptible to infection.

The presence of different pathogens initiates the formation of an inflammatory process, together with the production of innate proteins and activation of leukocytes. Polymorphonuclear leukocytes, during sepsis in preterm neonates, rapidly decrease in number, have delayed apoptosis and show potential to aggregate with decreased diapedesis function (23). Since their number in the medulla is depleted, immature and dysfunctional forms of leukocytes are released, and the process of phagocytosis is globally reduced (4). On the other hand, initiation of the inflammatory process results in innate immunity protein production, including CRP, PCT, collectins, lactoferrin and others. In addition, sepsis development induces the elevation of serum proteins with opsonization function (mainly IgM). Nevertheless, the total number of these proteins, as well as opsonization activity of blood plasma, is reduced in preterm neonates, compared to the term infants (24).

Pathogens and their products are sensed by transmembrane pattern recognition receptors (PRRs), including toll-like receptors (TLRs), which bind to the surface of the microorganisms. Up to now, there are 11 different TLRs in humans, and they play a key role in controlling the inflammation process (25). TLRs can recognize lipopolysaccharide endotoxins (LPS) on Gram-negative bacteria and byproducts of Gram-positive bacteria, mycoplasmas and yeast (26). The activation of TLRs leads to increased neutrophil activity, elevated cytokine and chemokine production and enhanced chemotaxis and immunoglobulin secretion. However, these mechanisms are significantly decreased in preterm neonates compared to the term infants (12).

Furthermore, it has been shown that sepsis development in preterm neonates results in markedly reduced expression of genes related to TLRs, suggesting the depleted innate immune response in preterm neonates (27). Similar findings revealed overexpression of genes related to innate immune response and inflammatory processes in preterm neonates, but fold changes have decreased further than those observed in term infants (28).

Other Immune Mechanisms

The TLRs activation leads to an immune response characterized by the production of pro-cytokines and chemokines (IL-1, IL-6, TNF-α, IL-12, IL-18, IL-8, MCP-1) via mitogen-activated protein kinases (MAPK) and the transcription nuclear factor κB (NF-κB) (29). The majority of cytokines are produced by activated lymphocytes and macrophages. Producing pro-inflammatory cytokines provides activation of endothelial cells and expression of cellular adhesion molecules, which results in increased leukocyte requirement and diapedesis. However, developed sepsis in preterm neonates markedly reduces production of most pro-inflammatory cytokines, mainly by decreased production of Myeloid Differentiation Factor (MyD88) (30). In line with previous findings, an earlier report showed reduced signaling through TLRs in preterm neonates, indicating reduced protection against different pathogens (31).

The characteristics of secreted cytokines and pathogens have a huge effect on the process of differentiation of T helper precursor cells (Th) toward Th1 or Th2 cells. IFN-γ, IL-2 and TNF-β are the main cytokines produced by Th1 cells, and they provide cellular and phagocytic activity. Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-13 and promote antibody production and humoral immunity. On the other hand, to control the intensity of inflammatory response, anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGF-β) are secreted by lymphocytes, Th2 cells and macrophages (4). Balanced control of cytokine secretion is crucial to control inflammatory activity and to prevent multiple organ dysfunction. Therefore, monitoring the total amount of these cytokines may provide a better understanding of sepsis development and treatment efficiency, since appropriate treatment would turn these cytokines to baseline levels. However, overexpression of the curtained component, named before, may lead to inconsistent inflammatory response in different populations, especially in preterm neonates, where all the components of the immune system show relative immaturity (3).

Conclusion

With high morbidity and mortality rates, neonatal sepsis in preterm neonates represents one of the leading major public health concerns around the world. Immaturity of the immune system in preterm neonates may contribute to increased susceptibility to infection. Innate immune system activation usually provides basic protective mechanisms against inflammatory response, initiated at the beginning of neonatal sepsis development. Production of different biomarkers during initiation of the immune response, secretion of various cytokines and chemokines, may serve as potential predictive factors for neonatal sepsis development. Additionally, better understanding the basic immune mechanisms, especially during innate immune system activation, may clarify sepsis development and simultaneously enable potent treatment efficiency. Further research and additional cohort studies are required to develop effective preventive methods for reducing the neonatal morbidity and mortality.

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IMUNOLOŠKI MEHANIZMI UKLJUČENI U RAZVOJ NEONATALNE SEPSE

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Razvoj neonatalne sepse, posebno kod preterminske novorođenčadi, predstavlja jedan od glavnih faktora značajnog morbiditeta i mortaliteta u neonatalnom periodu. Prevremeno rođena novorođenčad kod kojih imunološki sistem nije u potpunosti razvijen, pokazuju pojačanu osetljivost za razvoj sepse u odnosu na novorođenčad rođenu u terminu. Aktivacija urođenog imuniteta kod prevremeno rođene novorođenčadi predstavlja jedan od glavnih mehanizama koji se suprostavljaju razvoju sepse. Različite komponente urođenog imuniteta omogućavaju osnovnu zaštitu i mogu poslužiti kao rani biomarkeri za razvoj sepse. U ovom radu analizirani su bazični mehanizmi urođenog imuniteta na prisustvo patogena, kao i različiti biomarkeri koji su uključeni u pokretanje inflamatornog procesa. Bolje razumevanje mehanizama uključenih u nastanak sepse može biti od koristi za ranu predikciju sepse, što doprinosi efikasnijem terapijskom pristupu.

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***Ključne reči**: neonatalna sepsa, preterminska novorođenčad, imunološki sistem, urođeni imunitet*

Review article

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BREAST CANCER WITH LOW HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 EXPRESSION STATUS: A NEW THERAPEUTIC ENTITY

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Targeted human epidermal growth factor receptor 2 (HER2) therapies used in the treatment of HER2-positive early and metastatic breast cancer (mBC) include monoclonal antibodies such as trastuzumab, pertuzumab and margetuximab, as well as antibody-drug conjugates (ADC) trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), and tyrosine kinase inhibitors such as tucatinib, lapatinib, and neratinib. The introduction of these drugs into clinical practice has dramatically improved the course of treatment for HER2-positive breast cancer. However, the clinical evaluation of trastuzumab, pertuzumab, and T-DM1 in the HER2-low group of patients did not show significant benefits. Consequently, these patients were classified as HER2-negative cancers and treated in accordance with the expression of hormone receptors (HR) or other biomarkers. Trastuzumab deruxtecan, an ADC, which initially demonstrated its efficacy in the treatment of metastatic HER2-positive breast cancer, and subsequently in breast cancer with low HER2 expression, classified as immunohistochemistry IHC 1+ and IHC 2+ with a negative fluorescence *in situ* hybridization (FISH) introduced into clinical practice a new entity of HER2 breast cancers called HER2-low tumors. Following the publication of the DESTINY-Breast04 study results, it is clear that low HER2 positivity can be considered a rational target for the treatment of breast cancer. The results have changed clinical practice in both HR-positive and HR-negative HER2-low metastatic breast cancer. Further research is necessary in order to standardize HER2 testing, prevent T-DXd-related side effects and resistance to therapy, and identify the optimal sequence of available therapeutic options. Future research should also explore the role of these drugs in the treatment of early HER2-low breast cancer.

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Key words: *breast cancer, human epidermal growth factor receptor 2-low, antibody-drug conjugate, trastuzumab deruxtecan, sacituzumab govitecan*

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Introduction

Breast cancer (BC) expresses high levels of growth factors and their receptors. The HER2 receptor belongs to the HER 2/Neu receptor family, which includes four receptors: HER1-EGFR, HER2, HER3, and HER4. The natural ligand for the HER1 receptor is the EGFR—receptor for epidermal growth factor, while the natural ligand for the HER2 receptor is unknown. The natural ligands for HER3 and HER4 receptors are neuregulins. Receptors of the HER family belong to the transmembrane group of receptors, which consists of three subunits: the extracellular part, the transmembrane part, and the intracellular part of the receptor. A specific ligand binds to the receptor through its extracellular domain, which leads to receptor dimerization (either homo- or heterodimerization) and activates the intracellular tyrosine kinase domain. This is followed by a cascade of phosphorylation processes, ultimately resulting in the synthesis of proteins that influence cell growth, division, survival, motility, adhesion, and angiogenesis (1). The HER2 receptor is a glycoprotein the synthesis of which is encoded by the HER2 gene located on chromosome 17. In 20–30% of all breast cancers (15% in our country), a tumor disorder is present: increased synthesis of HER2 receptors on the cell surface and/or amplification of the HER2 gene in the nucleus. HER2-positive BC is characterized by a biologically aggressive clinical course, a shorter disease-free interval (DFI), as well as reduced overall survival (OS) compared to HER2-negative BC (2, 3). The presence of HER2 receptors on the surface of tumor cells is determined by the immunohistochemistry (IHC) and is expressed on a scale from 0 to 3+. A result labeled IHC 3+

(more than 10% of cells show intense and complete membrane staining) is considered HER2-positive BC. A HER2 result labeled IHC 2+ represents tumors with uncertain HER2 status, requiring retesting by means of chromogenic in situ hybridization (CISH) or fluorescence *in situ* hybridization (FISH). This determines whether gene amplification is present, which would confirm HER2-positive status (4).

The therapy, which has been in use for more than 15 years for the treatment of HER2-positive BC, such as trastuzumab or trastuzumab emtansine (T-DM1), has not shown benefits in HER2-low BC as shown in prospective randomized clinical studies (5, 6).

Trastuzumab deruxtecan (T-DXd), an antibody-drug conjugate (ADC), which initially demonstrated its efficacy in the treatment of metastatic HER2-positive BC, and subsequently in BC with low HER2 expression, classified as IHC +1 and IHC +2 with a negative FISH, introduced into clinical practice a new entity of HER2 breast cancers called HER2-low tumors (7, 8).

The Importance of the HER2 Signaling Pathway in Carcinogenesis and the Importance of HER2 Expression Levels

The HER2 receptor has been known to play a key role in the pathogenesis of BC since 1987 (9). In HER2-positive cancers, a specific ligand binds to the extracellular domain of the receptor, leading to homo- or heterodimerization of the receptor and subsequently the activation of the intracellular tyrosine kinase domain, followed by a cascade process of phosphorylation and the activation of signaling pathways via mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway. This results in the increased expression of certain cyclins, such as cyclin D1, E and CDK6, and the degradation of cell cycle inhibitors such as p27Kip1 (10, 11). The end result is the synthesis of proteins which affect cell proliferation, survival, motility, adhesion, as well as angiogenesis (12–15).

HER2-targeted therapies used in the treatment of HER2-positive early and metastatic breast cancer (mBC) include monoclonal antibodies such as trastuzumab, pertuzumab and margetuximab, as well as antibody-drug conjugates T-DM1 and T-DXd, and tyrosine kinase inhibitors such as tucatinib, lapatinib, and neratinib. The introduction of these drugs into clinical practice has dramatically improved the course of treatment for HER2-positive BC (2, 16–18). However, the clinical evaluation of trastuzumab, pertuzumab, and T-DM1 in the HER2-low group of patients did not show significant benefits. Consequently, these patients were classified as HER2-negative cancers and treated in accordance with the expression of hormone receptors (HR) or other biomarkers (5, 19–21).

HER2-Low as a Potential Target

For the past two decades, it has been believed that HER2 overexpression (IHC 3+ or IHC 2+/ISH positive) was necessary for the effectiveness of anti-HER2 therapies, but new data suggest that this has changed with the advent of newer and more potent agents, such as T-DXd, which is an antibody-drug conjugate. T-DXd consists of a humanized anti-HER2 monoclonal antibody (trastuzumab) linked to a topoisomerase I inhibitor (DX-895) via a cleavable linker. When T-DXd binds to the HER2 receptor, it enables the cytotoxic payload to act on both the tumor cell and the tumor microenvironment by means of a specific bystander effect, independent of the HER2 receptor expression level. This effect is the main difference between T-DXd and other HER2-targeted therapies, such as T-DM1 (22, 23).

The efficacy of as powerful a drug as T-DXd was first demonstrated in pretreated patients with HER2-positive mBC in phase 2 of the DESTINY-Breast01 study, and subsequently confirmed in phase 3 of the DESTINY-Breast02 study, in which the efficacy of T-DXd was statistically significantly better than conventional HER2 therapies in patients previously treated with multiple lines of anti-HER2 therapy and T-DM1. Another phase 3 of the DESTINY-Breast03 study, showed significantly longer survival with T-DXd versus T-DM1 in a patient population which was less heavily pretreated compared to those in DESTINY-Breast02 (24–26).

Efficacy of T-DXd in HER2-low mBC

Phase Ib and II studies initially demonstrated the activity of T-DXd in pretreated patients with HER2-low mBC, with an overall response rate (ORR) ranging from 33% to 38%, and progression-free survival (PFS) of 6.3–11.1 months (8, 27, 28). These results were the rationale behind the design of the phase 3 DESTINY-Breast04 study, which included 557 patients with HER2-low mBC (both hormone receptor positive HR+ and hormone receptor negative HR-) who had previously been treated for metastatic disease or had progressed during or within 6 months of adjuvant therapy completion. HR+ patients were required to have received ≥ 1 line of endocrine therapy. Patients were randomized 2:1 to receive T-DXd or the investigator's choice of therapy, including gemcitabine, capecitabine, paclitaxel, nab-paclitaxel, or eribulin. The primary aim was PFS in the HR+ patient population, while the secondary aims included PFS in the overall patient population and OS in the HR+ and overall population. The patient population had received an average of 3 lines of therapy for metastatic disease, with 88.7% of the patients being HR-positive and 11.3% HR-negative. The median follow-up time was 18.4 months. The ORR in the HR+ group of

patients treated with T-DXd was statistically significantly higher at 52.6%, compared to 16.3% in patients treated with chemotherapy. Moreover, the median PFS was almost doubled with T-DXd (10.1 vs. 5.4 months; HR: 0.51 [95% CI: 0.40–0.64]; p < 0.001), and OS was significantly longer (23.9 vs. 17.5 months; HR: 0.64 [95% CI: 0.48–0.86]; p = 0.003).

Similar results were observed in the HR-negative population, where ORR was 50% with T-DXd vs. 16.7% with chemotherapy, while PFS was 8.5 vs. 2.9 months (HR: 0.46 [95% CI: 0.24–0.89]). Overall survival was more than doubled, amounting to 18.2 months in the T-DXd group compared to 8.3 months in the investigator's choice chemotherapy group (HR: 0.48 [95% CI: 0.24–0.95]).

As for the adverse effects of the therapy, the most common ones in the T-DXd group were nausea (73.0%), fatigue (47.7%), and alopecia (37.7%). High-grade toxicity (grade 3 or higher) was significantly lower in the group of patients treated with T-DXd compared to those treated with chemotherapy (52.6% vs. 67.4%). The characteristic toxicity associated with T-DXd was pneumonitis or drug-related interstitial lung disease (ILD), which occurred in 12.1% of T-DXd-treated patients compared to only 0.6% of chemotherapy-treated patients. The majority of these events were grade 1 (3.5%) or grade 2 (6.5%) (7).

Based on all the aforementioned results, it can easily be concluded that low HER2 expression is indeed a good and reasonable target for ADC treatment. The results of the DESTINY-Breast04 study introduced HER2-low carcinoma into clinical practice as a completely new entity and changed the treatment paradigm for these patients.

New Treatment Algorithm for HER2-Low Patients

In the DESTINY-Breast04 study, the majority of patients with HR+ disease received ≥ 3 lines of systemic therapy and had endocrine-resistant mBC. Taking this into account, the use of T-DXd was considered rational in the fourth and subsequent lines of therapy (8). Another study, DESTINY-Breast06, the primary results of which were published at the latest ASCO conference, examines the efficacy of T-DXd versus the investigator's choice chemotherapy in HR-positive HER2-low and ultra-low mBC after progression on CDK4/6 inhibitors within 6 months or after the previous application of two lines of endocrine therapy, with or without targeted therapy, for metastatic disease. The primary aim of the study was PFS. A total of 866 patients were randomized, 90.4% of whom were previously treated with CDK4/6i. The control arm received capecitabine (59.8%), nab-paclitaxel (24.4%) or paclitaxel (15.8%). In HER2-low patients (HR, 0.62 [95% CI 0.51, 0.74], P < 0.0001), PFS was significantly longer in the T-DXd arm—13.2 months compared to 8.1 months in the chemotherapy arm, while the results were similar in the ultralow group. The median therapy duration was 11 months (T-DXd) versus 5.6 months (chemotherapy). Overall survival results are not yet mature, and a longer follow-up time is needed. Pneumonitis occurred in 11.3% of patients in the T-DXd-treated arm, but only in 1.4% was it grade 3 or higher. These results of the DESTINY-Breast06 study have established T-DXd as the standard treatment after one or more lines of endocrine therapy in patients with HER2-low and ultralow, HR+ mBC (29, 30).

The fact that ADC drugs are highly effective was confirmed by the results of another phase III study, TROPiCS-02, which examined the efficacy of sacituzumab govitecan (SG), an ADC targeting TROP2. The study enrolled pretreated patients with HR+ endocrine-resistant, locally recurrent, or mBC. Patients had previously been treated with CDK4/6 inhibitors and with 2–4 lines of chemotherapy. The experimental arm received SG, while the control arm received chemotherapy (CTX). After a median follow-up of 10.2 months, PFS in the SG arm was 5.5 months vs. 4.0 in the CTX arm (HR: 0.66 [95% CI: 0.53–0.83]; p = 0.0003). In these groups, OS was 14.4 and 11.2 months, respectively (HR: 0.79 [95% CI: 0.65–0.96]; p = 0.02), while ORR was 21% vs. 14%.

Subsequent subanalysis of the population of patients with HER2-low BC showed that the benefit of SG was comparable to that seen in the overall patient population. PFS interval was 6.4 months (SG) vs. 4.2 months with CTX (HR: 0.58 [95% CI: 0.42–0.79]; p < 0.001), and ORR was 26% with SG versus 12% with chemotherapy. The most common side effects of the therapy were neutropenia, which occurred in as many as 70% of patients, followed by diarrhea in 57% and nausea in 55%. Fatigue occurred in about one-third of patients (37%). There were no cases of pneumonitis in the group of patients treated with SG (31–33).

SG has been approved for the treatment of patients with metastatic triple-negative BC who have progressed on ≥ 2 previous lines of chemotherapy, including adjuvant therapy. It was approved after the results of the phase 3 ASCENT trial, which compared the effectiveness of SG to CTX of the investigator's choice. After a 17-month follow-up, the median PFS was 4.8 months with SG vs. 1.7 with CTX (HR: 0.43 [95% CI: 0.35–0.54]), and the median OS was 11.8 months with SG versus 6.9 months with CTX (HR: 0.51 [95% CI: 0.41–0.62]). A subanalysis of the study in HER2-low patients showed similar results to those in the overall patient population. The results were HR: 0.44 [p = 0.002] for PFS, HR: 0.43 [p < 0.001] for OS, and 32% vs. 8% for ORR. The safety profile of the drug was consistent with that in the TROPiCS-02 study (34, 35).

Optimal ADC Sequence

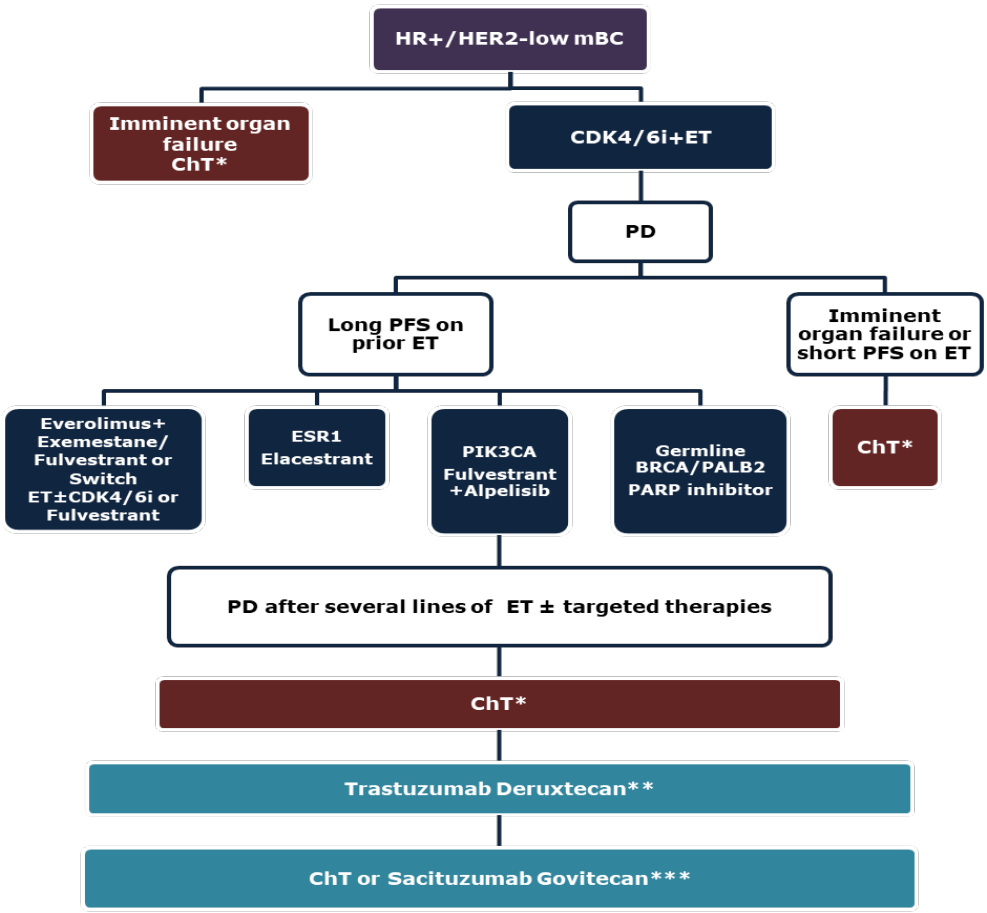
Based on the subanalysis of the ASCENT and TROPiCS-02 studies, it can be concluded that SG is another valid option for the population of

patients with low HER2 expression, although it is important to note that the optimal sequence of T-DXd and SG remains unknown.

In the aforementioned studies, the results showed HR for PFS of 0.51 and 0.46 with T-DXd in HR+ and HR- patients and HR for PFS of 0.58 and 0.44 for SG in HR+ and HR- patients, respectively. A direct comparison between the studies is not possible, given the differences in the study designs and patient populations. The studies examining the efficacy of SG involved more heavily pre-treated patients, with 71% having received 2 or more lines of therapy in the ASCENT study and 57% in TROPICS-02 more than 3 lines of therapy. In contrast, in DESTINY-Breast04, the majority of patients (60%) received only one line of therapy. Head-to-head randomized-controlled clinical trials are needed to determine the optimal sequence. In the absence of such studies, researchers currently favor T-DXd over SG for HR+/HER2-low patients who meet the inclusion criteria for the DESTINY-Breast04 and TROPiCS-02 trials. T-DXd is the preferred option due to the higher level of evidence in the HER2-low population, as the data for SG come from a post-hoc analysis, and because patients in DESTINY-Breast04 were treated with fewer lines of prior therapy. Needless to say, patient preferences, comorbidities and the risk the therapy carries based on adverse effects should also be taken into account (36–38).

The recommended algorithm in accordance with all valid clinical guidelines is shown in Figure 1 (16, 17, 39).

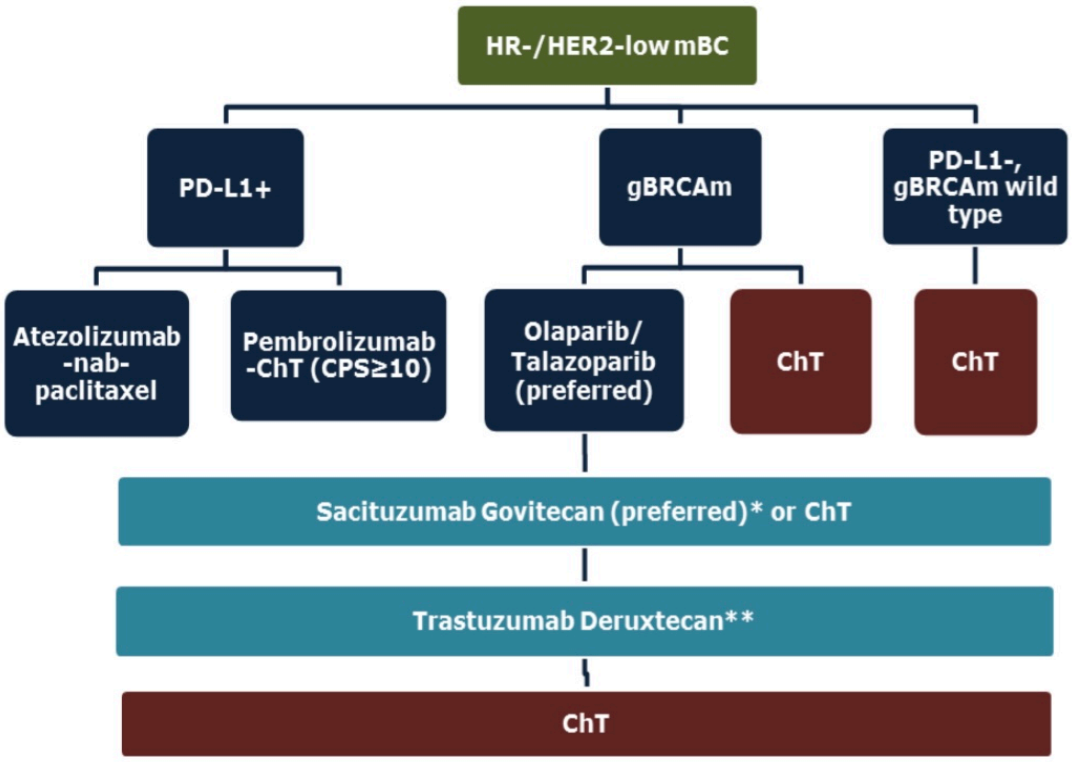
A) Recommended algorithm in treatment of HR+/HER2low mBC



MBC: metastatic breast cancer, HER2: human epidermal growth factor receptor 2; HR, hormone receptor, CDK4/6, cyclin-dependent kinase 4 and 6, ChT: chemotherapy; ESR1, estrogen receptor 1, m: mutation, PALB2: partner and localiser of BRCA2, PARP: poly (ADP-ribose) polymerase, PD: progressive disease, PIK3CA: phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, T-DxT: Trastuzumab deruxtecan, ChT: chemotherapy, gBRCAm: germline BRCA1/2 mutation, PD-L1, programmed death-ligand 1

Figure 1. The recommended algorithm in treatment of A) HR+HER2-low advanced/mBC and B) HR-HER2-low advanced/mBC with all valid clinical guidelines (16, 17, 39)

B) Recommended algorithm in treatment of HR-/HER2low mBC



A) *In the absence of imminent organ failure, the standard first line is CDK4/6 inhibitors with ET. Chemotherapy is the second-line therapy for endocrine-resistant cancers, at least after two ETs. The choice of therapy depends on the previous therapy, disease aggressiveness, toxicity and the patient's condition. **Trastuzumab deruxtecan should be considered for patients with HER2-low mBC after at least one line of ChT according to DESTINY-Breast04 study. ***Sacituzumab govitecan should be considered after at least two lines of chemotherapy, according to the TROPiCS-02 trial. It is recommended after Trastuzumab Deruxtecan therapy.
B) *Sacituzumab govitecan is the preferred treatment option after previous ChT according to the ASCENT III trial. **Trastuzumab deruxtecan should be considered for patients with HER2-low MBC after at least one line of ChT, according to the DESTINY-Breast04 study.

Ongoing Studies for the Treatment of HER2-Low BC

A number of agents, either alone or in combination, such as ADCs, immunotherapy, and cytostatics, are currently being investigated for the treatment of HER2-low BC. Table 1 presents the most important phase 2 and 3 studies, the preliminary results of which are either already available or expected soon. One of the studies with an interesting design is the phase Ib DESTINY-Breast08 study, which has 5 cohorts that differ based on HR expression and prior therapy. Preliminary results suggest that T-DXd can be safely administered in combination with endocrine therapy (40–42). A major problem in real-world clinical practice is the treatment of patients with CNS metastases. The phase 2 DEBBRAH study is currently examining the effectiveness of T-DXd in HER2-positive and HER2-low patients with CNS metastases. The results have been published for 2 cohorts of HER2-low patients: the cohort with untreated asymptomatic CNS lesions, where an intracranial ORR of 67% was noted, and for the cohort with metastases which had progressed after prior therapy, where the expected ORR is lower and amounts to 33% (43–46). The DAISY study also examined this patient population and found the ORR of up to 33% and PFS of up to 6.7 months. Other agents worth mentioning include active studies with margetuximab and disitamab vedotin (47–49).

After observing the effectiveness of anti-HER2 therapies in the treatment of metastatic HER2-low BC, a large number of studies are now examining their effectiveness in earlier lines of BC therapy (50, 51).

Table 1. Ongoing clinical trials of T-DXd and other therapies in HER2-low advanced/mBC

Study drug	Study name	Phase	Pts population	N of HER2-low	Results for HER2-low
T-DXd	DEBBRAH[43-45]	2	HER2+ OR HER2-low with untreated BMs or LMC (5 cohorts)	41	ORR Cohort 2 66.7% ORR Cohort 4 33.3% PFS (both): 5.7 mo
T-DXd	DAISY[27]	2	HER2+ HER2-low HER2 0	72	OR: 33.3% mPFS:6.7 mo
T-DXd + nivolumab	NCT03523572[28]	Ib	HER2+ HER2-low	16	ORR: 37.5% mPFS: 6.3 mo
T-DXd + durvalumab (+ others)	BEGONIA[46]	Ib/2	HER2-low TNBC	11	ORR: 100% (4/4; 7 pts still on therapy)
Disitamab vedotin	NCT04400695[52]	3	HER2-low	Recruiting	
Disitamab vedotin	NCT05331326[53]	2	HER2 + HER2-low	Recruiting	
Margetuximab	NCT01828021[48]	2	HER2-low	25	Not published yet

T-DXd: trastuzumab deruxtecan, ORR: overall response rate, HER2: human epidermal growth factor 2, PFS: progression-free survival, LMC: leptomeningeal carcinomatosis

Conclusion

After the publication of the DESTINY-Breast04 study results, it is evident that low HER2 positivity can be considered a rational target for the treatment of BC. The results have changed clinical practice in both HR-positive and HR-negative HER2-low metastatic BC. Further research is necessary in order to standardize HER2 testing, prevent T-DXd-related side effects and resistance to therapy, and identify the optimal sequence of available therapeutic options. Future research should also explore the role of these drugs in the treatment of early HER2-low BC.

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KARCINOM DOJKE SA HER2 STATUSOM NISKE EKSPRESIJE: NOVA TERAPIJSKA POJAVA

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Ciljane HER2 terapije koje se koriste u lečenju ranog karcinoma dojke pozitivnog na HER2 i metastatskog karcinoma dojke (engl. *metastatic breast cancer* – mBC) obuhvataju monoklonska antitela, kao što su trastuzumab, pertuzumab i margetuksimab, zatim konjugate antitela i leka (engl. *antibody–drug conjugate* – ADC), kao što su trastuzumab-emtazin (T-DM1) i trastuzumab-derukstekan (T-DXd) i inhibitore tirozin kinaze poput tukatiniba, lapatiniba i neratiniba. Uvođenje ovih lekova u kliničku praksu dramatično je popravilo tok lečenja karcinoma dojke pozitivnog na HER2. Uprkos tome, klinička evaluacija trastuzumaba, pertuzumaba i T-DM1 nije ukazala na njihove značajnije prednosti u grupi bolesnika sa slabo pozitivnim HER2, te su ovi bolesnici svrstani u grupu karcinoma negativnih na HER2 i lečeni na osnovu ekspresije hormonskih receptora (HR) ili drugih biomarkera. Trastuzumab derukstekan, konjugat antitela i leka, koji je najpre pokazao svoju efikasnost u lečenju metastatskog karcinoma dojke pozitivnog na HER2 a potom i u lečenju karcinoma dojke sa niskom HER2 ekspresijom, koji su prema imunohistohemijskom skoru klasifikovani kao IHC+1 i IHC+2 sa negativnom fluorescentnom *in situ* hibridizacijom (engl. *fluorescence in situ hybridization* – FISH), uveo je u kliničku praksu novi tip HER2 karcinoma dojke – tumore slabo pozitivnog HER2 statusa. Posle objavljenih rezultata studije DESTINY-Breast04 jasno je da se lečenje karcinoma dojke može usmeriti na nisku pozitivnost HER2. Rezultati su promenili kliničku praksu i u lečenju slabo pozitivnog HER2 metastatskog karcinoma dojke pozitivnog na HR i u lečenju slabo pozitivnog HER2 metastatskog karcinoma dojke negativnog na HR. Neophodna su dodatna istraživanja koja bi standardizovala HER2 testiranje, prevenirala neželjena dejstva koja ima T-DXd i rezistenciju na terapiju i odredila optimalnu dozu dostupnih terapijskih opcija. Takođe, potrebno je da buduća istraživanja pozicioniraju pomenute lekove i kada je reč o lečenju ranog karcinoma dojke slabo pozitivnog na HER2.

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***Ključne reči**: karcinom dojke, slabo pozitivni receptor humanog epidermalnog faktora rasta 2, konjugat antitela i leka, trastuzumab derukstekan, sacituzumab govitekan*

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CURRENT TREATMENT OPTIONS FOR CHILDREN AND ADOLESCENTS LIVING WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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The goal of human immunodeficiency virus (HIV) infection treatment in both adults and children and adolescents is to achieve a stable virological response, i.e., undetectable viral load in the blood for more than six months and recovery of immunity. Antiretroviral treatment of children and adolescents living with HIV (C/ALHIV) is even more specific and more difficult due to poorer therapeutic adherence, longer duration of infection and consequent toxic effects as well as chronic microinflammation. Another complicating factor is the lack of adequate pediatric pharmaceutical co-formulations depending on the region. With progress and the emergence of innovative types of therapy and strict guidelines, C/ALHIV are improving their quality of life and immune status. The most used official guidelines are the recommendations of the European AIDS Clinical Society (EACS), the World Health Organization (WHO), and the Center for Disease Control and Prevention (CDC). According to them, along with local conditions and opportunities, national guidelines are formed at the level of individual countries.

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Key words: *human immunodeficiency virus, children, adolescents, antiretroviral therapy, guidelines*

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Introduction

Human immunodeficiency virus (HIV) infection is one of the most serious infectious diseases in children. Approximately 3 million children and adolescents worldwide are infected with HIV. The principle of early and lifelong antiretroviral therapy (ART) is crucial for sustained viral suppression, recovery, sustenance of immunity and prevention of acquired immune deficiency syndrome (AIDS). This principle significantly reduces morbidity and mortality from HIV-related diseases and improves quality of life. However, there are many more difficulties in treating HIV-related diseases in pediatric patients than in adults. Access to therapy, especially in the youngest children (neonates and infants), remains a major challenge due to the limited number of formulations suitable for use (1–3).

Despite great efforts to prevent mother-to-child transmission of HIV, children continue to be infected. Mortality from AIDS-defining conditions in children and adolescents is significantly associated with failure of viral suppression, severe immune suppression underlying severe opportunistic infections (4). During growing up, children and adolescents living with HIV (C/ALHIV) can develop various organic damages as a consequence of the toxicity of the therapy, such as cardiovascular, renal, metabolic and neurological (5–7).

There is a legitimate concern about the consequences of lifelong HIV persistence in children with perinatally acquired HIV. Due to the longer duration of persistent HIV infection and the underlying microinflammation, adolescents and young adults who were infected with HIV in childhood are at an even greater risk of developing diseases associated with immunosenescence. Chronic inflammation and immune activation lead to non-AIDS-defining comorbidities, including impairments of neurocognitive functions and cardiovascular system and metabolic alterations. Although the therapy decreases immune activation in children, levels remain higher than in their uninfected peers. The long survival capacity of early infected cells through clonal expansion is a major obstacle to a cure. The effects of lifelong

HIV persistence and lifelong ART are complex, such that children living with HIV have a higher risk of developing non-AIDS comorbidities (8).

This review presents the basics of ART for C/ALHIV, currently available therapeutic options, dosing, and safety analyzes of therapy by drug class for all pediatric groups (newborns, children, adolescents).

Current Recommendations

The subject of current research is the development of new, safe, more effective drugs that are also easier to use in the pediatric population. At the same time, the topic is the right time to start ART, the consideration of therapeutic options after the failure of first-line therapy, as well as the prevention of opportunistic infections (9−11).

The well-known guidelines for the treatment of the disease caused by HIV in the pediatric population are the guidelines of the European AIDS Clinical Society (EACS), Center for Disease Control and Prevention (CDC), as well as the World Health Organization (WHO). Most of the countries of the European Union, as well as our country, mainly adhere to the EACS guidelines. At the end of 2024, under the auspices of the Ministry of Health of the Republic of Serbia, the National Guide for the treatment of people living with HIV was officially published (12, 13). What all the guides have in common is the standpoint that postponing therapy is no longer recommended. Prompt therapy initiation is recommended in all C/ALHIV. WHO recommends testing newborns at 4–6 weeks of age and prompt ART in all infected children (14).

European AIDS Clinical Society Recommendations

The European AIDS Clinical Society (EACS) guideline covers a large and diverse geographic territory that includes varying levels of therapy availability. For this reason, a wide range of recommendations is included in the aforementioned guide, which is the opposite of uniform national guides. EACS Guidelines version 11.1. October 2022 (15, 16).

1. Starting ART in children and adolescents

•It is recommended to start ART in all C/ALHIV infections, regardless of age, clinical stage, level of CD4 cells, or Viral Load (VL), i.e., the concentration of virus in the blood.

•Rapid diagnosis of HIV infection for infants born to HIV-infected women and rapid initiation of therapy for HIV-infected infants are necessary

•The "U=U" campaign (undetectable = untransmissible) regarding the sexual transmission of HIV is approved, which is of particular importance for sexually active adolescents. "Untransmissible" means VL < 200 copies/mL for more than 6 months.

2. Initial combination regimen for treatment—naïve C/ALHIV (children and adolescents who have not been on ART before).

•If accessible, baseline resistance testing should be performed.

•All regimens of the first-line regimens at present comprise 2 nucleoside reverse transcriptase inhibitors (NRTIs) along with a drug from another class (third agent)

•The combination of dolutegravir (DTG) plus 2NRTIs is the preferred option for all children older than 4 weeks and 3 kg.

•While "preferred options" are recommended, "alternative options" are permissible and persist as an important choice in settings where ART availability is restricted or in individuals at certain risk of specific toxicities or drug–drug interactions (DDIs).

•Whenever possible, "third agent of the first-line" with a high barrier to resistance is chosen, given the potential challenges with adherence in children and adolescents.

•Always consider the resistance transmission possibility, including exposure of both mother and infant to ART after failure to prevent vertical transmission.

•In infants younger than 4 weeks and/or under 3 kg, when nevirapine (NVP) was administered in pregnancy or there is a threat of passed NVP resistance, other than non-nucleoside reverse transcriptase inhibitors (NNRTI)-based ART is preferred, including raltegravir (RAL) from birth or ritonavir-boosted lopinavir (LPV/r) from the second week (16−18).

Preferred and alternative first-line options in C/ALHIV and antiretroviral formulations useful for dosing and administration in children and adolescents are listed in Table 1 and Table 2, respectively (16).

1. Due to long-term toxicity, any child on ZDV therapy should be switched to ABC (preferred for younger children) or TAF/TDF (alternative for younger children, with renal/bone toxicity monitored by TDF) when age and/or weight allow the use of licensed formulations. When ABC is contraindicated in young children, a choice between ZDV, TDF or TAF on an individual level is recommended

2. LPV/r should not be given to infants before a postmenstrual age of 42 weeks and a postnatal age of at least 14 days, although it may be considered if there is a risk of transmitted resistance to NVP and suitable INSTI formulations are not available. In these circumstances, the infant should be closely monitored for LPV/r-related toxicity (e.g., metabolic, endocrine, cardiac)

3. It is acceptable to continue therapy started with a 3rd agent other than DTG in the neonatal period. However, once the age of over 4 weeks and 3 kg is reached, switching to DTG is recommended if and when a suitable formulation is available

4. ABC should NOT be prescribed to HLA-B*57:01 positive individuals (where screening is available). ABC is not licensed for children under 3 months of age, but there are WHO-recommended dosing data for younger children

5. TDF is only approved for use from 2 years of age. Given concerns about potential effects on bone development and renal toxicity, TAF is recommended over TDF in all ages in settings where this is licensed and available.

6. DTG is allowed for use from 4 weeks and 3 kg. DTG is associated with excessive weight gain in adults, especially in combination with TAF. This has not yet been demonstrated in pediatric and adolescent observational studies or trials, but the possibility should be considered when using DTG. Families and youth should be counseled about this, and weight monitored

7. XTC indicates circumstances where FTC or 3TC can be used interchangeably

8. TAF is approved in Europe for the treatment of HIV in combination with FTC from 12 years of age and 35 kg in TAF/FTC and from 6 years of age and 25 kg in TAF/FTC/EVG/c. Since TAF is approved for use in younger ages and lighter weights, it can be included as a preferred option. TAF is associated with excessive weight gain in adults, especially when combined with DTG. This has not yet been demonstrated in pediatric and adolescent observational studies or trials; however, the possibility should be considered when using TAF. Families and youth should be counseled about this, and weight monitored

9. BIC is the preferred first-line option in adults. It has not yet been registered for use in children under 18 years of age, but may be considered in those under 18 years of age after professional consideration

10. Due to potential poor therapeutic adherence in adolescence, if preferred 3rd line agents (BIC or DTG) are not available/appropriate to possible alternative 3rd line agents, DRV/b resistance barrier should be preferred compared to EFV, RAL or RPV.

Table 1. Preferred and alternative first-line options in children and adolescents

	"Backbone'' therapy		**3rd agent (alphabetically)**	
Age	**Preferred**	**Alternative**	**Preferred**	**Alternative**
0–4 weeks	ZDV[(i)] + 3TC	-	LPV/r[(ii, iii)] NVP[(iii)] RAL[(iii)]	-
4 weeks–3 years	ABC[(iv)] + 3TC[(v)]	ZDV[(i)] + 3TC[(vi)] TDF[(vii)] + 3TC	DTG[(viii)]	LPV/r NVP RAL
3–6 years	ABC[(iv)] + 3TC[(v)]	TDF[(vii)] + XTC[(ix)] ZDV + XTC[(ix)]	DTG[(viii)]	DRV/r EFV LPV/r NVP RAL
6–12 years	ABC[(iv)] + 3TC[(v)] TAF[(x)] + XTC[(ix)]	TDF[(vii)] + XTC[(ix)]	DTG[(viii)]	DRV/r EFV EVG/c RAL
> 12 years	ABC[(iv)] + 3TC[(v)] TAF[(x)] + XTC[(ix)]	TDF[(vii)] + XTC[(ix)]	BIC[(xi)] DTG[(viii)]	DRV/b EFV[(xii)] RAL[(xii)] RPV[(xii)]

Abbreviations: ZDV—zidovudine, 3TC—lamivudin; LPV/r—lopinavir/ritonavir; NVP—nevirapine; RAL—raltegravir; ABC—abacavir; TDF—tenofovir disoproxil fumarate, DTG—dolutegravir; EFV—efavirenz; XTC—3TC or FTC; FTC—emtricitabine; EVG—elvitegravir; BIC—biktegravir; DRV/r—darunavir/ritonavir; TAF—tenofovir alafenamide

Table 2. Antiretroviral formulations useful for dosing and administration in children and adolescents

NRTI	
ABC	tablet (300 mg), solution (20 mg/mL)
FTC	capsule (200 mg), solution (10 mg/mL)
3TC	tablet (300, 150 mg), solution (10 mg/mL)
TDF	tablet (245, 204, 163, 123 mg), granules (33 mg/g)
ZDV	capsule (250 mg, 100 mg), solution (10 mg/mL) IV infusion: 10 mg/mL (20 mL/vial)
TAF/FTC	tablet (25/200 mg and 10/200 mg)
TDF/FTC	tablet (300/200 mg)
ABC/3TC	tablet (600/300 mg)
ZDV/3TC	tablet (300/150 mg)
NNRTI	
EFV	tablet (600 mg), capsule (200, 100, 50 mg)
NVP	tablet (200 mg), extended release tablet (400, 100 mg), suspension (10 mg/mL)
RPV	tablet (25 mg)
TDF/FTC/EFV	tablet (300/200/600 mg)
TAF/FTC/RPV	tablet (25/200/25 mg)
TDF/FTC/RPV	tablet (25/200/25 mg)
PI	
DRV	tablet (800, 600, 400, 150, 75 mg), solution (100 mg/mL)
DRV/c	tablet (800/150 mg)
LPV/r	tablet (200/50 mg and 100/25 mg), solution (80/20 mg/mL)
RTV	tablet (100 mg), powder for oral suspension (100 mg sachet)
TAF/FTC/DRV/c	tablet (10/200/800/150 mg)
INSTI	
DTG	tablet (50, 25, 10 mg), dispersible tablets (5 mg)
RAL	tablet (600 mg, 400 mg), chewable tablets (100, 25 mg,) granules for oral suspension (100 mg)
ABC/3TC/DTG	tablet (600/300/50 mg)
TAF/FTC/BIC	tablet (25/200/50 mg)
TAF/FTC/EVG/c	tablet (10/200/150/150 mg)
TDF/FTC/EVG/c	tablet (300/200/150/150 mg)

Abbreviations: ZDV—zidovudine; 3TC—lamivudin; LPV/r—lopinavir/ritonavir; NVP—nevirapine; RAL—raltegravir; ABC—abacavir; TDF—tenofovir disoproxil fumarate, DTG—dolutegravir; EFV—efavirenz; FTC—emtricitabine, EVG—elvitegravir; BIC—biktegravir; DRV—darunavir, DRV/r—darunavir/ritonavir; TAF—tenofovir alafenamide, RPV—rilpivirine, RTV—ritonavir, NRTI—nucleoside reverse transcriptase inhibitor, NNRTI—non-nucleoside reverse transcriptase inhibitor; PI—protease inhibitor; INSTI—integrase strand transfer inhibitor

World Health Organization Recommendations

The World Health Organization (WHO) has published updated *Consolidated guidelines on HIV prevention, testing, treatment, service delivery and monitoring: recommendations for a public health approach.* This publication brings together important clinical and programmatic additions by WHO since 2016, offering clear recommendations based on evidence and good clinical practice in relation to public health, with an individual-oriented approach and respect for human rights. This guide introduces the most recent recommendations on HIV testing strategies for entry points in HIV prevention and treatment and is composed of clear and comprehensive guidelines for diagnosis in children (19).

The diagnostic and therapeutic principles of caring for adults and C/ALHIV in Serbia are based on the EACS recommendations, taking into account the available diagnostic and therapeutic resources. WHO recommendations are also followed in circumstances of medication shortage (meaning that recommended drugs are in the process of registration or not yet). Preferred and

alternative first-line therapeutic regimens and preferred and alternative second-line therapeutic regimens are presented in Table 3 and Table 4, respectively. Considerations for switching to optimal treatment regimens for C/ALHIV regarded as steady on ART based on national guidelines are listed in Table 5 (19–31).

Table 3. Preferred and alternative first-line therapeutic regimens

Population	Preferred first-line regimen	Alternative first-line regimen	Special circumstances
Adolescents	TDF + 3TC (or FTC) + DTG[a]	TDF + 3TC + EFV 400 mg[b]	TDF + 3TC (or FTC) + EFV 600 mg[b] ZDV + 3TC + EFV 600 mg[b] TDF + 3TC (or FTC) + PI/r[b] TDF + 3TC (or FTC) + RAL TAF[c] + 3TC (or FTC) + DTG ABC + 3TC + DTG[a]
Children	ABC + 3TC + DTG[d]	ABC + 3TC + LPV/r ABC + 3TC + RAL[e] TAF + 3TC (or FTC) + DTG[f]	ABC + 3TC + EFV (or NVP) ZDV + 3TC + EFV[g] (or NVP) ZDV + 3TC + LPV/r (or RAL)
Neonates	ZDV + 3TC + RAL[h]	ZDV + 3TC + NVP	ZDV + 3TC + LPV/r[i]

Abbreviations: 3TC—lamivudine; ABC—abacavir; ZDV—zidovudine; DTG—dolutegravir; EFV—efavirenz; FTC—emtricitabine; LPV/r—lopinavir/ritonavir; NVP—nevirapine; PI/r—ritonavir-boosted protease inhibitor; RAL—raltegravir; TAF—tenofovir alafenamide; TDF—tenofovir disoproxil fumarate

a) Effective contraception should be offered to adolescent girls of reproductive age. DTG can be prescribed to adolescent girls of reproductive age or potentially pregnant or who are not using contraception if they are fully informed of the potential increased risk of neural tube defects (at conception and up to the end of the first trimester). If pregnancy is detected after the first trimester, DTG should be started or continued throughout the pregnancy

b) EFV-based ART should not be used in settings with national pre-treatment EFV resistance estimates of 10% or more. DTG-based ART is preferred, and if DTG is not available, a boosted PI-based regimen should be used. The choice of PI/r depends on the program characteristics;

c) TAF may be considered for people with established osteoporosis and/or impaired kidney function

d) For age and weight groups with an approved dosage of DTG

e) RAL should be used as an alternative regimen only if LPV/r solid formulations are not available

f) For age and weight groups with approved TAF dosing

g) EFV should not be used for children under the age of three

h) Infants initiated on ART with a RAL-based regimen should be switched to LPV/r solid formulation as soon as possible

i) LPV/r syrup or granules can be used if started after two weeks of age.

Table 4. Preferred and alternative second-line therapeutic regimens

Population	First-line regimen failure	Preferred second-line regimens	Alternative second-line regimens
Adolescents[a]	TDF[b] + 3TC (or FTC) + DTG[c] TDF + 3TC (or FTC) + EFV (or NVP) ZDV + 3TC + EFV (or NVP)	ZDV + 3TC + ATV/r (or LPV/r) ZDV + 3TC + DTG[c] TDF[b] + 3TC (or FTC) + DTG[c]	ZDV + 3TC + DRV/r[d] ZDV + 3TC + ATV/r (or LPV/r or DRV/r)[d] TDFb + 3TC (or FTC) + ATV/r (or LPV/r or DRV/r)[d]
Children and infants	ABC + 3TC + DTG[e] ABC (or ZDV) + 3TC + LPV/r ABC (or ZDV) + 3TC + EFV ZDV + 3TC + NVP	ZDV + 3TC + LPV/r (or ATV/r[f]) ZDV + 3TC + LPV/r (or ATV/r[f]) ZDV (or ABC) + 3TC + DTG[e]ABC + 3TC + DTG[e]	ZDV + 3TC + DRV/r[g] ZDV (or ABC) + 3TC + RALZDV (or ABC) + 3TC + LPV/r (or ATV/r[f])ABC + 3TC + LPV/r (or ATV/r[f] or DRV/r[g]

Abbreviations: 3TC—lamivudine; ABC—abacavir; ATV/r—atazanavir/ritonavir; ZDV—zidovudine; DRV/r—darunavir/ritonavir; DTG—dolutegravir; EFV—efavirenz; FTC—emtricitabine; LPV/r—lopinavir/ritonavir; NVP—nevirapine; RAL—raltegravir; TDF—tenofovir disoproxil fumarate

a) Sequencing is necessary if PIs are used in first-line ART: ATV/r (or LPV/r or DRV/r, depending on programmatic considerations) +

TDF + 3TC (or FTC), then AZT + 3TC + DTG in second-line therapy lines.

b) Effective contraception should be offered to adolescent girls of reproductive age. DTG can be prescribed to adolescent girls of reproductive age who can potentially become pregnant or who are not using effective contraception if they are fully informed of the potential increased risk of

neural tube defects (at conception and through the end of the first trimester). If pregnancy is detected after the first trimester, DTG should be started or continued throughout the pregnancy.

c) TAF can be used as an alternative NRTI in special situations for adolescents.

d) RAL + LPV/r can be used as an alternative second-line therapeutic regimen for adolescents.

e) The European Medicines Agency currently only approves DTG for children weighing at least 15 kg and more broadly for children weighing over 20 kg who can take the 50 mg film-coated tablets intended for adults. Dosing studies for younger children are ongoing, with approval expected in early 2020, but the 2016 WHO recommendations for second-line ART still apply (based on PI for children failing NNRTIs and RAL for children LPV/r has failed). TAF (tenofovir alafenamide) can be used as an alternative NRTI in children weighing at least 25 kg.

f) ATV/r can be used as an alternative to LPV/r for children older than three months, but there is limited availability of appropriate formulations for children younger than six years, a lack of a fixed-dose formulation, and the need for separate drug administration. Ritonavir booster should be considered when choosing this regimen.

g) DRV should not be used in children under three years of age and should be combined with an appropriate dose of ritonavir.

Table 5. Considerations for switching to optimal treatment regimens for children considered stable on ART based on national guidelines

Current regimen	Weight	Optimal regimen to switch	Considerations
ZDV + 3TC + NVP ZDV + 3TC + EFV ABC + 3TC + NVP	< 20 kg	ABC + 3TC + LPV/r	If stable, children can switch to DTG when they reach 20 kg
	20–30 kg	ABC + 3TC + DTG	If stable, children can switch to TDF + 3TC + DTG when they reach 30 kg
	> 30 kg	TDF + 3TC + DTG	–
ABC + 3TC + EFV	< 20 kg	No switches until they reach 20 kg, unless there is therapeutic failure	Switching to optimal regimens for these children is beneficial when they reach 20 kg, and DTG can be used to maintain once-daily administration
	20–30 kg	ABC + 3TC + DTG	If stable, children can switch to TDF + 3TC + DTG when they reach 30 kg
	> 30 kg	TDF + 3TC + DTG	–
ABC + 3TC + LPV/r ZDV + 3TC + LPV/r	< 20 kg	No switch until they reach 20 kg, unless there is therapeutic failure	Ensure pill use as soon as possible to reduce pill burden. Switching from ZDV + 3TC + LPV/r to ABC + 3TC + LPV/r may also be considered to reduce pill burden and preserve the antiviral advantage of NRTI sequencing
	20–30 kg	ABC + 3TC + DTG	If stable, children can be switched to TDF + 3TC + DTG when they reach 30 kg
	> 30 kg	TDF + 3TC + DTG	–

Abbreviations: 3TC—lamivudine; ABC—abacavir; ZDV—zidovudine; DTG—dolutegravir; EFV—efavirenz; LPV/r—lopinavir/ritonavir; NVP—nevirapine; TDF—tenofovir disoproxil fumarate; NRTI—nucleoside reverse transcriptase inhibitor

Centers for Diseasy and preventien Recommendations

The fixed-dose combination of bictegravir (BIC)/emtricitabine (FTC)/tenofovir alafenamide (TAF) is the preferred regimen based on the initial regimen with integrase inhibitors (INSTI) in children at least 2 years of

age who weigh at least 14 kg (previously preferred in children older than 6 years or whose weight is equal to or greater than 25 kg). FTC/TAF is recommended as the preferred combination with two NRTIs in children and adolescents weighing at least 14 kg when used with an INSTI or NNRTI (previously recommended for children and adolescents weighing 25 kg and more). Doravirine (DOR) is the recommended alternative to NNRTI for children and adolescents weighing at least 35 kg, either in a fixed combination of DOR/FTC/tenofovir disoproxil fumarate (TDF) or in combination with any two NNRTIs. This recommendation is based on efficacy and tolerability in adult and pediatric pharmacokinetic studies. A Centers for Diseasy and preventien (CDC) panel recommends use of abacavir (ABC) as the preferred NRTI component from birth in full-term infants after a negative HLA-B5701 test as part of a dual ABC/lamivudine (3TC) or ABC/FTC combination (previously ABC/3TC or ABC/FTC was the preferred NRTI combination after 3 months of age) (32–37).

When to start treatment?

ART consisting of 3 drugs from at least 2 classes should be started in all treatment-naïve C/ALHIV. Postponed treatment of HIV infection is discouraged. Prompt initiation of treatment (within 1–2 weeks of diagnosis) is suggested in all HIV-infected children older than 6 weeks but younger than 12 weeks. This rapid start should be well thought out in order to achieve and maintain adherence in children. Some of these infants will already be on prophylactic treatment (initiated as soon as possible in high-risk infants), and a change in this regimen may be considered. If initiation of ART in a child is unfeasible for any cause, the child's virological and immunological condition (HIV viral load and CD4+ T cells) should be supervised until the treatment start (38).

Historically, some antecedent drugs have been much more toxic and have been connected with enhanced emergence of resistance. Therefore, maintenance therapy used to be commonly advised in different age cohorts and in the initial phase of HIV infection. Nowadays, it has ceased to be the situation, and all C/ALHIV should be treated as soon as possible to evade disease advancement, to avoid infections, to secure growth and sexual maturation, to escape the neurocognitive repercussions of HIV disease, to help in obtaining a normal life span and possibly prevent additional HIV infection spread (treatment as prevention). All children on treatment should be monitored regularly for treatment efficacy and any toxicity associated with therapy (38, 39).

There is sufficient data on the dosage of zidovudine (ZDV), 3TC and NVP for therapy in premature infants. For term infants, there is also enough information for FTC, ABC, and raltegravir (RAL). ABC is not Food and Drug Agency-approved from birth. However, ABC is recommended by a CDC panel based on pharmacokinetics and safety data to be used from birth with administration for HLA-B5701 testing only (must be negative for HLA-B5701 to tolerate the drug). For full-term infants older than 2 weeks (but not preterm infants), there is sufficient data on LPV/r dosing. Triple therapy should be started as soon as the diagnosis is confirmed (40).

Preferred initial therapy in treatment-naïve infants and children consists of a backbone combination of two NRTIs plus an INSTI or NNRTI (preferred) or a protease inhibitor (PI) (alternative and requires boosting). Backbone NRTI options include the following: ABC plus 3TC or FTC preferred after negative HLA-B5701 testing, ZDV substitutes for ABC before HLA testing or if tolerated, effective, and prefers family not to switch. Although ZDV is not the preferred drug for children aged 6 years or more, it might be extended instead of switching to another ART drug if it effectively suppresses the viral load. It is also a replacement option for therapy start in children. NNRTI options comprise the following: for children aged less than 14 days, NVP is the preferred NNRTI. An alternative is rilpivirine therapy for children older than 12 years. Efavirenz is not proposed for children under 3 years of age and is not the preferred therapy because it has multiple side effects that affect sleep and cause neuropsychiatric symptoms (41).

INSTI therapy choices:

- Infants under 14 days of age but ≥ 2 kg: RAL (oral suspension or powder for suspension) can be used for treatment as prophylaxis in high-risk infants. It is an alternative after 4 weeks of age when dolutegravir becomes the preferred therapy.
- Children aged at least 4 weeks and ≥ 3 kg: initial therapy with DTG is preferred
- Children 2 years of age or older (≥ 14 kg): BIC in combination with a fixed dose (FDC) preferred initial therapy
- Children 3 years of age or older (≥ 25 kg): EVG/c is an alternative to INSTI
- Children aged 3 years or older (≥ 25 kg): EVG/c/FTC/TAF is an alternative fixed-dose combination therapy. PI options include the following:
- Children older than 14 days: LPV/r preferred PI therapy
- Children older than 3 months: ATV/r is an alternative therapy
- Children older than 3 years: DRV/r-twice daily is an alternative therapy
- Cobicistat (PI booster) and ritonavir- or cobicistat-boosted regimens can be used as an alternative to ritonavir boosting in children. Additionally, cobicistat-boosted atazanavir and cobicistat-boosted darunavir are suitable alternatives for children (39, 41, 42).

Conclusion

Previous forms of antiretroviral therapy had numerous toxic effects and interactions. Therefore it was justified to delay therapy initiation in order to have less negative impact over time on the health status of C/ALHIV. The new guidelines

currently include therapy that is comfortable for daily use without toxicity. For this reason, it is suggested to start therapy as soon as possible. This not only restores the immune status of the C/ALHIV and prevents opportunistic infections and malignancies, but also affects to some extent the reduction of the level of microinflammation and consequent immunosenescence.

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SAVREMENE OPCIJE LEČENJA DECE I ADOLESCENATA KOJI ŽIVE SA HIV INFEKCIJOM

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Cilj lečenja infekcije virusom humane imunodeficijencije (engl. *human immunodeficiency virus* – HIV) i kod odraslih i kod dece i adolescenata jeste postizanje stabilnog odgovora imunosistema na infekciju virusom, koji podrazumeva činjenicu da virus nije detektovan u krvi duže od šest meseci i oporavak imuniteta. Lečenje dece i adolescenata koji žive sa HIV-om (engl. *children and adolescents living with* HIV – C/ALHIV) specifičnije je i teže zbog slabijeg pridržavanja terapije, dužeg trajanja infekcije i posledičnih toksičnih efekata, kao i zbog hronične mikroinflamacije. Još jedan otežavajući faktor predstavlja nedostatak adekvatnih pedijatrijskih farmaceutskih koformulacija u zavisnosti od regiona. Napredak i pojava inovativnih vidova terapije i jasnih smernica doveli su do poboljšanja kvaliteta života i imunološkog statusa dece i adolescenata koji žive sa HIV-om. Kao najčešće korišćene zvanične smernice izdvajaju se preporuke Evropskog kliničkog udruženja za AIDS (engl. *European AIDS Clinical Society* – EACS), Svetske zdravstvene organizacije (SZO) i Centra za kontrolu i prevenciju bolesti (engl. *Center for Disease Control and Prevention* – CDC). Na osnovu pomenutih smernica i lokalnih uslova i mogućnosti formiraju se i nacionalni vodiči na nivou pojedinih država.

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***Ključne reči**: virus humane imunodeficijencije, deca, adolescenti, antiretroviralna terapija, smernice*

Review article

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SAFETY OF CONCURRENT INTRAVENOUS DRUG APPLICATION

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Simultaneous application of multiple intravenous pharmaceuticals, during which they come into direct contact, is common in clinical practice. This is done with both intravenous infusions and injections. While it can be practically and clinically justified, this practice can only be safe for patients if all the combined products are mutually compatible. Physical and chemical incompatibility, with precipitation being the most common and important phenomenon, presents a possible health risk. Intravenous drugs and simple intravenous liquids both have the potential for displaying incompatibility. Over time, many studies utilizing various analytical methods have uncovered numerous inadequate combinations. However, the methodology of these studies is very heterogeneous; it is not always clear whether the results are clinically relevant, and many combinations have not been tested yet. It has also been shown that healthcare providers who are involved in therapy management sometimes do not possess enough knowledge about drug compatibility, although this can be improved with appropriate interventions. Furthermore, a precisely defined protocol for compatibility studies could aid interpretation and comparison of future research data. On the other hand, easily accessible databases and knowledge of alternative application methods for therapy could prevent incompatibilities in everyday work.

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Key words: *intravenous infusions, intravenous injections, drug incompatibility, patient safety*

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Introduction

It is common in everyday clinical practice for patients to receive multiple drugs at the same time as part of their therapy. Furthermore, parenteral application of drugs is also common in this setting, especially in the form of intravenous injections and infusions (1). Ideally, these dosage forms should be applied separately using a new venous access site each time (2). However, it should be taken into account that intravenous therapy is a specific form of drug application that requires special tools and equipment, as well as trained personnel, time management and a favorable condition of the patient. With that in mind, it is clear why a need for simultaneous application of two or more intravenous drugs—infusions, injections or both—arises in practice (1–3).

The greatest advantage in combining these forms is in critical patients who require many drugs delivered in a short time span. Depending on the severity of a condition, it is often not possible to secure enough venous access sites in certain patients if each drug must be administered separately (1–2). Even when it is possible, using multiple venous accesses increases the risk of mechanical, infectious and thrombotic complications due to their association with intravenous drug application (4).

Of course, just like any intervention, this practice also carries its risks. The biggest problem lies in the mutual compatibility of the dosage forms being combined. Any unwanted interaction between the drug components being combined represents an incompatibility that manifests itself as a chemical or physical change (1). Naturally, microbiological contamination is also a possibility when dealing with sterile products (4). Looking at incompatibility through these types of changes is typical from the point of view of pharmaceutical technology. Pharmacologically speaking, one can speak of therapeutic incompatibility as well, which manifests itself in the body. However, this is outside of the scope of this paper. With that said, the direct contact between two drug formulations can lead to physical and chemical changes that

can further impact the efficacy and safety of the overall therapy. This is why having reliable information about the compatibility of two drugs is required to ensure their safe application (1).

Combining Intravenous Forms

It should be defined what exactly is meant by combining or mixing two or more intravenous drugs. Two methods that are used in practice fit this description. The first method is used in patients receiving an infusion to which new infusions or injections need to be added. Depending on the dosage form, they are connected or are directly injected in the infusion set carrying the primary infusion via injection and Y-site ports (1, 3). The connectors that are used are usually Y-shaped, which means that two formulations being applied meet at the intersection and are mixed before entering the bloodstream. Time of contact between them is relatively short (ranging from 1−2 minutes to an hour), which is why physical incompatibility is the more important negative outcome when utilizing this method compared to chemical incompatibility, which often requires more time to result in significant change. This compatibility is referred to as Y-site compatibility (1, 2).

The second method is more direct and involves adding a sterile drug to an infusion before application. Injections can be mixed in a similar way. These preparations are often done in a hospital pharmacy. Since in this instance time of contact is usually longer (measured in hours or days), more attention is given to chemical compatibility (1, 2, 5).

Regardless of which method is employed, the intravenous forms being combined are not just drug solutions, but also simple intravenous fluids. This is why incompatibility is not limited just to interactions between two or more active ingredients. In fact, intravenous fluids are often the primary infusion with the role (among others) of being the carrier of any additional therapy. Additionally, these solutions are used for diluting, reconstituting and directly mixing drugs. However, despite their simple composition (consisting of electrolytes, glucose and related compounds), intravenous fluids have the potential to interact with drug molecules and therefore cannot be considered inert (1, 3). In a larger sense, packaging material and excipients can also be a source of incompatibility. While most intravenous dosage forms are solutions, some emulsions can also be delivered in this manner. Compatibility considerations become even more complicated for complex pharmaceuticals such as parenteral nutrition – emulsion or not. The same can be said for blood products (3).

Forms of Incompatibility

Physical incompatibility most often presents as precipitation, turbidity, color change, gas formation or change in pH level. It can be visible and is often accompanied by chemical changes. Precipitation is the most important incompatibility-related phenomenon and oftentimes occurs as a result of pH change. Many parenteral forms are buffered, but their buffer capacity is limited. By mixing two solutions of significantly different pH levels, a change in drug ionization (in the case of weak acids and bases) occurs, leading to changes in solubility and consequently precipitate formation (3, 5).

Precipitates can also be formed in ion exchange reactions, e.g. when polyvalent cations from one solution displace monovalent cations from another. They can also form when the end result of post-mixing is a solvent in which one of the drugs is poorly soluble (3, 5). Precipitate formation is the most common manifestation of incompatibility and presents a serious problem, as it can lead to embolism if the particles formed enter the systemic circulation. Smaller particles can also cause organ damage due to the occlusion of small blood vessels. Overall, this leads to an increase in patient morbidity in cases where precipitation occurs during therapy. Local reactions such as phlebitis are less severe (6, 7). On the other hand, precipitates can also be a purely technical problem in instances where they cause mechanical blockage in an infusion set, which stops further drug delivery (7).

Changes in pH can happen without concomitant precipitation, but they are still an unwanted event. Gas formation can be dangerous as a potential cause of gas embolism. This frequently crops up in the form of carbon dioxide release in scenarios when one of the formulations being combined contains bicarbonates (2, 3, 8). In cases of incompatibility, emulsions display instabilities specific to their pharmaceutical form (creaming, sedimentation, phase separation and others) (2, 3).

Chemical incompatibility is frequently not visible and is a result of chemical degradation reactions typical for drug molecules—oxidation, reduction and hydrolysis. Packaging incompatibility can also be put in this category. Most of the time, drug degradation is considered significant when there is a loss of active ingredient larger than 10% or a toxic product has formed. As a consequence, this leads to a reduction in the therapeutic effect or possible toxicity (3, 6, 9). Changes in pH level and external factors, such as temperature, light and oxygen, can have a crucial effect on the rate of chemical change. Biologic drugs are especially sensitive to changes in internal and external conditions and, as a rule, are generally not mixed with other drugs (3).

Experiences in Clinical Practice

Many studies looked at various individual drug combinations and their compatibility (2, 6, 10−12). Likewise, research has been done on the occurrence of incompatibility in clinical practice,

with the results being highly variable. One study showed that 7.2% of all intravenous drug combinations used were incompatible in one clinical setting, while another gave a finding of 15% (13, 14). It should be clear that the drugs being looked at in these studies are those that are most often used in everyday clinical work—particularly in intensive care units, which require complex treatment plans. Table 1 lists certain examples of incompatibility along with the mechanisms of their formation.

Table 1. Examples of incompatibility encountered in practice

Drug-drug combination	Consequence of incompatibility
Heparin and many antibiotics (15)	Precipitate formation
Beta-lactams and vancomycin (16)	
Furosemide and low-pH solutions (3)	
Ringer's solution and many drugs (17)	
Metoclopramide and sodium bicarbonate (3)	Gas formation
Pantoprazole and many drugs (12)	Color change and Precipitate formation
Adrenaline and sodium bicarbonate (18)	Inactivation of adrenaline
Atracurium and high-pH solutions (3)	Inactivation of atracurium
Midazolam and hydrocortisone (11)	Loss of hydrocortisone
Diazepam and PVC (19)	Sorption to packaging
Propofol and lidocaine (20)	Droplet coalescence and phase separation of emulsion

It is also noticeable from systematic reviews in this area that certain drugs have a particular tendency to interact, such as vancomycin, hydrocortisone, pantoprazole and in general drugs whose solutions are highly acidic or basic (10−12). However, there is not always a strict distinction between "allowed" and "unallowed" combinations, as compatibility issues can sometimes be overcome with certain modifications in the drug mixing process. Time of contact, concentration of reacting components and type of solvent are all factors that determine whether a borderline incompatibility will manifest itself or not (2−3, 10). On the other hand, there are also situations when different studies give conflicting results about the compatibility of a certain drug combination (2, 12).

Parenteral nutrition products are a challenge of their own. Several types of them can be commercially acquired, but they can also be prepared in hospital pharmacies. It is useful to make a distinction between formulations without lipid components (solutions) and those that contain them (emulsions). Regardless of whether they are all-in-one or applied separately, they have a high potential for drug compatibility issues due to their complex composition. For example, trace elements can act as catalysts in degradation reactions, while any change in the calcium ion–phosphate ratio can result in precipitate formation. Of course, other components can be a source of instability as well. Special attention should be given to lipid formulations, as drugs that are stable in non-lipid forms can become unstable in the presence of lipids. Generally, mixing drugs and parenteral nutrition products should be avoided. Nevertheless, this practice is common in pediatric medicine when securing multiple venous access sites is not possible (21−22).

There is even less data in the literature about adding intravenous drugs to blood products. In fact, this practice is banned in many hospitals (23). For this reason, they are generally not mixed, and the same applies to biologic therapy (3, 23).

Uncovering Incompatibility

Although the term incompatibility is related to the term stability, stability guidelines laid out by the International Council for Harmonisation (ICH) do not require manufacturers to assess the compatibility of their drugs with other products, including intravenous drugs (24). However, since

many intravenous drugs require in-use stability testing, this process can be used to determine whether a drug is compatible with various intravenous solutions that are used as a solvent or diluent (25).

Data on intravenous compatibility has been gathered from experience, but also from formal studies of various drug combinations. These studies are based on monitoring physical and chemical stability. As there is no standardized protocol for them, researchers choose which parameters and analytical methods to employ during the study. Physical stability is usually determined by monitoring for precipitation formation, color change, gas bubbles and changes in pH level. Precipitates can be detected visually, but it is better to use nephelometry or turbidimetry. Color change is a visually qualitative change that can be quantified with certain instrumental methods, such as spectrophotometry. Changes in pH levels can be measured with a pH meter. Chemical changes are often detected with the help of HPLC. However, studies look at physical changes most of the time, as in practice it is more common to mix drugs through a concurrent infusion rather than directly, where chemical stability would be more important (2, 10, 11, 26).

The main downside of these studies is the heterogeneity of their methodologies. Each study makes its own choice on which parameters to follow and which analytical methods to utilize. Furthermore, it is not always clear whether the results are clinically relevant due to the way the study was conducted. One paper showed that researchers often monitor for physical changes after hours of mutual contact, even though in practice the time of contact is much less than that. The same paper showed that none of the studies reviewed had a blinded design, without which potential bias cannot be overlooked (2). The American Journal of Health-System Pharmacy recently published guidelines on stability and compatibility testing that touch on the area of intravenous incompatibility. The article deals with, among other things, sample preparation, variation of external factors and testing the impact of packaging on compatibility (26). Guidelines such as these could prove to be very useful for standardizing future research in the area of incompatibility.

Preventing Incompatibility

Actively preventing drug incompatibility is an imperative of safe clinical practice. A necessary precondition for this to be achieved is knowing ahead of time whether a certain combination of products is compatible. If an interaction is possible, and the combination of drugs is necessary and justified, an intervention can be made on several levels (27). When possible, an alternative, non-interacting dosage form should be used. Similarly, a drug can be switched with one that is compatible (3).

If the intravenous route is, in fact, needed, an attempt should be made to separate the two formulations by time or space. That can mean using a different venous access site to apply the second drug (2). However, nowadays, there is the option of using multi-lumen catheters that contain several spatially separated lumens in one tube, which enables the separate and simultaneous delivery of multiple intravenous drugs (3, 13). Another option is in-line filters built into infusion sets that prevent particles above a certain size (like those from precipitates) from passing into the bloodstream (28).

Conversely, incompatible drugs can sometimes also be separated by time of application. In those instances, the infusion set does not have to be changed if it is flushed with an appropriate sterile solution, before and after the incompatible drug is applied (29).

Healthcare workers who are involved in drug application are responsible for checking the compatibility of two or more products before combining them. However, hospital pharmacists also play an important role as they should be able to provide information on compatibility (12, 27). Several studies have shown that, in practice, knowledge about compatibility is not always satisfactory, although there is less data on the actual clinical consequences of incorrect practice (6, 11, 13, 14). Nevertheless, it has been shown that the frequency of compatibility issues can be significantly reduced after applying educational and preventative measures (11, 13, 14).

Official recommendations exist both on higher and lower levels. For example, the Royal College of Nursing in the United Kingdom has published guidelines that touch on intravenous therapy compatibility (29). On the other hand, hospitals can have their in-house guidelines and protocols that deal with this matter. These recommendations are often presented in the form of tables and graphs that are easily accessible to healthcare providers (30). Still, there are faster and simpler ways to obtain the necessary facts. For example, Micromedex and Lexicomp are online services that offer a compatibility database upon registration. Stabilis is an online database that anyone can access (9, 31, 32). The data in these databases are supported by references to the original papers where the combinations were tested. Of course, the quality of the data is directly correlated with the conception and methodology of the source studies. As mentioned before, there are certain issues with studies in this field. Another big problem is the fact that the number of possible drug combinations is huge, even when only considering two-part combinations of a limited number of drugs. With that in mind, many combinations have not been tested yet and therefore are generally not tried out in practice before sufficient information about them is generated (2, 10, 11).

Conclusion

Combining intravenous drugs can be safe in cases when this practice is justified, only when the precondition of mutual compatibility is met. A large number of guidelines and easily accessible databases allow for a fast check on the adequacy of a certain combination during everyday work. Simultaneously, raising awareness of the possibility of incompatibility, its types and ways to recognize and prevent it can decrease its frequency along with the associated health risks. However, as guidelines in this area are based on the results of individual research, there is a need to define a standard protocol for compatibility testing so that the data generated can be better evaluated and compared.

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BEZBEDNOST ISTOVREMENE INTRAVENSKE PRIMENE LEKOVA

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U kliničkoj praksi je uobičajena istovremena primena nekoliko lekova intravenskim putem, prilikom koje dolazi do njihovog direktnog kontakta. To se čini i sa intravenskim infuzijama i sa injekcijama. Mada ova praksa može biti opravdana iz praktičnih i kliničkih razloga, bezbedna je za pacijente samo ako je ispunjen preduslov da su preparati koji se kombinuju međusobno kompatibilni. Pojava fizičko-hemijskih inkompatibilnosti, od kojih je najčešća i najznačajnija pojava precipitacije, predstavlja mogući rizik po zdravlje. I kod lekova koji se primenjuju intravenski i kod intravenskih tečnosti jednostavnog sastava postoji potencijal za ispoljavanje inkompatibilnosti. U mnogobrojnim dosad sprovedenim ispitivanjima korišćenjem različitih analitičkih metoda utvrđen je veći broj nekompatibilnih kombinacija. Međutim, budući da je metodologija ovih ispitivanja veoma heterogena, nije jasno da li su dobijeni rezultati uvek klinički relevantni. Pritom, mnoge kombinacije još nisu ispitane. Ustanovljeno je i da zdravstveni radnici, koji su odgovorni za lečenje pacijenata, ponekad nemaju dovoljno znanja o kompatibilnosti lekova. Ipak, to se može prevazići odgovarajućim intervencijama. Takođe, precizno definisanje protokola ispitivanja kompatibilnosti moglo bi olakšati interpretaciju i upoređivanje podataka iz budućih studija. S druge strane, lako dostupne baze podataka i poznavanje alternativnih metoda primene terapije mogu sprečiti pojavu inkompatibilnosti u svakodnevnom radu.

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***Ključne reči**: intravenske infuzije, intravenske injekcije, inkompatibilnost, bezbednost pacijenata*

Case report

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ENZALUTAMIDE-INDUCED HEART FAILURE WITH REDUCED EJECTION FRACTION

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Prostate cancer is the second most common malignancy in men and the second leading cause of cancer death. Patients unfit for surgery, with locally advanced or metastatic disease, require androgen-deprivation therapy.

In the past decade, enzalutamide and abiraterone were established in prostate cancer treatment. Their safety profile and clinical evidence showed a significant possibility of cardiovascular complications. Heart failure is more common in patients treated with abiraterone. We report a case of a patient with heart failure induced by enzalutamide. In the review of the literature, enzalutmaide-induced heart failure was encountered in only two cases.

Further research on the influence of novel anti-androgen therapy on the cardiovascular system, personalized evaluation of the cardiovascular system, and risk stratification for potential cardiovascular adverse events before initiating therapy are needed.

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Key words: *enzalutamide, heart failure, prostate cancer*

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Introduction

Prostate cancer has one of the highest incidences among malignant diseases, and it has been estimated that more than 11% of males worldwide will have prostate cancer diagnosed during their lifetime. It is also responsible for nearly 50% of cancer deaths (1, 2). It is estimated that 288,300 men will be diagnosed with prostate cancer and 34,700 men will die of the disease by the end of 2025 in the United States of America. With approximately 2,300 to 2,400 newly diagnosed cases annually, prostate cancer is the second most common malignancy (after lung cancer) and the third leading cause of cancer death (after lung and colorectal cancer) in Serbia (3). Although prostate cancer is highly prevalent, the 5-year relative survival rate for men with localized or locally advanced disease is outstanding, nearing 100%, and yet men with metastatic disease have a 5-year survival rate of only about 30% (4). The risk of developing prostate cancer increases with age, and the median age at diagnosis is 67 years (2).

The etiology and pathogenesis remain unclear but are believed to be multifactorial and include: ageing, ethnicity, hormonal, and genetic factors. Androgens are crucial in the development, maintenance, and progression of prostate cancer. In 80–90% of cases, increased androgen activity is present in the early stage of the disease. The incorporation of androgen deprivation therapies into the treatment strategy for prostate cancer has provided numerous benefits, including enhanced survival for patients with clinically localized or locally advanced disease and improved symptom management for those with advanced stages of the condition (2). First-line androgen deprivation therapy (ADT) mechanism of action is blocking testosterone production by inhibition of the hypothalamic-pituitary-gonadal axis (5). There are different forms of ADT, and the most frequently used ones are gonadotropic releasing hormone (GnRH) agonists and antagonists. Most metastatic prostate cancer patients, treated with first-line

ADT, eventually develop castration resistance, as quickly as 7 months after therapy initiation. In that manner, metastatic or non-metastatic hormone-sensitive prostate cancer (HSPC) is becoming castration (hormone) resistant prostate cancer (CRPC), which is associated with a lower survival rate and quality of life deterioration (6). In the past decade, novel hormonal therapy has been introduced as the primary treatment of advanced prostate cancer to overcome castration resistance. Abirateron acetate, enzalutamide, apalutamide and darolutamide are the key novel hormonal therapies that inhibit androgen receptor (AR) function by blocking its dimerization, nuclear translocation, and binding AR-dimers to AR and have been shown to improve overall survival when added to first-line ADT (7).

Cardiac adverse drug reactions and mortality incidence are higher in patients treated with GnRH analogs, especially in individuals with pre-existing cardiac conditions. Incorporating novel agents, such as enzalutamide and abiraterone, into the treatment regimen further elevates cardiovascular risk (8). Abiraterone and enzalutamide are associated with a higher risk of hypertension, and abiraterone is also linked to an increased incidence of heart failure (9). Prescribing information for abiraterone cites risks of cardiac arrhythmias, chest discomfort, and heart failure, while enzalutamide cites only cardiac ischemia risk (10, 11).

Enzalutamide acts as a direct androgen receptor antagonist with greater affinity than first-generation antagonists. It also inhibits its nuclear translocation, thereby blocking the transcription of oncogenic genes essential for cancer growth and survival (12). It was shown that adding enzalutamide to ADT increases overall survival and quality of life in patients who progressed during ADT (13). The efficacy of enzalutamide was investigated in patients with non-metastatic CRPC (defined as prostate-specific antigen (PSA) doubling time ≤ 10 months during ADT) (14).

There is an urgent need for further research on the pharmacovigilance of novel hormonal therapy in prostate cancer patients due to the high mortality rate caused by cardiovascular disease, rather than malignancy itself. A recent study done in 2021 showed a higher incidence of cardiac events in patients treated with abiraterone in contrast to the patients treated with enzalutamide and concluded that enzalutamide has a much better safety profile than abiraterone, especially in patients with cardiac comorbidities (15).

In the review of the literature, enzalutamide-induced non-ischemic cardio-myopathy was encountered only in two previously published case reports (8).

Case Presentation

We present a case of a 75-year-old Caucasian man who was diagnosed with non-metastatic prostate cancer in 2010. He was experiencing dysuria for a couple of months before he scheduled an appointment with a urology specialist. The level of serum PSA was determined, and it was increased with an absolute value of 6.9 ng/ml. The urologist decided to perform a radical prostatectomy, as a primary diagnostic and treatment procedure, and it took place in December 2010. The pathology report indeed confirmed high-grade adenocarcinoma of the prostate gland, Gleason score 6, grade group 2, pT3N0 with positive lymphovascular invasion, impaired capsule, and infiltration of adipose tissue as well as seminal vesicles. Due to tumor size and impaired capsule with infiltration of surrounding tissue, the tumor board decided to conduct postoperative radiation therapy. After completion of the treatment, in May 2011, with a total of 45 Gy of radiation dose, the patient started a watchful waiting regimen. In May 2019, at his regular 6-month follow-up, an increased PSA of 12.76 ng/ml was noted. Radiological findings, including thoracic, abdominal, and pelvic compated tomograpy scans and bone scintigraphy, were negative for loco-regional and distant metastases. He started with androgen deprivation therapy (LHRH agonist), and his PSA level decreased. Nevertheless, in March 2024, the PSA level started to rise again, with an absolute value of 44.29 ng/ml. Imaging studies showed enlarged retroperitoneal lymph nodes (up to 32 mm in diameter) and bone metastases. Since he had a medical history of well-controlled hypertension and atrial fibrillation, and had undergone surgery for an aneurysm of the abdominal aorta back in 1997, echocardiography was performed before making a therapy decision. Initial findings were within normal, with an ejection fraction of 61% so we have decided to initiate enzalutamide along with LHRH agonist treatment. The patient started enzalutamide therapy in July 2024.

In August 2024, 40 days after the initiation of enzalutamide therapy, he presented to the emergency room with an acute onset of shortness of breath, pain in the middle of the chest, peripheral edema, and fatigue. An echocardiogram demonstrated new cardiomyopathy with a reduced left ventricular (LV) ejection fraction of 26% and diffuse hypokinesis. Coronary angiography showed no significant arteriosclerosis. Enzalutamide was discontinued because it was the only preceding event before the onset of heart failure. He started guided heart failure therapy with beta-blocker (bisoprolol), angiotensin-converting enzyme inhibitor (ramipril), mineralocorticoid receptor antagonist (spironolactone), and sodium-glucose transport protein inhibitor (dapagliflozin). A control echocardiogram after one month showed improvement in left ventricular systolic function with an ejection fraction of 36%. Four months after enzalutamide discontinuation, the ejection fraction was 39%. The patient was still

experiencing fatigue, but less intense compared to the onset of the symptoms. The tumor board decided to continue LHRH agonist as monotherapy since the patient was not symptomatic from his metastatic cancer and due to the possible cardiotoxicity of other hormonal and chemotherapy agents.

Discussion

In the majority of cancer patients, death is caused by reasons other than malignancy itself. Around 45% of prostate cancer patients die due to complications of anticancer therapy (16). In recent decades, prostate cancer treatment has advanced significantly, with the approval of numerous new therapies, including hormonal treatments (17). Resistance to androgen deprivation therapy prompted the development of novel androgen receptor blockers, such as enzalutamide, apalutamide, and darolutamide, which have proven effective in the CRPC setting. These agents primarily work by inhibiting the interaction between androgens and AR, preventing AR nuclear translocation, or blocking AR-dependent gene transcription. These mechanisms ultimately reduce prostate cancer cell proliferation and tumor size but increase the possibility of the development of serious cardiovascular complications (18). Increased cardiovascular risk is reported in prostate cancer patients treated with ADT, abiraterone or enzalutamide. Hypermineralocorticism can be induced by abiraterone, which further leads to hypokalemia, hypertension, and edema. As a result of that, heart failure and atrial tachyarrhythmia may occur in patients treated with abiraterone (9).

Enzalutamide, an androgen receptor inhibitor, exhibits a higher binding affinity to androgen receptors compared to older inhibitors like bicalutamide. This characteristic is linked to improved clinical outcomes in patients with non-metastatic or metastatic castration-resistant prostate cancer compared to bicalutamide (19). Pathophysiology of QT prolongation, the most common cardiac adverse event of enzalutamide, is delayed rectifier potassium and sodium current, longer action potential duration, and appearance of afterdepolarizations (20). In a recent meta-analysis of prospective studies, enzalutamide was found to have a much better cardiac safety profile compared to abiraterone (21). A study done in 2021 showed a higher incidence of cardiac events in patients treated with abiraterone in contrast to the patients treated with enzalutamide and concluded that enzalutamide has a much better safety profile than abiraterone, especially in patients with cardiac comorbidities (15). Nevertheless, the patient did have cardiac adverse events due to enzalutamide therapy. In the review of the literature, enzalutamide-induced heart failure with reduced ejection fraction was encountered in only two previously reported cases.

A meta-analysis from 2018, which included 8,660 patients with metastatic prostate cancer treated with enzalutamide, found an increased incidence of hypertension among those patients (22). Another study by Shrestha B confirmed that the most common cardiovascular complication in patients treated with enzalutamide was hypertension (10.6%), followed by ischemic heart disease (1.88%) and atrial fibrillation (0.39%) (23).

Given the relatively high prevalence of cardiovascular complications associated with prostate cancer treatment, every patient must undergo a thorough clinical evaluation before initiating any anticancer therapy, including novel hormonal treatments. Treatment decisions should consider the patient's age, type of therapy, current and prior cardiovascular status, existing comorbidities, and concurrent medications. Regular cardio-oncology assessments are highly recommended, and existing cardiovascular conditions should be effectively managed and treated. Before starting therapy, patients should be screened for hypertension, dyslipidemia, and prediabetes/diabetes. Baseline electro-cardiography and transthoracic echocardiography should be performed, with follow-up evaluations conducted before each new treatment phase. In the event of cardiovascular complications, management decisions should take into account the cancer prognosis (e.g., early-stage vs. metastatic disease), life expectancy, and the patient's preferences (24).

Conclusion

Prostate cancer has a very high prevalence among elderly men, and it could potentially be even higher due to the increasing elderly population worldwide. Anti-androgen therapy remains the standard of care in the majority of PC patients. In the past decade, several new hormonal agents were introduced in the treatment of prostate cancer. Although very efficient, new hormonal therapy is associated with an increased risk of many complications, of which cardiovascular complications are the most severe. One of the proposed mechanisms of the onset of hypertension, heart failmre, ischemic heart disease, rhythm disturbances, and venous thromboembolic disease is the exacerbation of the imbalance of the cardiovascular system due to hormonal and metabolic changes. Hereby, we report a case of likely rare cardiovascular toxicity due to enzalutamide therapy, which typically has a safer cardiovascular profile among novel AR blockers. Literature review shows that abiraterone has more cardiovascular side effects than enzalutamide, which more commonly causes hypertension. Further research on the safety profile of novel anti-androgen therapy, as well as their impact on the cardiovascular system, is needed and should encourage the development of comprehensive cardio-oncology programs. Our

findings underline the importance of baseline screening and personalized evaluation of the cardiovascular system, risk stratification for possible cardiac adverse events, fundamental analysis of potential risk or benefit, before therapy initiation.

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SRČANA INSUFICIJENCIJA SA REDUKOVANOM EJEKCIONOM FRAKCIJOM UZROKOVANA ENZALUTAMIDOM

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Karcinom prostate je drugo najčešće maligno oboljenje kod muškaraca i drugi vodeći uzrok smrti od malignih bolesti. Lečenje inoperabilnih pacijenata, pacijenata sa lokalno uznapredovalom ili metastatskom bolesti zahteva primenu terapije derivacije androgena.

U protekloj deceniji u terapiju karcinoma prostate uvedeni su lekovi enzalutamid i abirateron. Bezbednosni profil ovih lekova i podaci iz kliničke prakse ukazuju na to da pomenuti lekovi imaju značajan uticaj na pojavu kardiovaskularnih komplikacija. Srčana insuficijencija je češći neželjeni efekat primene abiraterona. U ovom radu se prikazuje slučaj pacijenta kod kojeg je pojavu srčane insuficijencije izazvao enzalutamid. Pregledom literature nađena su samo dva slučaja srčane insfucijencije uzrokovane enzalutamidom u svetu.

Neophodna su dalja istraživanja o uticaju nove antiandrogene terapije na kardiovaskularni sistem, kao i personalizovani pristup proceni funkcije kardiovaskularnog sistema i stratifikacija rizika od pojave potencijalnih kardiovaskularnih neželjenih efekata pre započinjanja terapije.

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***Ključne reči**: enzalutamid, srčana insufucijencija, karcinom prostate*

Case report

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EXPLORING BILIARY ILEUS: A RARE AND COMPLEX CLINICAL CASE

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Biliary ileus is a rare complication associated with cholelithiasis. Although it accounts for less than 4% of small bowel obstructions in patients under 65 years of age, its prevalence rises to 25% in patients over 65 years of age. Physicians are faced with the decision of whether to opt for immediate closure of a single- or two-stage cholecystoenteric fistula or to wait for natural closure.

A 62-year-old female patient presented to the surgical clinic with severe abdominal pain, predominantly on the right side, and a sensation of bloating. She reported symptoms of constipation, nausea, and vomiting. An emergency exploratory laparotomy was scheduled. During the surgical procedure, careful exploration revealed significant dilation of the proximal part of the intestine, and a gallstone was found in the jejunum. The gallstone was successfully removed, followed by closure of the enterotomy site.

This report highlights a condition that is rarely seen in practice. In older patients presenting with clinical manifestations of bowel obstruction and chronic issues with the gallbladder, this rare disease should be considered, particularly in women and elderly patients.

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Key words: *biliary ileus, Bouveret's syndrome, enterotomy, gallstone, vomiting*

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Introduction

Biliary ileus is an uncommon complication associated with cholelithiasis, characterized by mechanical intestinal obstruction caused by one or more gallstones within the gastrointestinal tract, often via a cholecystoenteric fistula (1–3). While gallstones can potentially obstruct any part of the gastrointestinal tract, the distal ileum is the most frequently affected site (1). Biliary ileus accounts for approximately 0.3–0.5% of complications arising from gallbladder diseases, translating to approximately 30–35 cases per million hospitalizations (1, 4, 5). The incidence of this condition as a cause of small bowel obstruction is less than 4% in patients under 65 years of age, but increases substantially to 25% in patients aged 65 and older (1). Bouveret's syndrome, a subtype of biliary ileus characterized by an impacted stone in the duodenum obstructing the gastric outlet, is observed in only 3% of cases (1).

Clinical manifestations of biliary ileus vary depending on the site of obstruction. Intestinal obstruction typically presents with symptoms such as abdominal pain, bloating, vomiting, reduced peristalsis, and constipation (1, 6). Additionally, patients may exhibit jaundice (1).

Diagnosis relies on a combination of basic laboratory and biochemical analyses, as well as additional diagnostic techniques. Abdominal X-rays in both supine and upright positions may reveal pneumobilia and distension of the intestinal loops (1).

In some cases, patients are presented with treatment options aimed at preemptively averting the emergence of symptoms and complications. Conversely, others are advised to adopt a watch-and-wait strategy, initiating active treatment only once the stones provoke symptomatic episodes (7). Selecting the most suitable surgical approach can pose challenges, especially when the patient's overall health is compromised. Physicians must navigate the decision between immediate one-stage or two-stage closure of the cholecystointestinal fistula or opting for a wait-

and-observe strategy, allowing for natural closure to occur (2).

Case Presentation

In our case, an emergency exploratory laparotomy was scheduled for a 62-year-old female patient who presented at the surgical clinic with severe abdominal pain, primarily localized on the right side, and a sensation of abdominal distension. The patient stated symptoms of constipation, nausea, and vomiting. Additionally, she reported being aware of gallbladder calculosis for the past seven years and occasionally experienced pain in the right side of the abdomen, which decreased with the use of analgesics.

Upon clinical examination, the abdomen exhibited distension in line with the chest, with tenderness and pain noted upon palpation in the right paraumbilical region. Laboratory blood tests revealed mild leukocytosis (13 x 109/L) and slightly elevated C-reactive protein levels (18 mg/L), while other blood parameters remained within the normal reference range. This intervention was considered essential for diagnostic and therapeutic purposes, given the findings during clinical examination and the presence of a positive history of chronic gallbladder calculosis with frequent exacerbations.

During the surgical procedure, after thorough exploration, significant dilation of the proximal intestine was observed. A biliodigestive fistula (Figure 1) and the presence of a gallstone in the jejunum (Figure 2) were identified. The gallstone was carefully manipulated distally using digital maneuvers (Figure 3), necessitating the creation of an enterotomy (Figure 3) to facilitate the extraction of a large biliary stone from the intestine. The gallstone, measuring approximately 3 cm in diameter, was successfully removed (Figure 4), and an enterotomy suture was meticulously performed. The patient was subsequently discharged from the hospital in full recovery, and her follow-up visits at the surgical clinic proceeded without any complications.

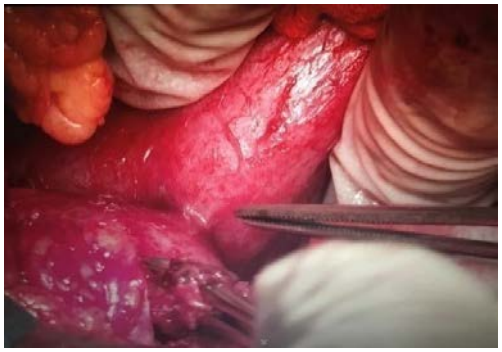
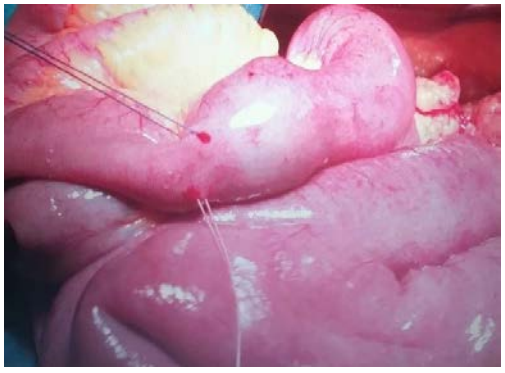


Figure 1. Identified part of the fistula

Figure 2. A gallstone was identified in the intestine



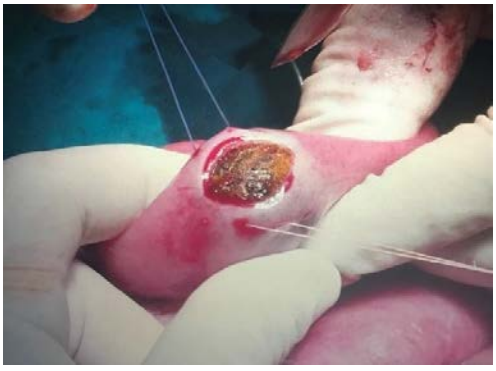
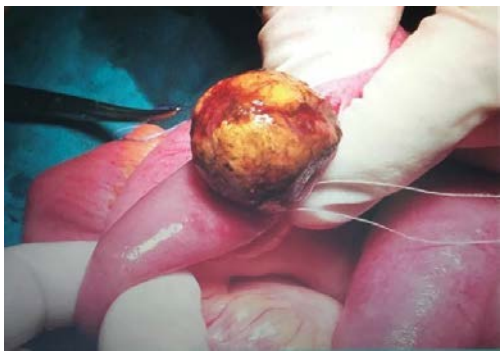


Figure 3. Support sutures are placed at the enterotomy site and the biliary stone in the lumen of the intestine



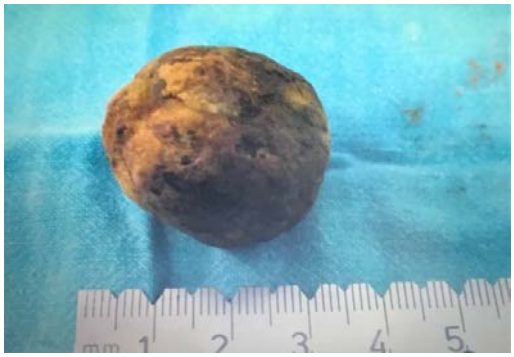


Figure 4. Gallstone after removal followed by measuring the size

Discussion

Cholecystoduodenal fistula typically arises from gallbladder inflammation (8). Surgical intervention remains the established standard for treating biliary ileus, with the primary objective being the resolution of intestinal obstruction through enterolithotomy (1, 4, 9). The key determinant in selecting the appropriate surgical approach is the duration of intestinal obstruction (1). Following an enterolithotomy procedure, the recurrence rate is estimated to be around 5% (1, 2). Potential sites of obstruction encompass the ileum (60%), jejunum (15%), stomach (15%), and colon (5%) (3). This condition predominantly affects women, with a prevalence ranging from 72% to 90%, typically occurring between the ages of 60 and 84 (10).

The case under discussion represents a rare cause of intestinal obstruction, mirroring similar clinical manifestations as reported by Mulita et al. (11), who presented a female patient with comparable symptoms in their clinical report. Additionally, the case described by Souiki et al. (12) involved an elderly woman, paralleling the circumstances in our case. In a review of the literature, right-sided abdominal pain and constipation emerge as characteristic clinical indicators, aligning with the clinical findings observed in the presented patient (13, 14). As a rare complication stemming from cholecystolithiasis, the clinical cases of biliary ileus discussed in this context reveal consistent symptoms of nausea and vomiting, which complement the clinical presentation of the patient in our case (15, 16).

When a patient is diagnosed with ileus caused by a gallstone, it becomes crucial to conduct a comprehensive examination of the entire biliary tree to pinpoint the gallstone's exit point (17).

The clinical case presented by Dai et al. (18) involving a patient with biliary ileus required an exploratory laparotomy, revealing the presence of a gallbladder fistula with the duodenum and proximal intestinal dilatation (19), mirroring the intraoperative findings in our case. In the cohort study on biliary ileus by Koliakos et al. (3), the operative technique employed across all patients involved enterotomy and the removal of biliary calculi, aligning with the operative approach utilized in our case.

Enterolithotomy entails the extraction of stones through enterotomy without performing any additional procedures on the gallbladder or enterobiliary fistula (20). The primary objective is to alleviate intestinal obstruction without subjecting the patient to procedures that could prolong the operation duration or increase morbidity (20).

Dunphy et al. (21) documented a case in which a 5 x 2.5 cm gallstone was removed via enterotomy, partially corresponding to the size of the stone extracted in our case.

Smaller gallstones can spontaneously traverse the normal gastrointestinal tract and pass through the stool without obstruction (21). This condition accounts for 1–4% of all hospital admissions related to small bowel obstruction, with 25% of cases occurring in individuals over the age of 65 (21).

In a systematic literature review conducted by Farkas et al. (14), it was concluded that this disease is more prevalent in women, a finding that aligns with the presented patient's demographic.

A limitation of this clinical case is the absence of a radiological scan report. The lack of diagnostic information stems from the hospital's current inability to perform abdominal scans due to technical reasons, and the urgency of the situation did not allow for postponing the surgical intervention.

Considering procedures in one or two stages provides additional perspective on our study regarding the surgical treatment of biliary ileus. A one-stage procedure may be more efficient in terms of reducing time spent in the operating room and patient recovery, while a two-stage procedure may offer greater precision and safety in more complex cases. Patient-specific characteristics and surgeon experience should be taken into account when deciding on the optimal treatment approach. Analyzing the advantages and disadvantages of each procedure is important

for achieving the best possible outcome for the patient.

In this case, the advantages of a one-stage procedure may include efficiency in terms of reducing overall time spent in the operating room and facilitating faster patient recovery. Additionally, the simpler organization of the operation may reduce the risk of complications related to the postoperative period. On the other hand, the benefits of a two-stage procedure may encompass greater precision in stone removal and a reduced risk of intraoperative complications, especially in cases with complex anatomical changes or the presence of adhesions.

As for the drawbacks, a one-stage procedure may be challenging in cases with large stones or serious patient health complications, which could increase the risk of intraoperative issues. Conversely, a two-stage procedure may prolong the overall treatment and recovery time for the patient, requiring additional surgeries and visits to the surgical team, which could pose additional burdens on the patient and the healthcare system.

Insights from this rare case underscore the importance of swift diagnosis and precise treatment of biliary ileus, with a particular focus on elderly patients with chronic gallbladder issues. This case report provides valuable guidance for better understanding and effectively managing biliary ileus, emphasizing that a multidisciplinary team approach can be key to successful treatment. While this is a rare condition, this case serves as a reminder of the need for ongoing education of healthcare professionals and patients about the symptoms and risks of biliary ileus.

Conclusion

The choice of the surgical approach for treating biliary ileus should be determined based on the specific site of intestinal tract obstruction. This case underscores the rarity of the condition in clinical practice. In elderly patients exhibiting clinical signs of bowel obstruction alongside chronic gallbladder issues, clinicians should consider the possibility of this rare disease, particularly among female and older patients. In conclusion, the successful intervention in this case offers hope and inspiration for patients facing this rare complication, simultaneously highlighting the importance of early diagnosis and proper treatment.

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Prikaz slučaja

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ISTRAŽIVANJE BILIJARNOG ILEUSA: REDAK I KOMPLEKSAN KLINIČKI SLUČAJ

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Bilijarni ileus je retka komplikacija povezana sa holelitijazom. Premda čini manje od 4% opstrukcija tankog creva kod bolesnika koji imaju manje od 65 godina, prevalencija bilijarnog ileusa raste na 25% kod bolesnika koji imaju više od 65 godina. Lekari treba da odluče da li će odmah zatvoriti jednostepenu ili dvostepenu holecistointestinalnu fistulu ili će sačekati prirodno zatvaranje.

Šezdesetdvogodišnja bolesnica je došla na Kliniku za hirurgiju sa jakim bolovima u stomaku, uglavnom sa desne strane, i sa osećajem nadutosti. Kao simptome je navela zatvor, mučninu i povraćanje. Zakazana je hitna eksplorativna laparotomija. U toku hirurškog zahvata je pažljivim istraživanjem uočeno značajno proširenje proksimalnog dela creva i pronađen je kamen u žuči koji je dospeo u jejunum. Kamen je uspešno uklonjen, a potom je zatvoreno mesto enterotomije.

U ovom radu je prikazano stanje koje se retko sreće u praksi. Kod starijih bolesnika kod kojih postoje kliničke manifestacije opstrukcije creva i hronični problemi sa žučnom kesom treba razmotriti ovu retku bolest; to posebno važi za žene i starije bolesnike.

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***Ključne reči**: bilijarni ileus, sindrom Bouveretou enterotomija, kamen u žči povraćanje*

Original article

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THE EFFECT OF AGEING ON MACROMORPHOMETRIC PARAMETERS AND HISTOLOGICAL CHARACTERISTICS OF BASOPHILIC AND ACIDOPHILIC PITUITARY CELLS: ANALYSIS OF MALE CADAVERS

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The aim of this study was to examine the macromorphometric parameters including weight, height, width, volume and length of the pituitary gland, as well as the histological characteristics of the hormone-producing cells. The focus was on basophilic (gonadotropic – luteinizing hormone (LH) producing cells and adrenocorticotropic hormone (ACTH) producing cells) and acidophilic (somatotropic – growth hormone (GH) cells and mammotropic – (prolactin PRL) cells) cells in male cadavers, aiming to assess the characteristics of the pituitary gland in living individuals during ageing. The research included 15 male cadavers of different ages (44 and 89 years), which were divided into three groups. In the first group (I) there were cadavers aged 30 to 49, in the second (II) 50 to 69 years, and in the third (III) 70 years and older. The pituitary cells were immunohistochemically identified by the PAP method using the appropriate antibodies: LH (βLH 1:100), ACTH (hACTH 1:200), GH (hGH 1:200), and PRL (hPRL 1:300). Our results show that the width, height, weight and volume of the pituitary gland did not change significantly ($p > 0.05$) with ageing, while the length of the gland showed statistically significant changes between groups ($p < 0.05$). The length of the pituitary gland was significantly ($p < 0.05$) greater in age groups II and III, compared to group I. In conclusion, the results of the examined macromorphometric parameters showed that only the length of the pituitary gland changed significantly during ageing.

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Key words: *ageing, men, macromorphometric parameters, immunoreactive pituitary cells*

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Introduction

Ageing is a physiological process resulting from the accumulation of a wide range of molecular and cellular damage over time, which leads to a gradual decline in mental capacity, physical abilities and an increasing risk of disease (1, 2). Accumulated damage can manifest itself in health problems characteristic of advanced age, such as deterioration of urinary tract function, prostate hyperplasia, erectile dysfunction and reduced fertility, osteoporosis and general weakness (3). The pituitary gland in humans is located at the base of the brain, in a depression in the sphenoid bone called the sella turcica. It consists of hormone-producing cells that secrete hormones: gonadotropic (luteinizing hormone – LH), thyrotropic (thyroide-stimulating hormone – TSH), adrenocorticotropic (adrenocorticotropic hormone – ACTH), somatotropic (growth hormone – GH), mammotropic (prolactin – PRL) cells, as well as folliculostellate (FS) cells, which are non-endocrine cells (4). With ageing, dysregulation of the hypothalamic-pituitary-gonadal axis occurs (5), which is reflected in a decrease in the secretion of gonadotropic-releasing hormone (GnRH) from the hypothalamus, the maximum and average amplitude of LH, and a decrease in the negative feedback mechanism mediated by testosterone. This process is referred to as gonadopause or late hypogonadism (6, 7). Ageing causes functional changes in the hypothalamic-pituitary-cortical axis (8). Previous studies indicate that during ageing, the synthesis and secretion of corticotropin-releasing hormone (CRH) from the hypothalamus decreases, and the sensitivity of ACTH from the adenohypophysis and adrenal cortex increases (9), which results in the inability

to quickly terminate glucocorticoid secretion stimulated by acute stressors (10). With the ageing process, a deficiency in the secretion of growth hormone-releasing hormone (GHRH) and/or ghrelin is observed, as well as an increase in the secretion of somatostatin from the hypothalamus, both of which lead to a reduction in growth hormone secretion (11). This process is called somatopause (12) and is associated with numerous problems such as mental, metabolic and musculoskeletal (13). In people aged 70 and over, GH levels drop significantly and are approximately 1/3 of those in later puberty (14). The World Health Organization has established that people live longer and that by 2030 every sixth person in the world will be 60 years old or older, and that by 2050 the number of people over 60 will double, and the number of people over 80 will triple from 2020 to 2050 (15). Because all of these can disrupt healthy ageing, the United Nations (UN) General Assembly has declared the period 2021–2030 as the Decade of Healthy Ageing. Based on the above, this work aims to examine the macromorphometric parameters of the pituitary gland and the histological characteristics of the hormone-producing immunopositive LH, ACTH, GH, and PRL cells within the pituitary gland.

Materials and Methods

The material for this stydy was taken from 15 male cadavers, in a routine autopsy at the Centre for Forensic Medicine in Niš, Serbia, with the approval of the Ethics Committee of the University of Niš, Faculty of Medicine (Decision No. 12-2307-2/8 dated 10.03.2016 described in detail in our previous work). The cadavers used were free of previously diagnosed neurological, psychiatric or endocrine disorders during their lifetime. No visible damage to the brain or pituitary gland was observed during the autopsies. Additionally, the pathohistological evaluation of the brain and pituitary gland ruled out the presence of any hidden or misdiagnosed diseases. Cadavers were classified into three age groups: Group I - from 30 to 49 years, Group II - from 50 to 69 years, and Group III - 70 years and over.

Macromorphometric Parameters

The weight of the pituitary gland, expressed in grams, was determined using a Denver Instrument Company AA-200 DS analytical balance, the precision of which is measured to 4 decimal places. The height of the pituitary gland was the mean value of the height of the central and both lateral parts of the pituitary gland. The three listed parameters are expressed in mm and measured with a "Kennon" vernier calliper, with a precision of 1/20 (0.05 mm). The width of the pituitary gland was the largest distance between the points on the lateral parts of the pituitary gland. The volume of the pituitary gland, shown in mm^3, was determined by measuring the volume of the displaced liquid in a glass beaker with a total volume of 10 ml (16). The length or sagittal diameter represented the mean value of the same at the level of the central and two lateral parts (wings). The same person measured all five parameters.

Histological Procedure

The histological processing of material taken from cadavers was described in detail in earlier reports (17–21). For immunohistological visualisation of hormone-producing cells, primary antibodies for gonadotropic LH cells (βLH 1:100; NIH, Bethesda, Md., USA) (17, 18), ACTH (hACTH 1:200; DAKO A/S, Glostrup, Denmark) (8), GH (hGH 1:200; DAKO A/S, Glostrup, Denmark) (19, 20, 22), and for PRL (hPRL 1:300; DAKO A/S, Glostrup, Denmark) (23) are used.

Statistical Analysis

The statistical analysis of the data was performed using SPSS v. 15.0. Given that these are small samples and that the continuous variables deviate from the normal distribution (as determined by the Shapiro–Wilk test), they are presented as medians, along with the minimum and maximum values. The dependence of these variables in relation to age (belonging to an age group) was determined by the Kruskal–Wallis test, and the Mann–Whitney test determined the difference in values between individual groups. As a threshold of statistical significance in the conclusion, the level of the error of estimation lower than 5% ($p < 0.05$) was used.

Results

Macromorphometric Parameters

The values of the macromorphometric parameters of the pituitary gland of male cadavers are shown in Figure 1. Our results show that the weight, height, width and volume of the pituitary gland did not change significantly ($p > 0.05$) during ageing (Figure 1A-1D), while the length of the gland showed statistically significant changes between groups ($p < 0.05$) (Figure 1E). The length of the pituitary gland was significantly ($p < 0.05$) greater in age groups II and III by 15.4% and 14.6%, respectively, compared to age group I.

Histological Characteristics

In group I, gonadotropic LH cells were polygonal or oval in shape, either in groups or as single cells, with an eccentrically positioned nucleus (Figure 2A). In cadavers of the third group, LH cells were darker in colour, more often oval, single with an eccentric, smaller, hyperchromatic nucleus and larger in volume

compared to younger cases (Figure 2B). In younger cadavers, ACTH cells were numerous, oval, polygonal or stellate with extensive cytoplasm (Figure 2C). In cases belonging to age group III, these cells did not change shape but were observed to be distributed in larger irregular groups or in smaller oval regular groups (Figure 2D). GH cells in younger cadavers were predominantly polygonal, with an eccentric euchromatic nucleus (Figure 2E). In age group III, GH cells were less numerous, larger, and exhibited somewhat stronger immunoreactivity with sporadic clear cytoplasmic vacuoles observed (Figure 2F). PRL cells of younger cadavers were spherical or irregularly polygonal in shape, with acidophilic granules in the cytoplasm. They were either single or present in small groups (Figure 2G). In older cadavers, PRL cells showed no difference in shape and distribution compared to young cadavers, but darker colored granules were visible (Figure 2H).

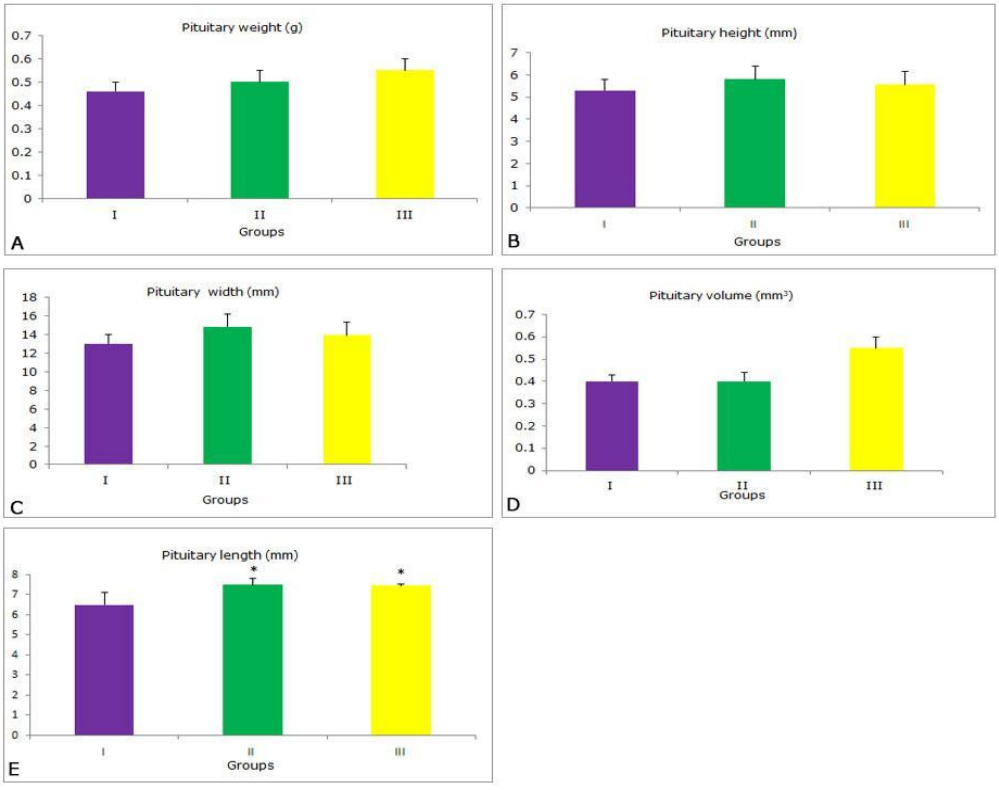

Figure 1 A–E. Macromorphometric parameters of pituitary gland in men cadavers in I (30 to 49 years), II (50 to 69 years), and III (70 years and older) groups. All values are provided as the mean ± SD; *p < 0.05, II and III vs. I group.

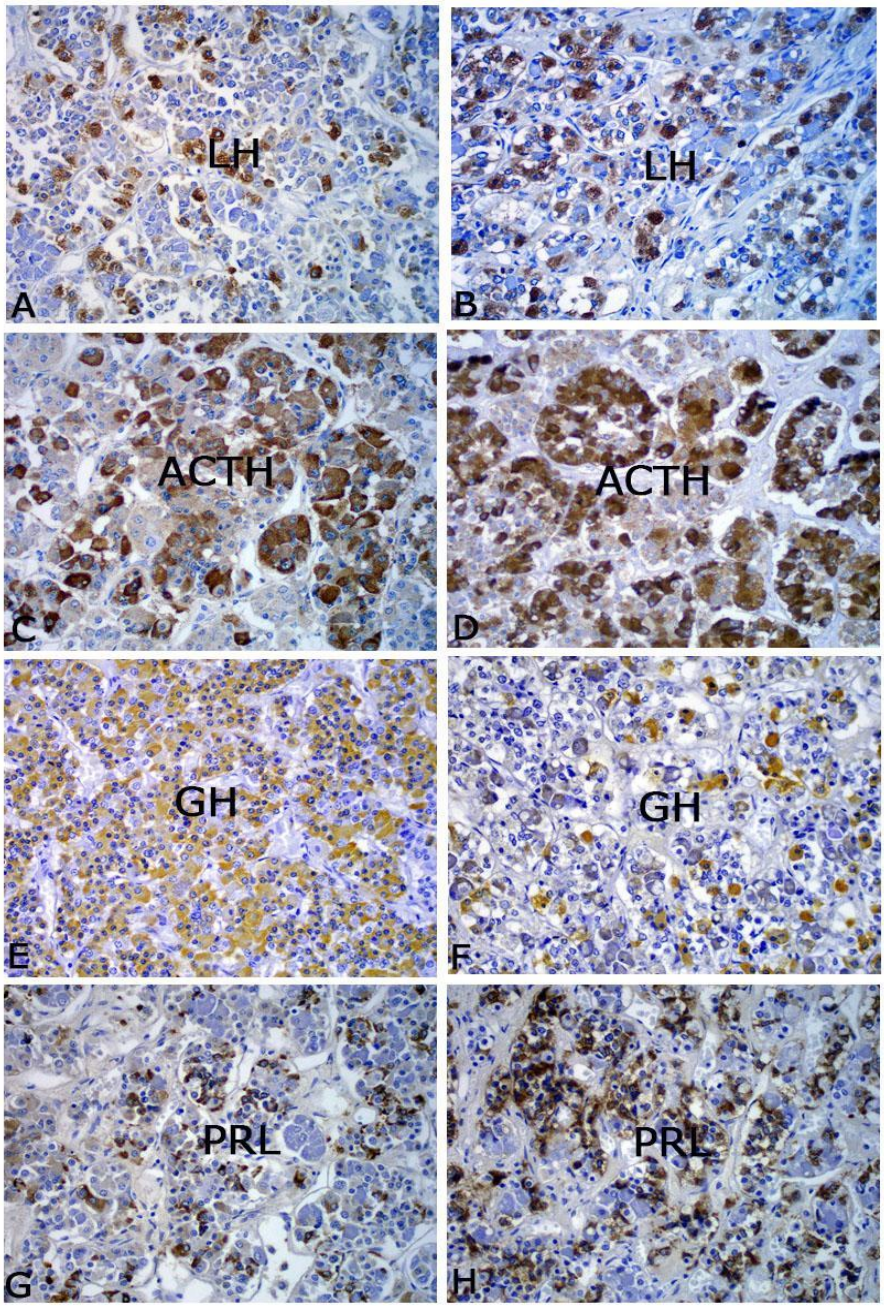

Figure 2 A–H. Representative micrographs of immunopositive pituitary cells in man cadavers. LH cells in I group (A), large immunopositive LH cells with small eccentric hyperchromatic nuclei in III group (B), ACTH I group (C), in III group (D); GH cells in: a-41-year-old man (E), an 87-year-old man (F), PRL cells in: a-41-year-old man (G) and an 87-year-old man (H). I (30 to 49 years), II (50 to 69 years) and III (70 years and older) groups. PAP,objective magnification 40x

Discussion

It has been observed that the size and shape of the pituitary gland change throughout life, which can be highly important for the diagnosis and possible treatment of diseases affecting this gland (8).

In our study, no statistically significant change in the weight of the pituitary gland was observed with age. Pituitary gland weight of cadavers of both sexes in Asia (24) was equal to (0.5 ± 0.1 g), similar to values reported in Japan (25), Chicago US (26), and India (27), which is similar to our results on male cadavers. Our results show that the peak height of the pituitary gland was recorded in the II age group, although the differences between individual age groups are not statistically significant. Similar values of hypophysis height to ours were recorded by Denk et al. (28) and Ikram et al. (29). Earlier research by Singh et al. (30) indicated that the height of the pituitary gland differed significantly between the sexes, while Ibinaiye et al. (31) found no statistically significant differences. The width of the pituitary gland was the largest in the II age group; however, there were no statistically significant differences between the groups, as previously shown in the population of north-western Indians (32). In our research, no significant difference was found in the volume of the pituitary gland between age groups. The results of the study by Ibinaiye et al. (31) are consistent with our results. Determining the volume of the pituitary gland is crucial in various pathological conditions of this gland, as traumatic brain injuries have been noted to involve the pituitary gland in both the early and chronic phases (33). The length of the pituitary gland was significantly increased in male cadavers in group II (50−69 years) by 15.4% and by 14.6% in group III (70 years and older) compared to group I. An examination of the length and peak that the pituitary gland reaches in both sexes in India was recorded in men in the fifth decade and in women after 50 years (30) which corresponds with our results. Literature data show that on magnetic resonance, the length of the pituitary gland differed significantly between the sexes in India (30), while this difference was not found in Nigeria (31).

Histological changes occurring on basophilic gonadotrope LH cells in old male cadavers compared to young cadavers agree with those described in earlier works (17, 18, 20, 21). Histological characteristics of basophilic ACTH cells in young male cadavers: cells were numerous, oval, polygonal or star-shaped with extensive cytoplasm, without visible changes in older cases, which is in agreement with earlier works (8, 10). Acidophilic GH cells in younger cadavers were numerous, polygonal, with acidophilic granules and an eccentric euchromatic nucleus. In older cases, they were fewer, larger, and exhibited somewhat stronger immunoreactivity, with sporadic clear cytoplasmic vacuoles observed. Similar histological characteristics of younger and older cadavers were recorded in earlier works (17, 19–21). Immunopositive acidophilic PRL cells of younger and older corpses are spherical or irregularly polygonal in shape, with acidophilic granules in the cytoplasm. They were either single or present in small groups. Similar properties of PRL cells were described in earlier works (34).

To live long and minimize health problems in old age, one should, as much as possible, adopt the way of life of centenarians in the "blue zones" (Okinawa, Japan; Sardinia, Italy; Ikaria, Greece; Nicoya, Costa Rica; and Loma Linda, California, USA) (35). A proper and healthy diet based on the highest possible intake of polyphenols (fruits, vegetables, legumes, two glasses of red wine), socializing, physical activity, spiritual fulfilment and a stress-free life are the basis of recommendations for healthy ageing and living over 100 years (36).

Conclusion

Given that we determined in this study that there were changes in some of the examined macromorphometric parameters and histological characteristics of the examined pituitary cells, further and more extensive research is necessary to confirm with certainty whether these parameters undergo irreversible processes during ageing.

Study limitations

In this study, we focused on assessing macromorphometric parameters and their association with histological changes in hormone-producing (LH, ACTH, GH and PRL) cells of the pituitary gland during the ageing process. The main limitation of this research was the small sample size - a total of 15 cases, divided into three age groups. Future studies should include a larger number of cases and use immunofluorescence staining of cells for histological analysis, followed by examination under a confocal microscope.

Acknowledgements

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UTICAJ STARENJA NA MORFOMETRIJSKE PARAMETRE I HISTOLOŠKE KARAKTERISTIKE BAZOFILNIH I ACIDOFILNIH ĆELIJA HIPOFIZE: ISPITIVANJE MUŠKIH KADAVERA

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Cilj ove studije bio je da ispita makromorfometrijske parametre, uključujući težinu, visinu, širinu, zapreminu i dužinu hipofize, i histološke karakteristike ćelija koje proizvode hormone. Ispitivanje je bilo usmereno na bazofilne (gonadotropne ćelije koje proizvode luteinizirajući hormon (engl. *luteinizing hormone* – LH) i adrenokortikotropne ćelije koje proizvode adrenokortikotropni hormon (engl. *adrenocorticotropic hormone* – ACTH)) i acidofilne (somatotropne ćelije koje proizvode hormon rasta (engl. *growth hormone* – GH) i mamotropne ćelije koje proizvode hormon prolaktin (engl. *prolactine* – PRL)) ćelije muških kadavera i njihovu povezanost sa starenjem. Istraživanje je obuhvatilo petnaest muških kadavera različite starosti (od 44 godine do 89 godina), koji su podeljeni u tri grupe. U prvoj grupi (I) bili su kadaveri muškaraca starih od 30 do 49 godina, u drugoj (II) kadaveri muškaraca koji su imali između 50 i 69 godina, a u trećoj (III) kadaveri muškaraca starijih od 70 godina. Ćelije hipofize su imunohistohemijski identifikovane PAP metodom i korišćenjem odgovarajućih antitela: LH (βLH 1 : 100), ACTH (hACTH 1 : 200), GH (hGH 1 : 200) i PRL (hPRL 1 : 300). Rezultati su pokazali da se širina, visina, težina i zapremina hipofize nisu značajno menjale ($p > 0,05$) u toku starenja. S druge strane, uočena je statistički značajna promena dužina žlezde pri poređenju kadavera iz pomenutih grupa ($p < 0,05$). Dužina hipofize je bila statistički značajno ($p < 0,05$) veća u grupama II i III nego u grupi I. Rezultati dobijeni ispitivanjem makromorfometrijskih parametara pokazali su da se samo dužina hipofize značajno menja u toku starenja.

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***Ključne reči**: starenje, muškarci, makromorfometrijski parametri, imunoreaktivne ćelije hipofize*

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AN IMPACT OF RADIOTHERAPY ON PSYCHO-EMOTIONAL CHARACTERISTICS OF CANCER PATIENTS

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The psychological distress that cancer patients endure can greatly affect their treatment experience and quality of life, making it crucial to understand the psycho-emotional effects of radiotherapy, an established cornerstone in cancer treatment, as this knowledge is vital for creating comprehensive care strategies that address both physical and mental health needs. The study investigated the psycho-emotional effects of radiotherapy in cancer patients with a focus on the interplay of radiotherapy with hormone therapy, biological therapy, and chemotherapy. Our results indicate that while psychological distress is prevalent among patients undergoing radiotherapy, changes in emotional competence, stress reactions, depression, and mature religiosity are similar to those experienced by healthy individuals. Notably, patients receiving radiotherapy exhibited significantly lower scores on the Interpersonal Reactivity Index (IRI), altruism, and the Beck Anxiety Inventory (BAI) compared to healthy controls, suggesting that, while anxiety and empathy fatigue did not increase, reduced altruism could reflect a preoccupation with personal health challenges.

A higher externality score among radiotherapy patients indicates a search for external justifications for their illness. The introduction of hormone therapy significantly increased religiosity scores, enhancing emotional acceptance of illness, while biological therapy resulted in diminished religiosity, likely due to its unfamiliarity and associated skepticism. Importantly, chemotherapy did not significantly alter radiotherapy-induced psycho-emotional effects, reinforcing the notion that familiarity with treatment modalities can foster emotional resilience and a sense of control in patients. In conclusion, this study underscores the importance of addressing psycho-emotional well-being in comprehensive cancer care of radiotherapy-treated patients.

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Key words: *radiotherapy, psycho-emotional characteristics, altruism, emotional competence, stress*

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Introduction

The psychological distress experienced by cancer patients can significantly influence their overall treatment experience and quality of life (1). Patients may grapple with a multitude of emotional challenges, including anxiety, depression, and fear of recurrence, which can be exacerbated by the uncertainties associated with their diagnosis and treatment journey (1).

Radiotherapy is a cornerstone in the treatment of various malignancies (2). Its role in cancer management is well-established, yet the psycho-emotional implications for patients undergoing this treatment are often less thoroughly examined (3). Understanding the psycho-emotional effects of radiotherapy is essential for developing comprehensive care strategies that address both physical and mental health needs (3, 4). When radiotherapy is administered as a standalone treatment, patients often exhibit psychological responses that reflect their coping mechanisms in the face of cancer (4, 5). The stress of receiving a cancer diagnosis, coupled with the physical side effects of treatment, such as fatigue, skin irritation, and changes in body image, can lead to significant emotional burden (4). Moreover, the experience of undergoing radiotherapy can create

feelings of vulnerability and loss of control, which may fuel anxiety and depressive symptoms (4). Thus, it is crucial to explore how patients psychologically adapt to the rigors of radiotherapy and which supportive measures can be implemented to enhance their emotional well-being (5).

The combination of radiotherapy with other treatment modalities, such as hormone therapy and biological therapy, introduces additional layers of complexity to the psycho-emotional landscape (3, 6). Hormone therapy, often prescribed for hormone-sensitive cancers, can induce significant psychological changes (6). While some patients may feel a sense of empowerment from actively participating in their treatment, the side effects associated with hormone therapy, such as mood fluctuations and cognitive changes, can also contribute to increased psychological distress (6). This duality necessitates a deeper exploration of how the combined effects of radiotherapy and hormone therapy shape patients' emotional experiences, especially considering the potential for enhanced religious or spiritual engagement as a coping mechanism (6).

Similarly, the integration of biological therapy with radiotherapy presents a unique challenge (7, 8). Biological or immunotherapies, which often employ novel mechanisms to target cancer, are not as familiar to patients as traditional treatments like chemotherapy or radiation (7). This unfamiliarity can lead to skepticism, anxiety, and a diminished sense of control over their treatment process (8). As patients navigate the complexities of receiving combined biological and radiotherapy, their psychological responses may vary widely, reflecting a spectrum of coping strategies influenced by their understanding of these therapies and their potential outcomes (7, 8).

Furthermore, patients receiving combined chemotherapy and radiotherapy face a distinct set of psycho-emotional challenges (9, 10). Chemotherapy is known for its systemic side effects, which can significantly impact a patient's quality of life (9). The physical toll of chemotherapy, coupled with the localized treatment of radiotherapy, can exacerbate feelings of fatigue, anxiety, and helplessness (9, 10). Understanding the interplay between the physical side effects of chemotherapy and the psychological impacts of radiotherapy is crucial for providing holistic care and support (9).

An investigation of the psycho-emotional effects of radiotherapy, either as a standalone treatment or in combination with hormone therapy, biological therapy, or chemotherapy, offers valuable insights into the emotional challenges faced by cancer patients (3, 4). By examining these dynamics, researchers can better inform clinical practices and interventions aimed at mitigating psychological distress, ultimately enhancing the quality of life for individuals navigating the complexities of cancer treatment (1, 3). Accordingly, this study tended to illuminate the intricate relationship between treatment modalities and their combined effects on patients' emotional well-being, paving the way for more integrated approaches to cancer care.

Materials and Methods

Participants

Participants in this study were patients suffering from malignant disease who underwent radiotherapy alone or in combination with biological therapy or hormonal therapy at Clinical Center Kragujevac, Faculty of Medical Sciences, University of Kragujevac, Serbia (n = 156). All patients had a complete medical history, including physical examination, laboratory tests and diagnostic imaging (chest X-ray, abdominal ultrasound, abdominal computed tomography scan and endoscopy).

Patients were divided into several groups, depending on the therapy that they received (radiotherapy alone, combined radiotherapy and hormone therapy, combined radiotherapy and biological therapy and combined radiotherapy and chemotherapy). The groups were homogenized in number, gender, age, socio-economic status and cultural background. Participants did not differ in occupation, physical assets, social position or area of residence. The control group consisted of 50 healthy individuals. A control group was matched with the experimental groups based on gender, age, socio-economic status and cultural background.

All participants gave their informed consent to participate in this study. An adherence was made to the Principle of Good Clinical Practice and the Declaration of Helsinki at all times. The study was approved by the Ethical Committee of the Faculty of Medical Sciences, University of Kragujevac, Serbia and the Ethical Committee of Clinical Center Kragujevac, Serbia.

Study Design

The psychological tests, previously standardized for the Serbian population, were given to study participants. They volunteered for the study after the tester briefly explained its purpose and assured them that anonymity would be maintained. Trained assistants collected the data under the supervision of a PhD staff member, an associate professor at the Department of Psychology, Faculty of Medical Sciences, and the University of Kragujevac.

Participants responded to standardized psychological tests: Interpersonal Reactivity Index (IRI), Altruism Scale, Externality Scale, Emotional Competence Questionnaire, Coping Inventory for Stressful Situations, and the Religious Maturity Scale (11).

Interpersonal Reactivity Index (IRI)

Interpersonal Reactivity Index (IRI) was used to assess empathy of participants (11, 12). IRI is a multidimensional questionnaire that measures both cognitive and affective aspects of empathy. It is a measure of dispositional empathy which assumes that empathy consists of a set of separate but reciprocally related constructs. The IRI questionnaire contains 28 items answered on a 5-point Likert scale ranging from “Does not describe me well” to “Describes me very well”. The measure has 4 subscales, each made up of 7 different items. These subscales are: Perspective Taking—the tendency to spontaneously adopt the psychological point of view of others; Fantasy—taps respondents' tendencies to transpose themselves imaginatively into the feelings and actions of fictitious characters in books, movies, and plays; Empathic Concern—assesses "other-oriented" feelings of sympathy and concern for unfortunate others; Personal Distress—measures "self-oriented" feelings of personal anxiety and unease in tense interpersonal settings. A total score of empathic responsiveness of the participant, named "Interpersonal Reactivity Index—total (IRIT)" is obtained as the sum of the points scored on these subscales.

Altruism Scale

The Altruism Scale (11, 13) was used for the assessment of altruism. This scale measures pro-social behavior and the tendency to behave in an altruistic way in everyday situations. The Altruism Scale consists of 17 items that describe different behaviors towards friends or strangers, with disregard of the subject’s personal interests and with specific personal sacrifice (example: “I have shown school assignments to a friend who was sick" or “I offered my seat on the bus to an older person"). On a scale of zero to four (0—never; 4—very often), the participant describes how often they behave in the stated manner. Results can range from 0–68 points, and a higher score indicates a higher degree of altruism.

Externality Scale

The Externality Scale (11, 14) was used for determining the externality. This scale measures one dimension of Rotters’ concept of the locus of control. Locus of control can be external or internal. External locus of control reflects a fatalistic orientation of the person who believes that exclusively fate, fortune and predestination have the power to determine the outcome of events. Internal locus of control reflects internal orientation and the belief that individual has the power and control over life events. The Externality Scale consists of 10 items, answered on a 5-point Likert scale ranging from 1 = strongly disagree to 5 = strongly agree. Results vary from 10 to 50, and higher results reflect external orientation or external locus of control, while the lower results indicate internal orientation or internal locus of control.

Emotional Competence Questionnaire

Emotional Competence Questionnaire (11, 15) was used for the assessment of emotional intelligence (competence). Participants evaluate how some claims relate to them on a scale from 1—not at all to 5—totally (example: ''I can express my emotions well''). This Scale is one-dimensional; a higher score indicates greater emotional competence.

Coping Inventory for Stressful Situations (CISS)

The Coping Inventory for Stressful Situations (CISS), which analyzes styles of coping as stable personality characteristics, was used to assess the behavior of participants in stressful situations (11, 16). The questionnaire contains 48 items, three subscales with 16 statements and is used for measuring three major types of coping styles: Task-Oriented, Emotion-Oriented and Avoidant Coping. It also identifies two types of avoidance patterns: Distraction and Social Diversion. It helps in identifying an individual’s preferred coping style and contributes to the overall understanding of the relationship between that coping style and their personality. The task of the participant is to assess on a scale from one to five (1—not at all to 5—completely) to what extent they practice a certain type of activity, and how they act in a difficult, stressful or upsetting situation. Coping styles play an important role in physical and psychological well-being.

Religious Maturity Scale

The Religious Maturity Scale (11) was used to assess religiosity of participants. This scale consists of eight items with two statements that refer to the same aspect of religiosity, but express different maturity and intellection. In particular, one statement reflects the religiosity of the second or third stage of Fowler's model (for example: “I think only my faith offers an insightful look into what God wants from us"), while content of the other statement is in accordance with the Fowler's fourth or fifth stage (“Even though my faith has a lot to offer, I think that other religions can provide important religious knowledge"). In each particle, the participant chooses the statement that better reflects their way of thinking, and gets one point if they choose one that reflects a more mature religiosity. The final score is in the range 0–8, where a higher score reflects a more mature religiosity.

Statistical Analysis

All statistics were carried out using SPSS 19.0 for Windows software. Results were analyzed

using the Student's t-test or Mann–Whitney test depending on normal distribution determined by the Kolmogorov–Smirnov test. The data were expressed as mean ± standard error (SEM). Values of $p < 0.05$ were considered statistically significant.

Results

Radiotherapy affects altruism, empathy, anxiety and externality

Detection of psychological distress is often seen among cancer patients, including those undergoing radiotherapy. Results obtained in healthy individuals were similar to results of patients who underwent radiotherapy, indicating that changes in emotional competence (Figure 1A), reaction to stress (Figure 1B), depression (Figure 1C) and mature religiosity (Figure 1D) were not related to the radiation therapy. IRI index (Figure 1E; $p < 0.05$), altruism score (Figure 1F; $p < 0.05$) and BAI score (Figure 1H; $p < 0.05$) were significantly lower in the group of radiotherapy-treated patients compared to healthy controls. Reduced anxiety and empathy scores indicate that radiotherapy didn't induce progress of anxious attachment and empathy fatigue, while reduced altruism may be a response to cancer development since radiotherapy-treated patients are constantly worrying about the future and trying to focus on getting better. On the other hand, the externality score was significantly higher in radiotherapy-treated patients than in healthy subjects (Figure 1G; $p < 0.001$). An increase in externality score may be linked to radiotherapy-treated patients seeking external justification for their illness, rather than possible internal reasons and causes.

There was no significant difference in UEK- 15 (A), CISS (B), BDI (C) and religiosity (D) scores between radiotherapy-treated patients and healthy individuals. IRI index (E), altruism score (F) and BAI score (H) were significantly lower while externality score (G) was significantly lower in the group of radiotherapy-treated patients compared to healthy controls. Values are presented as mean ± SEM; *$p < 0.05$, ***$p < 0.001$.

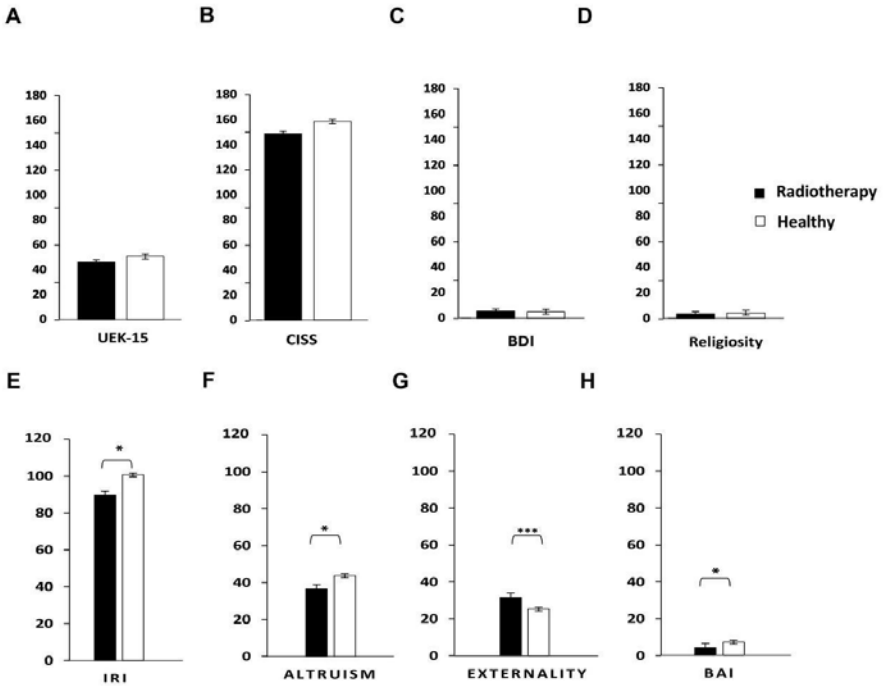

Figure 1. Results of psychological tests obtained in healthy individuals and cancer patients who underwent radiotherapy

Hormone therapy enhances radiotherapy-based effects on religiosity

To analyze the impact of hormone therapy on radiotherapy-induced effects on psycho-emotional well-being, we compared psycho-emotional traits of cancer patients who received radiotherapy with those who underwent combined hormone and radiotherapy. Significantly increased religiosity score was observed in patients treated with both hormone and radiotherapy compared to patients who received only radiotherapy (Figure 2A; $p < 0.05$). These findings suggest that the addition of another treatment approach encouraged emotional orientation towards religion, helping cancer patients to accept illness better. In contrast to religiosity, addition of hormone therapy did not significantly alter radiotherapy-induced effects on IRI, UEK-15, CISS, externality, altruism, BDI and BAI scores of cancer patients (Figure 2B–H).

Religiosity score was significantly increased in cancer patients who received both hormone and radiotherapy compared to patients who received only radiotherapy (A). There was no significant difference in IRI (B), UEK-15 (C), CISS (D), externality (E), altruism (F), BDI (G) and BAI (H) scores between cancer patients treated with radiotherapy and those who received both hormone therapy and radiotherapy. Values are presented as mean ± SEM; *$p < 0.05$.

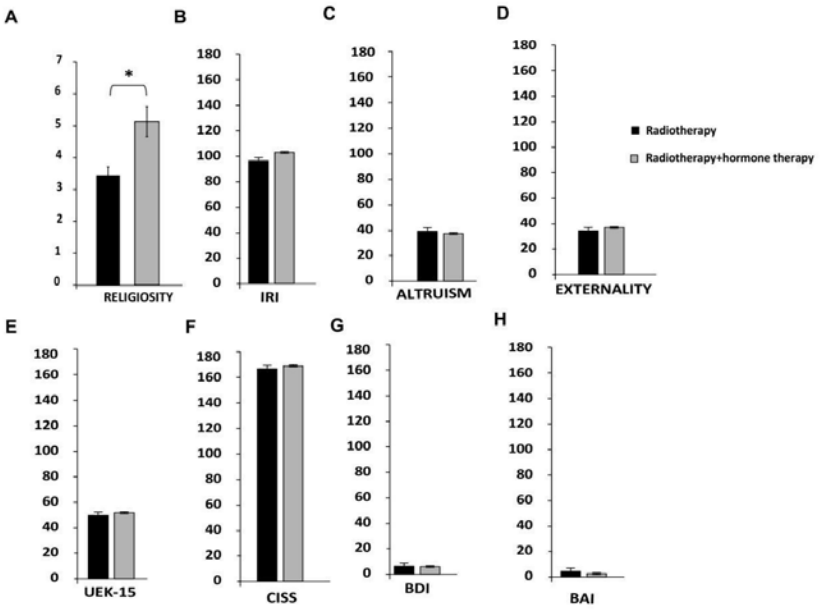

Figure 2. Comparison of psycho-emotional traits of cancer patients who received radiotherapy or combined hormone and radiotherapy

Biological therapy reduces radiotherapy-based effects on religiosity

To assess the influence of biological therapy on the psycho-emotional effects of radiotherapy, we compared the psycho-emotional characteristics of cancer patients who received radiotherapy alone with those who underwent a combination of biological therapy and radiotherapy. Patients treated with both therapies showed a significantly lower religiosity score compared to those receiving only radiotherapy (Figure 3A; $p < 0.05$). Biological therapy is less familiar to patients than radiotherapy and other conventional treatments for malignant diseases, such as surgery and chemotherapy. This lack of knowledge regarding the effectiveness and advantages of biological therapy leads to skepticism about potential improvements. Additionally, the understanding that prior treatments did not cure their cancer, coupled with the need to adopt an unfamiliar approach like biological therapy, contributes to a decrease in religiosity among these patients. In a similar manner as it was observed in patients who received combined hormone and radiotherapy, addition of biological therapy did not significantly alter radiotherapy-induced effects on IRI, altruism, externality, UEK-15, CISS, BDI and BAI scores of cancer patients (Figure 3B–H).

Religiosity score was significantly decreased in cancer patients who received both biological and radiotherapy compared to patients who

received only radiotherapy (A). There was no significant difference in IRI (B), altruism (C), externality (D), UEK-15 (E), CISS (F), BDI (G) and BAI (H) scores between cancer patients treated with radiotherapy and those who received both biological therapy and radiotherapy. Values are presented as mean ± SEM; *p < 0.05.

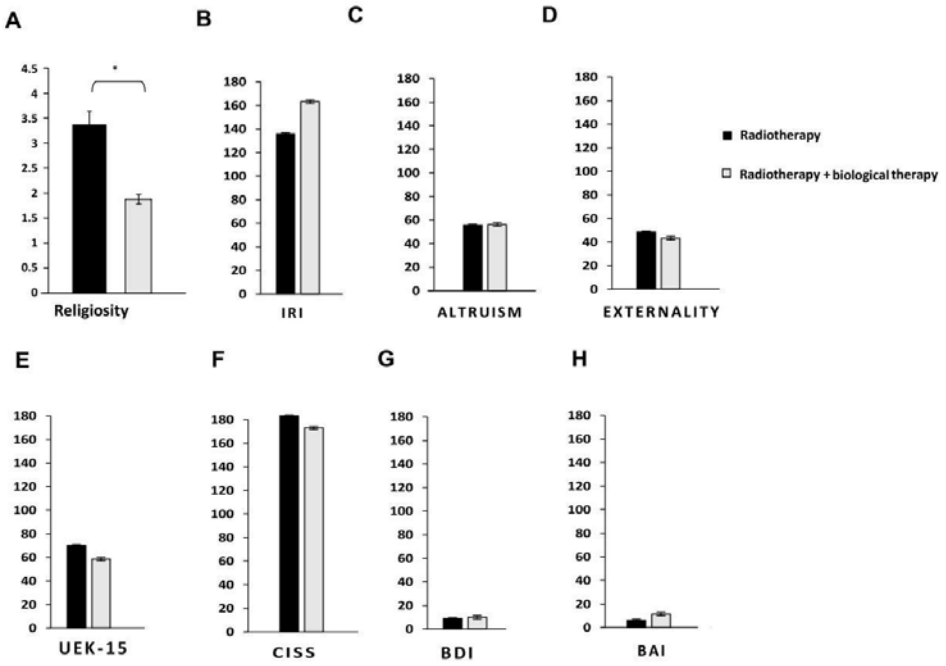

Figure 3. Comparison of psycho-emotional traits of cancer patients who received radiotherapy or combined biological and radiotherapy

Chemotherapy did not significantly alter radiotherapy-induced effects on the psycho-emotional well-being of cancer patients

As it is shown in Figure 4, there were no differences in IRI (Figure 4A), altruism (Figure 4B), externality (Figure 4C), UEK-15 (Figure 4D), CISS (Figure 4E), BDI (Figure 4F), BAI (Figure 4G) and religiosity scores (Figure 4H) of cancer patients treated with radiotherapy and cancer patients who received combined chemotherapy and radiotherapy. We assume that patients tend to be familiar with the beneficial effects of chemotherapy and radiotherapy, since these two therapeutic approaches have been widely used for the treatment of malignant diseases. This familiarity often stems from the extensive discussions between healthcare providers and patients regarding the expectations and possible adverse effects of these therapeutic approaches. Greater awareness and understanding of these therapeutic strategies can significantly enhance the mental well-being of cancer patients, as they are more prepared for the possible outcomes and side effects associated with chemotherapy and radiotherapy. When patients know what to anticipate, they will experience a greater sense of control over their treatment journey.

There was no significant difference in IRI (A), altruism (B), externality (C), UEK-15 (D), CISS (E), BDI (F), BAI (G) and religiosity (H) scores between cancer patients treated with radiotherapy and those who received combined chemotherapy and radiotherapy. Values are presented as mean ± SEM.

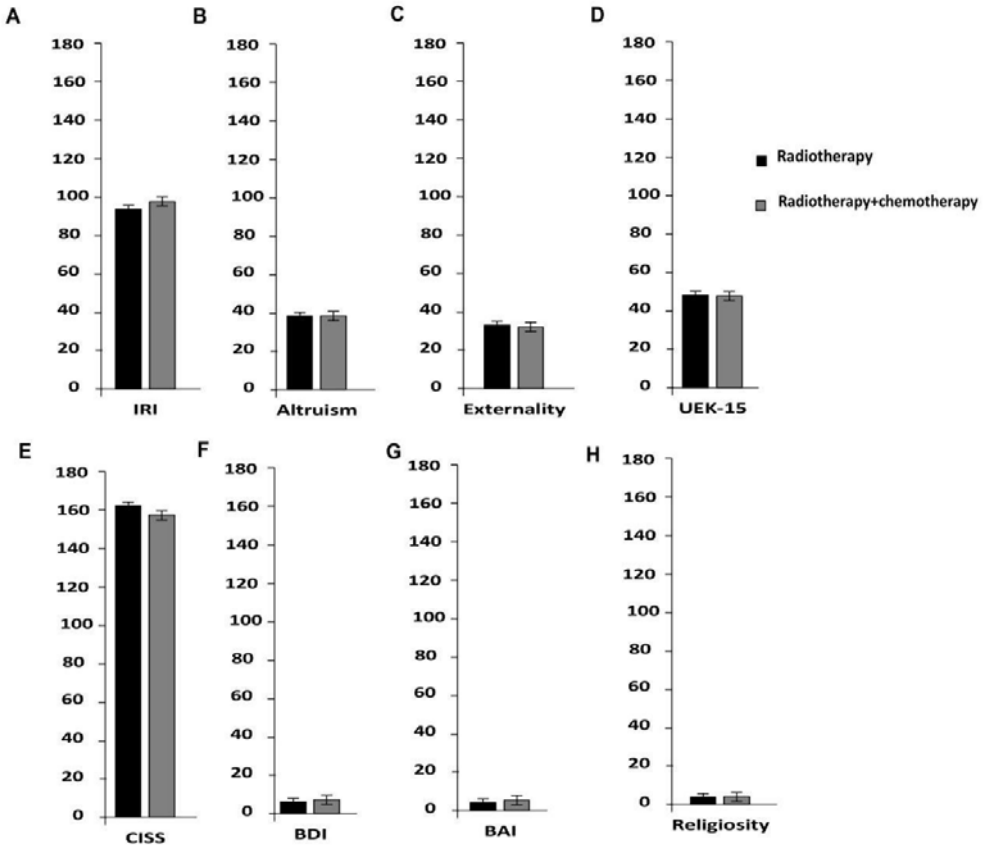

Figure 4. A comparison of the psycho-emotional characteristics of cancer patients who underwent either radiotherapy or a combination of chemotherapy and radiotherapy

Discussion

The results of our study reveal a complex picture of psychological distress among cancer patients undergoing radiotherapy. Notably, the detection of psychological distress is a common experience for these individuals, echoing the findings observed in healthy control subjects (1, 3). The similarities in emotional competence, stress reactions, depression levels, and mature religiosity between the two groups suggest that the emotional challenges faced by patients undergoing radiotherapy may not be directly attributable to the treatment itself. Instead, these changes may reflect broader psychological responses to the stress of a cancer diagnosis and the accompanying uncertainties (3). The significant differences highlighted in the IRI index, altruism score, and BAI scale indicate that while radiotherapy patients do not exhibit increased anxiety or empathy fatigue, they do demonstrate a notable decline in altruism. This reduction in altruism may stem from the intense focus on their illness and the associated need for self-preservation (17). As patients navigate their cancer journey, their worries about the future and the quest for recovery can overshadow their capacity for altruistic behaviors. This suggests a psychological adaptation process, where the immediate survival instinct and the emotional toll of the disease take precedence over empathy and connection with others (17). Conversely, the heightened externality score among radiotherapy-treated patients compared to healthy controls points to a significant psychological coping mechanism (18, 19). This increase may signify an external locus of control, where patients seek to rationalize their illness by attributing it to external factors rather than internal shortcomings (17, 18). The pursuit of justifications for their condition can indicate a psychological struggle to make sense of their circumstances, reflecting a broader human tendency to look for reasons behind adverse events (18, 19). This search for external explanations may serve as a coping strategy, allowing patients to distance themselves from the responsibility of their illness, albeit potentially leading to a sense of helplessness in managing their health (18, 19).

The findings from our analysis shed light on the nuanced effects of hormone therapy when combined with radiotherapy on the psycho-emotional well-being of cancer patients. Notably, the significant increase in religiosity scores among

those receiving both hormone and radiotherapy indicates a potential emotional and psychological benefit derived from the integration of these treatment modalities (6). This enhancement in religiosity may reflect a coping mechanism that helps patients find meaning and solace in their illness, suggesting that the combination of therapies fosters a greater emotional orientation towards spirituality (6). Such a shift can play a crucial role in how patients navigate their cancer journeys, providing them with a support system that transcends the physical aspects of treatment (1, 3). The observed increase in religiosity among patients receiving hormone therapy alongside radiotherapy highlights the multifaceted nature of coping with cancer (6). It suggests that the introduction of hormone therapy may motivate patients to seek comfort and strength in their faith, enhancing their ability to accept and manage their illness (6). This emotional orientation towards religion can have profound implications, as it may bolster resilience, foster community support, and ultimately contribute to improved mental health outcomes (6, 18). As patients integrate their treatment experiences with their spiritual beliefs, they may find a renewed sense of purpose and hope, which can be critical in facing the challenges of cancer (6, 18). Despite the positive impact on religiosity, it is noteworthy that the addition of hormone therapy did not significantly alter other psycho-emotional measures (IRI, UEK-15, CISS, externality, altruism, BDI and BDA). This lack of change suggests that while hormone therapy may enhance certain aspects of psycho-emotional well-being, it does not comprehensively address other emotional domains affected by cancer and radiotherapy (6). The stability of these scores indicates that the underlying psychological stressors associated with cancer diagnosis and treatment remain predominant, and that hormone therapy alone may not be sufficient to alleviate these emotional burdens (1, 6).

The results of our study indicate a noteworthy shift in psycho-emotional characteristics among cancer patients receiving a combination of biological therapy and radiotherapy, particularly in terms of religiosity. The significant decrease in religiosity scores for patients treated with both therapies compared to those receiving radiotherapy alone suggests that the introduction of biological therapy may engender feelings of uncertainty and skepticism (7, 8). This decline in religiosity is particularly striking, as spiritual beliefs often provide a crucial support system for individuals facing significant health challenges (20). The unfamiliarity of biological therapy, when compared to more traditional treatments such as chemotherapy and surgery, likely contributes to patients' hesitance in embracing this new approach, leading to diminished spiritual engagement (7, 20). The skepticism surrounding biological therapy may stem from a lack of comprehensive understanding of its mechanisms and benefits. Unlike established treatments that patients may have heard about or experienced previously, biological therapy is often perceived as a novel and complex option. This unfamiliarity can lead to increased anxiety and uncertainty, ultimately resulting in a diminished sense of control and a weakened connection to spiritual beliefs (20). As patients grapple with the reality that prior treatments may not have been curative, the introduction of an unfamiliar therapy can feel overwhelming, causing them to withdraw from their spiritual practices that typically provide comfort and hope (20, 21). In contrast to the impact on religiosity, it is notable that the addition of biological therapy did not significantly affect empathy, altruism, depression and anxiety in cancer patients. This consistency in scores suggests that while the psycho-emotional landscape changes in some areas, other emotional responses remain relatively stable despite the introduction of biological therapy (20, 22). It underscores the complexity of emotional responses in cancer treatment, indicating that traditional measures of empathy, altruism, and depression may not be as susceptible to change with the addition of a new treatment modality (7, 22).

The absence of significant differences in the scores of evaluated psycho-emotional characteristics between patients receiving radiotherapy and those undergoing combined chemotherapy and radiotherapy suggests that both treatment modalities are similarly understood and accepted by patients, allowing them to maintain consistent emotional landscapes regardless of the specific combination of therapies they are receiving (9). The familiarity that patients possess regarding the effects of both chemotherapy and radiotherapy likely contributes to this uniformity in psycho-emotional responses (9, 23). Given that these treatments have been extensively discussed in medical settings and are widely recognized as standard approaches to combatting cancer, patients may feel more equipped to cope with the complexities of their treatment regimens (9, 23). This increased awareness can lead to greater emotional resilience, as patients who are informed about potential benefits and side effects are better prepared to manage their expectations (24, 25). The familiarity with these therapies can foster a sense of control, which is particularly important in the often tumultuous experience of cancer treatment (23, 24). Importantly, our findings emphasize the importance of effective communication between healthcare providers and patients. When medical teams engage in thorough discussions about treatment options, including the anticipated effects and possible adverse reactions, patients are likely to develop a clearer understanding of their therapeutic journey (9, 25). This understanding not only enhances their preparedness but also empowers them to navigate their emotional responses more effectively (9, 23). As patients feel more in control, their overall mental well-being can improve, leading to a more positive treatment experience (24, 25).

Conclusion

In summary, while radiotherapy itself may not directly exacerbate psychological distress, the emotional landscape of cancer patients is profoundly influenced by their diagnosis and treatment journey. The observed patterns of reduced altruism and increased externality underscore the need for supportive interventions that address the psychological impacts of living with cancer. While hormone therapy may provide specific emotional benefits, particularly in terms of enhancing religiosity, it is crucial to recognize that cancer patients may require more comprehensive support to address the full spectrum of their psycho-emotional needs. Also, there is a critical need for comprehensive educational initiatives aimed at improving patient understanding of biological therapies. By fostering a deeper knowledge of the benefits and side effects of these innovative approaches, healthcare providers can help mitigate skepticism and anxiety in cancer patients. Additionally, integrating psychological support that addresses the emotional and spiritual needs of patients as they navigate their treatment options becomes essential. Such support could facilitate a stronger connection to spirituality, potentially countering declines in religiosity and enhancing overall emotional well-being. Future research should continue to explore the intricate relationship between treatment familiarity and psycho-emotional health, aiming to develop tailored interventions that support radiotherapy-treated patients in embracing all aspects of their cancer care.

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UTICAJ RADIOTERAPIJE NA PSIHOEMOCIONALNE KARAKTERISTIKE PACIJENATA SA MALIGNIM BOLESTIMA

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Stres može značajno uticati na kvalitet života pacijenata sa malignim bolestima i na njihovo iskustvo u vezi sa primenjenom terapijom. Budući da je radioterapija jedan od najvažnijih terapijskih pristupa u lečenju malignih bolesti, neophodno je da se razume njen uticaj na psihoemocionalno zdravlje kako bi se mogle osmisliti sveobuhvatne strategije za očuvanje i poboljšanje psihičkog i fizičkog zdravlja pacijenata.

U ovom istraživanju ispitivali su se efekti radioterapije na psihoemoconalne karakteristike pacijenata sa malignim bolestima. Takođe, analizirani su efekti primene radioterapije kombinovane sa hormonskom terapijom, biološkom terapijom i hemoterapijom. Dobijeni rezultati ukazuju na to da, uprkos prisustvu stresa kod pacijenata koji primaju radioterapiju, postoje promene u njihovoj emocionalnoj kompetenciji, reakcijama na stres, depresiji i intrinzičnoj religioznosti slične onima koje doživljavaju zdrave osobe. Pacijenti podvrgnuti radioterapiji imali su značajno niži rezultat na Indeksu interpersonalne reaktivnosti (engl. *interpersonal reactivity index* – IRI), na Skali altruizma i na Bekovom indeksu anksioznosti (engl. *Beck anxiety inventory* – BAI) u poređenju sa zdravim ispitanicima.

Porast nuspojava kod pacijenata koji primaju radioterapiju ukazuje na traženje spoljašnjih opravdanja za postojeću bolest. Uvođenje hormonske terapije značajno je povećalo stepen religioznosti i poboljšalo prihvatanje bolesti u emocionalnom smislu. Nasuprot tome, biološka terapija je dovela do smanjenja stepena religioznosti verovatno zbog nepoznavanja mogućih ishoda i posledica primene ove metode lečenja. Važno je pomenuti da hemoterapija nije značajno promenila psihoemocionalne karakteristike izazvane radioterapijom, što može učvrstiti shvatanje da poznavanje terapijskih modaliteta može podsteći emocionalnu otpornost i osećaj kontrole kod pacijenata. U zaključku ovog istraživanja naglašena je važnost očuvanja psihoemocionalnog blagostanja za lečenje i sveobuhvatnu negu pacijenata sa malignim bolestima koji su bili podvrgnuti radioterapiji.

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***Ključne reči**: radioterapija, psihoemocionalne karakteristike, altruizam, emocionalna kompetencija, stres*

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PREDICTIVE FACTORS FOR MAJOR ADVERSE CARDIAC EVENTS AFTER CAROTID ENDARTERECTOMY

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Carotid endarterectomy (CEA) is a standard surgical procedure for stroke prevention in patients with carotid artery stenosis but carries a significant risk of major adverse cardiovascular events (MACE).

By integrating clinical risk biomarkers, we aim to improve preoperative risk stratification and contribute to the development of personalized perioperative care strategies in this high-risk patient population.

A total of 110 patients undergoing elective CEA in 2017 were prospectively enrolled. Preoperative clinical data, including soluble urokinase plasminogen activator receptor (suPAR), urea, and left ventricular ejection fraction (LVEF), were collected. MACE, defined as myocardial infarction, arrhythmias, heart failure, stroke, or cardiovascular death, was monitored for 30 days postoperatively. Statistical analysis included univariate and Cox regression modeling to assess predictors of MACE.

Within 30 days post-CEA, 10 patients (9.1%) experienced MACE. These patients had significantly higher suPAR levels (7.04 ± 1.81 vs. 3.15 ± 1.01 ng/mL, $p < 0.001$), elevated serum urea (7.69 ± 2.25 vs. 6.14 ± 1.89 mmol/L, $p = 0.024$), and lower LVEF (48.9 ± 5.43% vs. 55.17 ± 7.8%, $p = 0.007$). Cox regression analysis identified suPAR as an independent predictor of 30-day MACE (HR = 2.144, $p < 0.001$).

Elevated preoperative suPAR, increased serum urea, and reduced LVEF are associated with higher risk of MACE following CEA. Integrating these biomarkers into preoperative assessment may enhance cardiovascular risk stratification and guide perioperative management in high-risk patients.

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Key words: *carotid endarterectomy, major adverse cardiovascular events, soluble urokinase plasminogen activator receptor, ejection fraction, urea*

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Introduction

Carotid endarterectomy (CEA) is a commonly performed surgical intervention aimed at reducing the risk of stroke in patients with significant carotid artery stenosis (1). As the aging population continues to grow and surgical techniques advance, the frequency of major vascular procedures such as CEA has increased substantially, particularly among elderly patients (2). Despite its benefits, CEA remains associated with notable perioperative cardiovascular risk (3). According to the European Society of Cardiology (ESC) and the European Society of Anaesthesiology and Intensive Care (ESAIC), major vascular surgery is classified as high-risk due to the elevated incidence of perioperative myocardial infarction and cardiac arrest, exceeding 5% in this population (4). Given that atherosclerosis is a systemic and progressive disease, fewer than 10% of patients undergoing major vascular surgery have angiographically normal coronary arteries (5). This underscores the critical need for comprehensive cardiovascular risk assessment in the perioperative setting.

Cardiac biomarkers play a pivotal role in the evaluation and prognostication of patients undergoing CEA. The identification of patients at heightened risk for myocardial injury and major adverse cardiovascular events (MACE), a composite endpoint encompassing cardiovascular death, myocardial infarction, stroke, and heart failure, is essential for optimizing clinical outcomes (6). In recent years, both conventional and novel biomarkers have been investigated to enhance the precision of preoperative risk stratification.

Among these, soluble urokinase plasminogen activator receptor (suPAR) has emerged as a promising candidate. suPAR is a stable circulating marker that reflects chronic immune activation and systemic inflammation, key processes implicated in the pathophysiology of atherosclerosis (7). Elevated suPAR levels have been associated with adverse cardiovascular outcomes in various clinical settings, suggesting potential utility in identifying patients at increased risk for postoperative complications (8). In addition to suPAR, traditional markers such as serum urea, an indicator of renal function and systemic catabolic stress, and left ventricular ejection fraction (LVEF), a widely used measure of cardiac performance, may also provide valuable prognostic information in vascular surgery (9, 10).

Early and accurate identification of high-risk patients could enable more tailored perioperative management, thereby reducing the incidence of MACE and improving long-term prognosis (11). However, data on the combined predictive utility of suPAR, urea, and LVEF in patients undergoing CEA remain limited.

Therefore, the objective of this study is to evaluate the predictive value of preoperative suPAR levels, serum urea, and LVEF in identifying patients at increased risk for MACE following carotid endarterectomy.

Aim of Study

By integrating these biomarkers, we aim to improve preoperative risk stratification and contribute to the development of personalized perioperative care strategies in this high-risk patient population.

Material and Methods

The study was approved by the Ethics Committee of Medical Faculty University of Niš, Serbia. During 2017, we prospectively enrolled all 110 patients scheduled for major open elective vascular surgery,specifically carotid endarterectomy in Clinic for Cardiovascular and Transplantation Surgery, Clinical Center Niš, Niš, Serbia. Exclusion criteria were: 1) patients younger than 21 years, 2) unstable coronary disease and 3) decompensated heart failure. All procedures were performed during general anesthesia.

All patients initially underwent detail evaluation of medical history, physical examination, routine hematologic and biochemical blood analysis, 12-lead electrocardiogram, and chest radiography. Preoperative risk was assessed using the online V-POSSUM risk calculator (http://www.riskprediction.org.uk/vascindex.php). During the 30-days following the procedure, major adverse cardiac events, including myocardial infarction, ventricular arrhythmias, decompensating heart failure, and new onset atrial fibrillation were recorded.

Statistical Analysis

The collected data were analyzed using standard descriptive statistical parameters, including arithmetic mean, standard deviation, minimum and maximum values, absolute numbers, and relative frequencies (percentages). Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. Numerical variables were compared between two groups using the t-test or Mann–Whitney U test, depending on data distribution. Cox regression analysis was performed for survival analysis. A significance level of $\alpha = 0.05$ was used to test the null hypothesis. Statistical analyses were conducted using the R statistical software package.

Results

A total of 110 patients were included in the study (54 males and 56 females). The mean age of the study population was 67.43 ± 5.62 years (range: 48–79 years). A history of prior stroke was reported in 44.5% of patients, and diabetes mellitus (DM) was present in 35.5%. Most patients were receiving beta-blockers (77.3%) and ACE inhibitors (65.5%) (Table 1).

Within the first 30 postoperative days, 10 patients (9.1%) experienced a major adverse cardiovascular event (MACE). These events included 4 myocardial infarctions, 4 ventricular arrhythmias, 3 cardiopulmonary resuscitations, 6 episodes of decompensated heart failure, 4 new episodes of atrial fibrillation, 1 stroke, and 1 neurological complication.

Coronary artery disease and prior percutaneous coronary intervention (PCI) were significantly more common in patients who experienced MACE within the first 30 days ($p = 0.021$ and $p = 0.041$, respectively). Conversely, a history of stroke was significantly more frequent among those patients who did not experience MACE ($p = 0.040$). Antithrombotic therapy was significantly more common among patients who developed MACE ($p = 0.017$). Additionally, the frequency of MACE differed significantly based on the severity of dyspnea ($p = 0.029$). No significant association was observed between ASA score and MACE occurrence ($p = 0.334$) (Table 2).

Patients who experienced MACE within the first 30 days had significantly higher levels of urea ($p = 0.024$), sUPAR ($p < 0.001$), as well as lower left ventricular ejection fraction ($p = 0.007$) compared to those without events (Table 3).

Cox regression analysis demonstrated that elevated preoperative sUPAR levels were significantly associated with the occurrence of MACE within 30 days (HR: 2.144, $p < 0.001$). No significant associations were observed for age, sex, the American Society of Anesthesiologists (ASA) score, or the New York Heart Association (NYHA) class (Table 4).

Table 1. Demographic and Clinical Characteristics of the Study Population

Variable	N (%) / Mean ± SD	Range
Age†	67.43 ± 5.62	48–79
Sex		
Male	54 (49.1%)	49.1
Female	56 (50.9%)	50.9
Atrial fibrillation	3 (2.7%)	2.7
Prior stroke	49 (44.5%)	44.5
Coronary artery disease	21 (19.1%)	19.1
Cardiomyopathy	11 (10.0%)	10
Prior PCI	4 (3.6%)	3.6
Prior myocardial infarction	18 (16.4%)	16.4
Prior CABG	1 (0.9%)	0.9
Diabetes mellitus	39 (35.5%)	35.5
Insulin-dependent DM	22 (20.0%)	20
Hyperlipidemia	20 (18.2%)	18.2
Smoking	36 (32.7%)	32.7
Positive family history	37 (33.6%)	33.6
Beta-blockers	85 (77.3%)	77.3
ACE inhibitors	72 (65.5%)	65.5
Calcium channel blockers	24 (21.8%)	21.8
Antithrombotic therapy	57 (51.8%)	51.8
Statins	62 (56.4%)	56
Diuretics	24 (21.8%)	51.8
Nitrates	8 (7.3%)	7.3

† Mean ± Standard Deviation, Minimum–Maximum

Table 2. Demographic and Clinical Characteristics by 30-Day MACE Status (Selected rows shown for brevity)

Variable	No Event (N, %)	MACE (N, %)	P
Age	67.27 ± 5.63	69.00 ± 5.52	0.356[2]
Sex (Male)	49 (49.0%)	5 (50.0%)	1.000[1]
Prior stroke	48 (48.0%)	1 (10.0%)	0.040
Coronary artery disease	16 (16.0%)	5 (50.0%)	0.021
Prior PCI	2 (2.0%)	2 (20.0%)	0.041
Antithrombotic therapy	48 (48.0%)	9 (90.0%)	0.017
NYHA Class III	23 (23.0%)	6 (60.0%)	0.029

[1] Fisher's exact test; [2] t-test

Table 3. Laboratory Parameters by 30-Day MACE Status

Variable	No Event	MACE	P
Urea (mmol/L)	6.14 ± 1.89	7.69 ± 2.25	0.024
sUPAR (ng/mL)	3.15 ± 1.01	7.04 ± 1.81	< 0.001
LVEF (%)	55.17 ± 7.8	48.9 ± 5.43	0.007

*Mann-Whitney U test

Table 4. Cox Regression Analysis of Predictors for 30-Day MACE

Variable	B	HR	95% CI	p
Age	0.026	1.027	0.887–1.188	0.725
Sex	-0.308	0.735	0.154–3.514	0.700
ASA Score	-0.558	0.572	0.067–4.884	0.610
Urea	0.159	1.172	0.850–1.616	0.333
sUPAR	0.763	2.144	1.561–2.944	< 0.001
NYHA III	-0.078	0.925	0.105–8.114	0.944

*B – Regression coefficient; HR – Hazard Ratio; 95% CI – 95% Confidence Interval

Discussion

The interpretation of MACE in the context of CEA remains complex due to the lack of a standardized, universally accepted definition. While MACE is commonly defined as a composite of non-fatal myocardial infarction, non-fatal stroke, and cardiovascular death, considerable heterogeneity exists across studies. Some definitions additionally include heart failure, arrhythmias, urgent revascularization, or hospital readmission (12). This variability hinders direct comparison between studies and complicates meta-analytic interpretations. Moreover, inconsistencies in outcome timeframes (e.g., 30-day vs. long-term) and diagnostic methods further limit comparability. Given the dual cerebrovascular and cardiovascular risks associated with CEA, a procedure-specific, harmonized MACE definition is warranted to improve evidence-based perioperative care.

In our cohort, the mean patient age was 67.4 ± 5.6 years, reflecting an inherently high-risk population. Age is a well-established predictor of perioperative complications, due in part to increased arterial stiffness, decreased physiologic reserve, and a higher prevalence of comorbid conditions such as coronary artery disease (CAD), atrial fibrillation (AF), and heart failure (13, 14). A meta-analysis by Nantakool et al. showed significantly increased rates of stroke, myocardial infarction, and mortality in patients ≥ 75 years, especially among octogenarians (15). These outcomes are likely driven by age-related endothelial dysfunction, frailty, and impaired autonomic regulation.

Our population exhibited a high burden of comorbidities,most notably, a history of cerebrovascular events in 44% of patients. Such individuals are at increased risk for cerebral hypoperfusion and impaired autoregulation, making them more susceptible to perioperative ischemia (16). Additionally, patients with previous ischemic heart disease and prior percutaneous coronary intervention (PCI) were overrepresented among those who developed MACE. Although PCI is intended to stabilize coronary pathology, it is also a marker of advanced atherosclerosis and residual ischemic burden, and it introduces complexities related to dual antiplatelet therapy and perioperative bleeding risk (17).

Interestingly, patients receiving antiplatelet therapy had a higher incidence of MACE, which may reflect confounding by indication, i.e., antiplatelets being prescribed more frequently to those with established cardiovascular disease (18). This emphasizes the need for careful interpretation of medication effects in observational studies.

Postoperative arrhythmias, especially atrial fibrillation, were among the most frequent complications, consistent with existing literature (19). Pathophysiologic drivers include hemodynamic stress, autonomic imbalance, and systemic inflammation. Elderly patients with structural heart disease are particularly vulnerable. We also observed cases of ventricular arrhythmia and three instances requiring cardiopulmonary resuscitation, highlighting the severity of cardiac events following CEA. Previous reports by Hertzer et al. and Hannan et al. identified arrhythmias as independent predictors of perioperative morbidity and mortality (20, 21).

Diabetes mellitus, present in 35.5% of our cohort, was another significant contributor to adverse outcomes. Diabetic patients exhibit endothelial dysfunction and systemic inflammation, both of which increase susceptibility to ischemia and adverse cardiovascular events (22). Pharmacologic management, including beta-blockers and ACE inhibitors, was prevalent. While beta-blockers are known to reduce sympathetic activity and prevent ischemia, their association with cerebral hypoperfusion and increased intraoperative shunting has been reported (23). The role of ACE inhibitors remains debated, though some studies suggest perioperative benefits in stroke and mortality reduction (24).

Among novel risk markers, suPAR and serum urea have emerged as promising biomarkers. Elevated preoperative suPAR levels reflect systemic immune activation and are associated with increased risk of adverse outcomes in vascular surgery (25, 26). Its stability and chronic disease sensitivity make it an attractive tool in risk stratification. Likewise, elevated serum urea, indicative of renal dysfunction and catabolic stress, has been independently linked with increased postoperative myocardial infarction, stroke, and death (9).

Our findings also validated the utility of the NYHA functional classification, as higher NYHA

classes were associated with increased MACE risk. NYHA status reflects the extent of heart failure symptoms and functional capacity, both critical in predicting cardiovascular vulnerability in the perioperative period (4).

Left ventricular ejection fraction, another cornerstone of cardiovascular evaluation, was a robust predictor in our study. Reduced LVEF (< 40%) significantly correlated with higher rates of MACE, including myocardial infarction and arrhythmias. LVEF dysfunction signals poor myocardial reserve and electrical instability, mandating optimized pharmacologic therapy and hemodynamic management in the perioperative setting (10).

In conclusion, our findings underscore the multifactorial nature of cardiovascular risk in patients undergoing CEA. Advanced age, comorbid burden, arrhythmias, and emerging biomarkers such as suPAR and urea collectively inform risk stratification. A comprehensive, individualized approach, combining clinical history, functional classification, and biomarkers, is critical for improving outcomes in this high-risk population.

Conclusion

Major adverse cardiovascular events remain a significant cause of morbidity and mortality following CEA, underscoring the need for improved perioperative risk stratification. Our findings support the utility of a multimodal biomarker approach incorporating suPAR, serum urea, and LVEF to identify patients at elevated cardiovascular risk. Each of these markers offers distinct yet complementary insights into the pathophysiological processes underlying postoperative complications, such as chronic inflammation, renal dysfunction, and impaired cardiac performance.

Soluble urokinase plasminogen activator receptor serves as a robust indicator of systemic inflammatory burden and atherosclerotic disease activity, while elevated serum urea reflects metabolic stress and possible cardiorenal dysfunction. Reduced LVEF, a well-established predictor of adverse cardiac outcomes, highlights underlying myocardial vulnerability. The integration of these parameters into a unified risk assessment model may enhance the precision of perioperative management strategies and improve patient outcomes.

Further prospective studies are warranted to validate this triad of biomarkers and assess its performance in predictive algorithms tailored to the CEA population. Ultimately, such an approach may facilitate personalized perioperative care, enabling timely interventions that mitigate the risk of cardiovascular complications in high-risk surgical patients.

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PREDIKTIVNI FAKTORI ZA NASTANAK VELIKIH NEŽELJENIH SRČANIH DOGAĐAJA NAKON KAROTIDNE ENDARTEREKTOMIJE

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Karotidna endarterektomija (engl. *carotid endarterectomy* – CEA) predstavlja standardnu hirurško proceduru u prevenciji moždanog udara kod pacijenata sa stenozom karotidne arterije. Međutim, pri izvođenju ove procedure postoji značajan rizik od nastanka velikih neželjenih kardiovaskularnih događaja (engl. *major adverse cardiovascular events* – MACE).

Cilj ovog rada bio je da unapredi preoperativnu procenu rizika i da doprinese razvoju personalizovanih perioperativnih strategija u ispitivanoj populaciji kod koje postoji visok rizik od nastanka MACE-a.

U studiju je u toku 2017. godine prospektivno uključeno ukupno sto deset pacijenata koji su bili podvrgnuti elektivnoj CEA. Prikupljeni su preoperativni klinički podaci, koji su obuhvatili i nivoe suPAR-a (engl. *soluble urokinase plasminogen activator receptor*), uree i ejekcione frakcije leve komore (engl. *left verticular ejection fraction* – LVEF). Pojava MACE-a, koji podrazumeva infarkt miokarda, aritmije, srčanu slabost, moždani udar ili kardiovaskularnu smrt, praćena je trideset dana posle operacije. Statistička analiza, zasnovana na univarijantnoj analizi i Koksovoj regresionoj analizi, izvršena je radi procene prediktora MACE-a.

U toku trideset dana praćenja nakon CEA, MACE je zabeležen kod deset pacijenata (9,1%). Ovi pacijenti su imali značajno više nivoe suPAR-a (7,04 ± 1,81 naspram 3,15 ± 1,01 ng/mL; $p < 0,001$), povišene vrednosti uree (7,69 ± 2,25 naspram 6,14 ± 1,89 mmol/L; $p = 0,024$) i niži LVEF (48,9% ± 5,43% naspram 55,17% ± 7,8%; $p = 0,007$). Koksova regresiona analiza identifikovala je suPAR kao nezavisan prediktor za pojavu MACE-a u roku od trideset dana (HR = 2,144; $p < 0,001$).

Povišeni preoperativni nivoi suPAR-a, povećana urea i smanjena ejekciona frakcija povezani su sa većim rizikom od pojave MACE-a nakon CEA. Integracija ovih biomarkera u preoperativnu procenu može unaprediti stratifikaciju kardiovaskularnog rizika i pomoći u donošenju odluke o načinu na koji će se tretirati pacijenti kod kojih postoji visok rizik od nastanka MACE-a pre operacije.

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***Ključne reči**: karotidna endarterektomija, veliki neželjeni kardiovaskularni događaji, soluble urokinase plasminogen activator receptor, ejekciona frakcija, urea*

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THE EFFECTS OF COCAINE USE ON THE COURSE AND OUTCOME OF PREGNANCY

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The epidemic of drug addiction is a significant public health issue worldwide. Through easy availability of cocaine, its use by childbearing women may have deleterious effects on the course and outcome of pregnancy.

Cocaine use during pregnancy causes: intrauterine growth retardation, abruptio of placenta, increased frequency of premature birth, premature rupture of the membranes, precipitous labor, meconium-stained amniotic fluid, stillbirth, neonatal necrotizing enterocolitis, neonatal bowel perforation, complete bilateral absence of the diaphragm, neonatal respiratory disorders, neonatal subcutaneous fat necrosis, sudden infant death syndrome, intimal fibromuscular dysplasia of numerous blood vessels, hearing impairment, retinal hemorrhages, fetal or maternal intracranial accident, rupture of the uterus, ruptured tubal ectopic pregnancy, placenta previa, changes in neonatal behavior, neonatal cardiac arrhythmias, various congenital anomalies, maternal myocardial infarction and long QT interval in a parturient.

Retrospective studies have shown that intrauterine exposure to cocaine causes major malformations in newborns: congenital heart defects, genitourinary, brain and skull anomalies, absence of limbs, ankyloglossia, hypothalamic hamartoblastoma, and Poland-Möbius syndrome.

Some authors suggest that there is a specific fetal cocaine syndrome and a link between the high incidence of autism and exposure to cocaine *in utero*. Cocaine is a neurobehavioral teratogen, as long-term monitoring of prenatally exposed children has shown deleterious effects of cocaine on their psychophysical development. Cocaine use during pregnancy can result in a wide range of adverse effects on pregnancy and its outcome.

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Key words: *cocaine, pregnancy, neurobehavioral teratogen, congenital anomalies*

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Introduction

Cocaine is a local anesthetic, central nervous system stimulant and appetite suppressor. It primarily acts on the presynaptic level by blocking reuptake of monoamine neurotransmitters dopamine, norepinephrine and serotonin through specific plasma membrane transporters. In this way, it causes tachycardia, peripheral vasoconstriction, increase in blood pressure, agitation, euphoria and excitation.

The name of this drug is derived from the word “khoka” meaning “divine tree” in the language of Inca. Cocaine is produced by extraction from evergreen leaves of *Erythroxylum coca* (Bolivian coca) and *Erythroxylum novogranatense v. truxillense* (Peruan coca), as well as other *Erythroxylum* species originating from South America. Spanish conquistadors observed in XVI century that natives could work for days and move without rest and food in the high Andes, when chewing these plants leaves (1).

Cocaine (molecular weight is 303.4) is an ester of benzoic acid and ecgonine aminoacid (methylbenzoylecgonine – $C_{17}H_{21}NO_4$). It is a fine, colorless or white crystalline powder with bitter taste, highly soluble in alcohol and ether (insoluble in water). Cocaine melting point is 96–98°C. About 1 kg of crude cocaine may be produced out of 150 kg of coca leaves. The end-product of coca

leaves extraction is 99% cocaine in the form of cocaine hydrochloride. It is often "cut" with other substances of similar color: starch, powder, lactosis, sacharosis, mannitol, lidocaine, procaine and amphetamine. Cocaine hydrochloride – $C_{17}H_{21}NO_4HCl$ (molecular weight is 339.8) is a fine, colorless or white, bitter tasting crystalline powder, highly soluble in water and alcohol, insoluble in ether. Its melting point is 197°C, when it begins to decompose.

From South America and Asia, cocaine is exported world-wide and illegally sold as a white powder of uncertain purity. In the street (illegal) market, cocaine is found in various concentrations (30−60%) and by different names: "c", "charlie", "coconut", "coke", "cola", "crack", "dust", "ice", "king", "lady", "lady snow", "rock(s)", "snow", "snowflake", "toot", "white", etc.

A large number of people world-wide use cocaine occasionally or regularly. About 41 million people aged ≥ 18 years reported lifetime use of cocaine, and 5.4 million people reported having used cocaine in 2019 among hospitalized patients in the United States of America (USA) (2). According to the report by the United Nations Office on Drugs and Crime, annual prevalence of cocaine use expressed as a percentage of the population in Serbia (age 15−64) was 2.0% in 2018 (3).

There are various ways to consume cocaine: orally, intranasally, sublingually, vaginally, rectally, intravenously, by inhaling smoke or rubbing it into mucosas. The slang terms for these are: chewing, draw up, sniffing, smoking, etc. Cocaine is sniffed into nostrils and absorbed through the nasal mucosa. The effects start in 3−5 minutes, and last for 30−60 minutes. Intravenous cocaine application is followed with almost immediate and much stronger effects (in 6−8 seconds) which lasts 10−15 minutes. Cocaine abuse causes severe psychological dependence. Quitting cocaine may lead to occurrence of various withdrawal symptoms: the urge for cocaine, depression, apathy, paranoia, suicidal thoughts, loss of sexual desires, insomnia, etc.

"Crack" is the street name for freebase cocaine produced by mixing powder cocaine hydrochloride with ammoniac or sodium bicarbonate and water, and later by heating it until hydrochloride evaporates. Such a substance has a form of small, wax-colored, hard blobs, and it is ready for smoking. The term "crack" is onomatopoeic, and comes from crackling that can be heard while smoking it. When smoking "crack", euphoria occurs within 10 seconds. Such a quick and powerful effect is the reason for "crack's" popularity during mid-80's of the 20th century. Psychological and physical addiction is developed quickly, sometimes already after the first dose. Long-term "crack" abuse leads to complete degradation, both psychologically (loss of self-control and self-esteem) and physically. The "crack" addict's personality is dominated by: depression, extreme mood swings and the feeling of persecution.

In the case of simultaneous cocaine and alcohol abuse, cocaethylene is synthesized in the liver. This metabolite emphasizes euphoric effects of cocaine, but is more toxic and increases the risk of sudden death.

Cocaine addicts usually consume both cocaine and heroin. This combination is called the "speedball" because the effects of these two substances potentiate each other. This is especially dangerous because heroin takes away the unpleasant "edge" effect of cocaine, giving the addict a false sense of security. Therefore, more heroin or cocaine can be taken, which may lead to overdose and a fatal outcome.

Cocaine Effects on Gestation in Animals

Extremely low concentrations of cocaine are detected in fetal tissues after administration to pregnant mice (4). Cocaine application during organogenesis results in the increased frequency of intrauterine resorptions, as well as skeletal defects, exencephaly, eye anomalies, hydronephrosis, cryptorchism and delayed ossification of cranial bones and the bones of the paws (5). Administration of prazosin (a selective α1-blocker) reduces, while administration of diltiazem (a calcium channel blocker) increases the incidence of fetal anomalies in the offspring of mice exposed to cocaine *in utero* (6). In rats, during gestation, cocaine increases the possibility of resorption or fetal edema, and can also cause a significant decrease in fetal weight (7), as well as focal necrosis, necrobiosis, hemorrhages, and inflammatory reactions in the gastrointestinal tract of the embryo (8).

It has been shown that the changes that occur in rat offspring after intrauterine exposure to cocaine (bilateral necrosis and cavitation of the cerebral cortex, hemorrhage and ectopic outgrowths in the corpus striatum, vacuolization in the lens of the eye) can also be induced by temporary uterine artery occlusion, or directly by pressuring gravid uterus with fingers (9).

Intraperitoneal application of cocaine to pregnant rats during gestation causes dose-dependent development of the fetal soft tissue malformations, predominantly in the genitourinary system (10). Doses corresponding to "recreational" doses taken by humans (0.5−1.0 mg/kg) may cause the following changes in sheep during gestation: increase in maternal blood pressure (32% and 37%, respectively) and fetal blood pressure (12.6%), decrease in uterine blood flow (36% and 42%, respectively), and increase in catecholamine concentrations (210%). The maximum effect is achieved after 15 minutes, with a subsequent rapid decline (11). Prenatal cocaine exposure can have long-term dose-dependent effects on dopamine D3 receptor function in adult rhesus monkeys (12).

Cocaine Effects on Gestation in Women

Cocaine is widely distributed throughout the body after administration, and is rapidly metabolized by esterase enzymes in the circulation and in the liver. The main metabolites are benzoylecgonine, ecgonine, ecgonine methyl ester, and norcocaine, which is highly hepatotoxic. There are three different metabolic pathways of cocaine biotransformation: when hydrolyzed by hepatic and plasma esterases, benzoyl group is lost, forming ecgonine methyl ester (the most important metabolic pathway); spontaneous hydrolysis (probably non-enzymatic) leads to the formation of benzoylecgonine which is then degraded to ecgonine; and by N-demethylation forming norcocaine. Therefore, the most important metabolites of cocaine are ecgonine methyl ester, benzoylecgonine and ecgonine, all inactive metabolites. Norcocaine is an active metabolite and may have an important role in the acute poisoning with cocaine. In the presence of alcohol, cocaine undergoes a process of transesterification, forming a psychoactive metabolite, cocaethylene. This metabolite is more toxic than cocaine itself (13). There are great individual differences in cholinesterase activity, which may explain some obscurities in cocaine effects and metabolism. Plasma cholinesterase activity is significantly decreased during pregnancy (14, 15). Fetus has low plasma cholinesterase activity (14) and cocaine is metabolized very slowly in the fetus. By the end of the fourth hour after the use of cocaine, the most of the drug has already been eliminated from plasma, but metabolites (ecgonine methyl ester, benzoylecgonine and ecgonine) may still be detected even after 144 hours (16). Depending on the liver and kidney function, 1–9% of unmetabolized cocaine (there is more unmetabolized cocaine in acidic urine) and cocaine metabolites may be detected in the urine (17). Unmetabolized cocaine can also be found in saliva, and is eliminated by feces (17, 18). The placenta is no barrier to the transfer of cocaine and its pharmacologically active derivatives (norcocaine and cocaethylene) to the fetus (19). Both cocaine and benzoyl cocaine (benzoylecgonine) can still be detected in the urine of the newborn after 5 days (20).

The pharmacological effects of cocaine are associated with reduced catecholamine uptake and corresponding activation of the sympathetic nervous system. Chronic excess of circulating catecholamines in the mother who uses cocaine induces downregulation of catecholamine receptors. Vasoconstriction of the uterine and umbilical arteries causes a decrease in the delivery of oxygenated blood to the fetus, followed by hypoxemia, hypertension, and tachycardia.

The etiology of recurrent hypoxic insults to the placenta and fetus in humans lies in cocaine-induced vasoconstriction of the uterine artery (11, 21, 22). The human umbilical artery also shows intense vasoconstriction *in vitro* in the presence of cocaine, which can be prevented by the administration of diltiazem (23). *In vitro*, the human placenta increases thromboxane synthesis and decreases prostacyclin production (24). After analysis of 69 placentas from women who used cocaine during pregnancy, its reduction (in size and weight) was evident. In addition, ablation was observed in 25% of cases, inflammation/infection (41%), ischemic changes (29%), obliteration of the intervillous space (29%), and placental membrane anomalies (16%) (25). Cocaine readily crosses the placental barrier by simple diffusion because it is highly soluble in both water and fat and has a low molecular weight. At physiological pH, cocaine is poorly ionized, but since the pH of fetal blood is lower than that of maternal blood, significantly higher concentrations of cocaine in the fetal circulation can be expected.

Cocaine and its most important metabolite benzoylecgonine have been detected in fetal brain, liver, kidneys, heart, blood and hair. The highest concentrations were found in the liver (as for cocaine), and the brain (as for benzoylecgonine) (26). The cocaine plasma half-life is around 30 to 90 min, but metabolites are present in the urine for a longer period (2 to 5 days). After intravenous application, cocaine elimination halftime is 30–40 minutes, but its metabolites may be detected even after more than a week. Elimination halftime of benzoylecgonine with newborns is 14.6 hours, twice as long as with adults (27). In one case of a mother's severe poisoning, the concentration of this metabolite measured in the newborn, was 41 000 ng/ml (27).

The prevalence of cocaine use during pregnancy varies worldwide. The percentage established were 4.4% (of 353 newborn meconium samples tested) in Barcelona (Spain) (28), 5.3% (of 1625 pregnant women tested) in Toronto, Canada (29), 11.5% (of 1111 anonymous postnatal maternal urine samples from New York (USA) (30). An alarmingly high 13.6% of newborns (966 of 7083 consecutive births) were reported to have suffered from intrauterine cocaine exposure in Oakland, California (31).

Long-term use cocaine during pregnancy causes (21, 30–128): intrauterine growth retardation (21, 30, 38–41, 45, 49, 54, 56, 58, 63, 75–80, 86, 87, 90, 108, 110, 120), abruptio of placenta (26, 33, 34, 49, 54, 63, 74–77, 89), increased frequency of premature birth (21, 30, 33, 34, 42, 49, 54, 56, 59, 63, 76, 87, 90), premature rupture of the membranes (36, 59, 63, 91, 101), precipitous labor (21, 63), meconium-stained amniotic fluid (63), stillbirth (37, 76), neonatal necrotizing enterocolitis (43, 70, 71, 81, 113), neonatal bowel perforation (56, 80, 82, 112), complete bilateral absence of the diaphragm (105), neonatal respiratory disorders (50), neonatal subcutaneous fat necrosis (102), sudden infant death syndrome (31, 34, 37, 94), intimal fibromuscular dysplasia of numerous blood vessels (122), hearing impairment (46, 119), retinal

hemorrhages (114, 115), fetal or maternal intracranial accident (36, 44, 48, 49, 57, 69, 90, 103, 109, 126), rupture of the uterus (57, 95, 111, 125), ruptured tubal ectopic pregnancy (55), placenta previa (76, 107, 115), changes in neonatal behavior (34, 53, 76, 98), neonatal cardiac arrhythmias (36, 51, 56, 100, 127), various congenital anomalies (32, 34, 35, 38, 41, 52, 56, 62, 64–68, 72, 73, 80, 83–86, 89–94, 96–99, 117–121), and maternal myocardial infarction (104, 105, 123, 129).

Retrospective studies have shown that intrauterine exposure to cocaine causes major malformations in newborns with a risk of 8–10% (37, 38). Some authors have linked the increasing number of women and men using cocaine occasionally or regularly to an increase in the incidence of congenital heart defects (32, 51). Cocaine exposure can lead to the development of congenital cardiovascular (38, 56, 65–68, 89, 91, 94, 120), genitourinary (34, 35, 41, 49, 53, 65, 67, 83, 92, 99, 119), brain and skull anomalies (32, 64, 83, 96–98, 117), and absence of limbs (73, 83, 85, 86, 116, 117). Interestingly, the incidence of ankyloglossia in newborns is 3.5 times higher in those exposed prenatally to cocaine (80). A case of a newborn with hypothalamic hamartoblastoma, postaxial polydactyly in both hands and the left foot, and heart defects (Pallister-Hall syndrome) after intrauterine exposure to cocaine, marijuana, and methaqualone has been reported (32). The development of Poland-Möbius syndrome with calcifications in the medulla oblongata and unilateral defects of the right pectoral muscle, thorax, and hand has been described in a newborn exposed to cocaine during the first trimester of gestation. In addition, central apnea, bilateral cranial nerve palsies, and dysphagia have been reported after birth (130).

Cocaine has been shown to induce bilirubin metabolic pathways, thereby reducing the risk of hyperbilirubinemia in neonates. Bilirubin concentrations in neonates exposed to cocaine *in utero* are half that of controls (55 ± 26 μmol/L vs. 110 ± 32 μmol/L) (131).

After birth, neonates exposed to cocaine *in utero* may exhibit the following signs: jittery/tremor, high pitched cry, irritability, excessive suck, hyperalertness and autonomic instability (124).

During the first days of a newborn, minute volume and stroke heart volume are decreased, while mean arterial pressure is increased (132). Hypertension or borderline hypertension was observed in 6 of 12 children exposed prenatally to cocaine, without any renal, cardiovascular, or endocrine abnormalities (133).

Mechanism of Action of Cocaine on Fetus

Cocaine impairs neurotransmission in the central nervous system during embryonic development, especially in the first trimester. It can therefore be considered a neurobehavioral teratogen for humans (49). These changes most likely occur in the central dopaminergic system of infants exposed to cocaine *in utero*, since concentrations of homovanillic acid (a major metabolite of dopamine) are significantly reduced in the cerebrospinal fluid (134). High concentrations of the circulating catecholamine precursor dihydroxyphenylalanine, and consequently increased catecholamine activity, have also been found in children exposed prenatally to cocaine. These changes may play an important role in the pathogenesis of neurobehavioral disorders (78).

The pathogenesis of these impairments at the cellular level includes dysfunctional myelination, disrupted dendritic architecture, and synaptic alterations. Prenatal exposure to cocaine is also associated with disruption of the hypothalamic-pituitary-adrenal axis hormones that mediate neuroendocrine responses (135).

Children exposed prenatally to cocaine have shown specific language and cognitive deficits, and impaired social development (136, 137). There is also a significant depression of organizational responses to external stimuli according to the Brazelton scale in such children (34, 37), as well as transient electroencephalographic abnormalities (138). Exposed children have a 2.8 times higher risk of showing learning difficulties, compared with unexposed children (139). Prenatal cocaine exposure slows postnatal growth at seven and ten years of age (140) and predicts poorer perceptual reasoning IQ at the age of nine (141).

In a long-term follow-up study of 70 children exposed to cocaine *in utero*, a number of significant neurodevelopmental abnormalities were reported: delayed speech (94%) and an extremely high incidence of autism (11.4%) (79). The authors suggest that the high incidence of autism is specific to intrauterine cocaine exposure, as it is not observed in those exposed to other opiates or alcohol. They also described various optical anomalies in children exposed to cocaine *in utero* (142, 143): frequent refractive errors, optic nerve anomalies, microphthalmia, development of retinopathy, and prolonged eyelid edema. At age 21, young adults who were prenatally exposed to cocaine had lower mean full-scale (83.7 ± 10.4 vs. 87.3 ± 12.5, $p < 0.1$) and perceptual reasoning IQ (87.3 ± 11.5 vs. 91.4 ± 13.9, $p < 0.02$), lower high school completion rates (75% vs. 86%, $p < 0.02$), and were slightly more likely to have been on probation than non-cocaine exposed young adults, but did not differ in Verbal IQ, self-reported problematic substance use or incarceration. Young adults with prenatal cocaine exposed in foster/adoptive had similar lower IQ scores but had better verbal skills and high school graduation rates that did not differ from non-cocaine exposed young adults (80.6 vs 86.2%, $p > .05$) (144).

In 1985−1986, the cost of postpartum care in the United States was 5,200$ higher for newborns prenatally exposed to cocaine than for babies who were not. The increased cost was primarily due to longer hospital stays (by an average of four days), admissions to special care units, and various medical procedures and therapies, among other factors (145). In another study, data showed that intrauterine exposure to cocaine or some other illicit drug in 1991−1992 increased hospital stay by seven days and increased costs by $7,731 compared to controls (146). The United States spends about $500 million annually to address this growing and serious problem (1991 data), which means that about 100,000 newborns are exposed to cocaine *in utero* annually (145).

Exposure to cocaine during the development of the nervous system can lead to permanent changes in brain structure and function and later produce altered responses to environmental or pharmacological challenges (147). Cocaine use during pregnancy has been associated with a higher risk of heart failure 10 years later (148) and may be associated with a risk of cerebrovascular disease and cerebrovascular interventions more than 15 years later (149).

Cocaine exposure during nervous system development can lead to lasting changes in brain structure and function, resulting in altered responses to environmental or pharmacological challenges later in life (147).

Numerous studies suggest that cocaine causes changes in the fetus, which can vary significantly in intensity and frequency, depending on the amount of cocaine used, duration and timing of exposure during pregnancy, potential interactions with other drugs (e.g., alcohol), habits, as well as the possibility of obtaining and introducing cocaine into the pregnant woman's body.

The identification of “fetal cocaine syndrome” was not possible until recently, unlike the identification of “fetal alcohol syndrome” and “fetal hydantoin syndrome”. However, attempts have been made, and the most comprehensive description of fetal cocaine syndrome to date includes the following: neurological irritability, large fontanelles, prominent glabella, marked periorbital and palpebral edema, low nasal bridge with a transverse crease, short nose, lateral soft tissue nasal build-up and small toenails (90).

Conclusion

Cocaine use during pregnancy may have deleterious effects on both pregnancy and its outcome. It has been suggested that cocaine abuse causes: intrauterine growth retardation with low birth weight and microcephaly, placental ablation, increased frequency of premature delivery, premature rupture of the membranes, stillbirth, neonatal necrotizing enterocolitis, neonatal bowel perforation, neonatal respiratory disorders, neonatal subcutaneous fat necrosis, sudden infant death syndrome, fetal or maternal intracranial accident, ruptured tubal ectopic pregnancy, placenta previa, changes in neonatal behavior, neonatal cardiac arrhythmias, maternal myocardial infarction and various congenital anomalies. It has not been proven that these effects depend on the dose used, the time of exposure, and the simultaneous use of other drugs with cocaine. Some authors suggest that there is a specific fetal cocaine syndrome and a link between the high incidence of autism and exposure to cocaine *in utero*. Cocaine is a neurobehavioral teratogen, as long-term monitoring of prenatally exposed children has shown deleterious effects of cocaine on their psychophysical development.

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EFEKTI KOKAINA NA TOK I ISHOD TRUDNOĆE

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Epidemija zavisnosti od nedozvoljenih droga predstavlja značajan problem javnog zdravlja širom sveta. Upotreba kokaina, do kojeg se lako može doći, kod žena u fertilnom periodu može imati štetne uticaje na tok i ishod trudnoće.

Upotreba kokaina u trudnoći uzrokuje: usporen intrauterini rast ploda, abrupciju placente, povećanu učestalost prevremenog porođaja, prerano pucanje plodnih ovojnica, prisustvo mekonijuma u amnionskoj tečnosti, mrtvorođenost, neonatalni nekrotizirajući enterokolitis, neonatalnu perforaciju creva, kompletno odsustvo dijafragme, neonatalne respiratorne poremećaje, neonatalnu nekrozu potkožnog masnog tkiva, sindrom iznenadne smrti novorođenčeta, fibromuskularnu displaziju intime brojnih krvnih sudova, oštećenje sluha, krvarenje u mrežnjači, intrakranijalna krvarenja fetusa ili majke, rupturu materice, rupturu jajovoda zbog vanmaternične trudnoće, placentu previju, promene u ponašanju neonatusa, neonatalne srčane aritmije, različite kongenitalne anomalije, infarkt miokarda trudnice i dug QT interval kod porodilje.

Retrospektivne studije su pokazale da intrauterino izlaganje kokainu izaziva *major* malformacije kod novorođenčadi: urođene srčane mane, anomalije genitourinarnog sistema, mozga i lobanje, odsustvo udova, ankiloglosiju, hamartoblastom hipotalamusa i Poland–Möbius sindrom.

Pojedini autori ističu da postoji veza između kokainskog sindroma specifičnog za fetus i visoke incidencije autizma i izloženosti kokainu *in utero*. Kokain je neurobihevioralni teratogen. Naime, dugotrajno praćenje dece koja su bila izložena kokainu pre rođenja ukazalo je na štetne efekte kokaina na njihov psihofizički razvoj. Upotreba kokaina u toku trudnoće može imati mnogobrojne štetne uticaje na trudnoću i njen ishod.

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***Ključne reči**: kokain, trudnoća, neurobihevioralni teratogen, kongenitalne anomalije*

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