УНИВЕРЗИТЕТ У БЕОГРАДУ
МЕДИЦИНСКИ ФАКУЛТЕТ
UNIVERSITY OF BELGRADE
FACULTY OF MEDICINE

MEDICINSKA ISTRAŽIVANJA | MEDICAL RESEARCH

Časopis Medicinskog fakulteta Univerziteta u Beogradu | *The Journal of the Faculty of Medicine University of Belgrade*

Vol 57 | Sv. 2 | 2024

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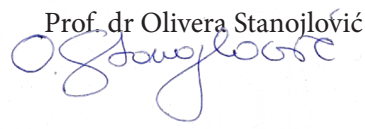

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Medicinska istaživanja 2024; 57(2):1-5 | DOI: 10.5937/medi57-47911

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Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Risk factors in the occurrence of cholelithiasis in children and adolescents: a single-center experience

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Recived: 28 November 2023

Revised: 04 January 2024

Accepted: 10 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Cholelithiasis is etiopathogenetically very heterogeneous and, in terms of frequency, a rare disease in the period of growth and development. The aim of the study was to analyze risk factors for the occurrence of cholelithiasis in that period of life.

Material and Methods: This retrospective study included 80 children and adolescents, 50 females and 30 males, aged 4-18 (14±5.55) years, with symptomatic cholelithiasis who were operated on at the University Children's Hospital in Belgrade in the period from 2000 to 2016. The diagnosis of the disease was based on ultrasound findings. Data on risk factors for the development of biliary calculosis were obtained from medical history.

Results: Predisposing risk factors for cholelithiasis were verified in 46 (57.5 %) patients. One risk factor was identified in 34 patients, two risk factors were identified in 10 patients, three risk factors were found in two patients, while in others risk factors were not identified. Family predisposition to cholelithiasis was registered in 28 (35%) patients, obesity in 10 (12.5%), pre-obesity in nine, rapid weight loss in four, hereditary hemolytic disease in two, and premature birth combined with parenteral nutrition and sepsis as a complication were found in two patients. Apart from those who were born prematurely, another 10 patients had combined risk factors for cholelithiasis, six patients had family predisposition and obesity, and four patients had obesity and self-initiated rapid weight loss program.

Conclusion: According to our research, the most common risk factors for cholelithiasis in children and adolescents are family predisposition and excess body weight. Most of the patients were adolescents and females.

Keywords: cholelithiasis, risk factors, children, adolescents

Cite this article as: Radlović V, Jovanović B, Leković Z, Dučić S, Radulović S, Đurićić G, Pavićević P, Janković J, Nikolić D, Radlović N. Risk factors in the occurrence of cholelithiasis in children and adolescents: a single-center experience; Medicinska istaživanja 2024; 57(2):1-5 DOI: 10.5937/medi57-47911

INTRODUCTION

Cholelithiasis is a rare disease in children and adolescents. According to literature data, it occurs in 0.13 to 0.22% of members of this age group (1-4). It occurs as a consequence of various etiopathogenetically clear pathological conditions or in idiopathic form (1). This condition is extremely rare prenatally and it can be revealed in the first years after birth, but it is usually diagnosed in late childhood and adolescence (2, 5, 6). With the onset of puberty, cholelithiasis is more common in girls than in boys (2). From a clinical point of view, it can be symptomatic and asymptomatic (1, 6, 7). Possible complications of the disease are cholecystitis, acute cholangitis, and pancreatitis (1, 6-12). Symptomatic cholelithiasis requires treatment, most often and most reliably cholecystectomy, while asymptomatic cholelithiasis, due to the possibility of spontaneous resolution, especially in infants, requires an appropriate follow-up (1, 6, 9). All modern guidelines recommend laparoscopic cholecystectomy as the treatment of symptomatic uncomplicated cholelithiasis (6, 13).

The aim of this study was to analyze risk factors for the occurrence of cholelithiasis in children and adolescents.

MATERIAL AND METHODS

This retrospective study included a group of 80 children and adolescents, 50 females and 30 males, aged 4-18 (14±5.55) years, with symptomatic cholelithiasis who had undergone cholecystectomy at the University Children's Hospital in Belgrade in the period from 2000 to 2016. Cholecystectomy was performed laparoscopically in 77 patients while three patients required open approach due to choledocholithiasis. The approval for conducting the study was obtained from the Institutional Review Board of the University Children's Hospital in Belgrade (the approval number: 017 16/48).

Diagnosis of the disease was based on the association of recurrent abdominal pain, characteristic for biliary colic, and ultrasound findings of echogenic foci in the gallbladder lumen or choledochus with acoustic shadowing.

Data related to risk factors in all patients concerning the occurrence of cholelithiasis were obtained from the available medical documentation, according to the objectives. Beside the anamnestic data of the presence of cholelithiasis in first and second degree relatives, personal medical history and clinical and laboratory findings of each patient were analyzed for the existence of additional factors that could cause this pathological condition. Those factors included being overweight (obesity and pre-obesity), a rapid loss of body weight, parenteral nutrition, congenital or acquired hemolytic disease, hepatobiliary diseases, premature birth, etc. The criteria for obesity, pre-obesity and rapid weight loss were determined. For obesity, it was body mass index (BMI) ≥95th percentile for age and sex, for pre-obesity the BMI ≥85th percentile but <95th percentile on the Centers for Disease Control and Prevention's (CDC) specific growth charts. The criterion for excessively fast weight loss was the loss of >1 kg per week (14, 15). In addition to adequate clinical indications and ultrasound findings, complete preoperative evaluation of the patients was done by using corresponding laboratory blood parameters, such as complete blood count, sedimentation rate, C-reactive protein, liver function tests, the level of amylase, lipase, as well as the levels of amylase and bile pigments in the urine. In the absence of hepatocellular damage in patients with unconjugated hyperbilirubinemia, the additional tests to verify or rule out a hemolytic state were the determination of the number of reticulocytes, the analysis of morphology of erythrocytes, Coombs's test and the measurement of osmotic resistance of erythrocytes.

RESULTS

Most patients were older than 10 years (90%) and females (62.5%). In 46 (57.50%) cases, cholelithiasis-predisposing risk factors were identified. In 34 (42.5%) patients there was one risk factor, in 10 patients (12.5%) there were two risk factors and in two patients there were three risk factors, while in the rest of the patients no risk factors were found **(Table 1)**. In the group of patients with more than one cholelithiasis-predisposing risk factor, six of them had an association of obesity and familial predisposition, four patients were obese and experienced too rapid weight loss, and two patients had premature birth (31 and 32 weeks of gestation), parenteral nutrition and sepsis. Four patients with rapid weight loss were adolescent girls who underwent an excessive hypocaloric diet on their own and without any professional supervision, while two patients had required parenteral nutrition due to prematurity, which had been complicated by sepsis as an additional cholelithiasis-risk factor.

Table 1. Frequency of risk factors for the occurrence of cholelithiasis in our group of patients (n= 80)

Risk factors	Frequency
Familial predisposition*	28 (35,0%)
Obesity and pre-obesity**	19 (23,75%)
Rapid loss of body weight	4
Hereditary hemolytic disease***	2
Premature birth	1
Parenteral nutrition	1
Sepsis	1

Notes: *First degree relatives 12, second degree relatives 16; **Obesity 10, pre-obesity 9;
***Spherocytosis 1, elliptocytosis 1

DISCUSSION

Cholelithiasis, a mixture of cholesterol, calcium salts of bilirubinate or palmitate, proteins, and mucin, is etiopathogenetically a very heterogeneous disease that occurs with varying frequency in all population groups and ages. It is much less common in children and adolescents than in adults (1-4, 16). Based on their composition, gallstones are classified into pigment stones, cholesterol stones, and mixed stones. Both in adults and in pediatric patients, their prevalence increases throughout life (2, 6, 8, 17, 18). Due to the cholelithiasis-favoring effect of estrogens, from puberty to the end of the reproductive period, it is significantly more common in women than in men (2, 4, 6, 17). According to some studies in humans and animals, the basis of this phenomenon lies in the fact that estrogens have such an effect on the liver that it produces supersaturation of bile with cholesterol. This is due to simultaneous increase in its secretion while the synthesis of bile acids is decreased (7, 19, 20, 21). These facts are also true for the group of our respondents, in which the ratio of representation of children over and under ten years of age was 9 vs 1, and the representation of girls compared to boys was 1.7 vs 1.

Apart from the mentioned factors, important risk factors for the occurrence of cholelithiasis are family predisposition and various diseases accompanied by destabilization of the solubility of biliary contents, such as obesity, hemolytic diseases, Crohn's disease, ileal resection, cystic fibrosis, hepatobiliary diseases, hyperlipidemia, insulin resistance and others (2, 6, 7, 19, 23, 24, 25). Additional risk factors for biliary stone formation are excessively fast weight loss, prolonged parenteral nutrition, and systemic infections (2, 6, 8, 19, 23). In a significant number of cases, several factors are involved in the etiopathogenesis of cholelithiasis (8). Also, in many cases, the cause of the occurrence of cholelithiasis remains unknown (8, 9). Some of the risk factors for the occurrence of cholelithiasis were registered in slightly more than half of our respondents, out of which a quarter had two or three risk factors. One third of the patients had first or second degree relatives with cholelithiasis, a quarter were obese or pre-obese, while in a smaller number of them cholelithiasis-predisposing factors included too rapid weight loss, hereditary hemolytic disease and association of premature birth, parenteral nutrition and sepsis. Family history and obesity play a critical role in the incidence of gallstone disease (4, 20). Genetic factors account for the occurrence of approximately 25%–30% of symptomatic gallstones (4, 19). Familial predisposition to gallstone disease is linked to variations (polymorphism) or mutations of genes that encode regulatory proteins responsible for biliary cholesterol solubility, such as ATP-binding cassette transporters ABCG5, ABCG8 and ABCB4 and farnesoid X receptor (19). Coding genes for ABCG5 and ABCG8 transporters, responsible for hepatobiliary cholesterol secretion, are located on chromosome 2p21, for ABCB4 transporter, the regulator of hepatic phospholipid secretion, on chromosome 7q21.12 and for farnesoid X receptor, the inducer of reabsorption of bile acids, on chromosome 12q23.1. (7, 19, 20, 21, 26-29). Also, it is thought that alteration of mucin gene expression (11p15.5) is involved in the pathogenesis of gallstone formation (30). Being overweight (obesity and pre-obesity), as a highly prevalent and continuously growing health problem of modern man, is also a frequent risk factor for biliary lithogenesis (7, 13, 21). The disturbance of the solubility of biliary content in obese and pre-obese patients is a consequence of hypersaturation of bile caused by either increased hepatic cholesterol uptake or increased de novo cholesterol synthesis (4, 7). This especially applies to the central (abdominal or visceral) type of obesity, which is characterized by pronounced insulin resistance and the consequent high hepatic cholesterol secretion (21). The basis of biliary lithogenesis during rapid weight loss on very low-calorie diets is cholesterol supersaturation of bile due to its mobilization from adipose tissue and cholecystic hypomotility as a consequence of reduced cholecystokinin stimulation (7, 13, 21, 24). Cholestasis due to insufficient cholecystokinin induced gallbladder contraction and accompanying hyperconcentration of bile is the cause of biliary lithiasis as a complication of prolonged total or subtotal parenteral nutrition (5, 24). The pathogenesis of gallstone formation in premature infants is of a multifactorial nature and is a consequence, not only of the necessity of prolonged parenteral nutrition, as well as often accompanying catheter related recurrent sepsis, i.e. hemolysis and bacterial translocation and cholangitis, but also of immaturity, reduced gallbladder motility and the bile acid enterohepatic circulation (5, 6, 23, 31-35). Gallstones in hemolytic diseases are the result of an excess of unconjugated bilirubin in the bile, which binds with calcium and builds up insoluble calcium bilirubinate (6, 23, 24).

CONCLUSION

Slightly more than half of children and adolescents with cholelithiasis have some of the known risk factors for its occurrence, and of those two thirds have one risk factor whereas one third have two or three risk factors. The most common cholelithiasis risk factors, which are found in around half of the patients, are family predisposition and/or excess body weight. Nine tenths of the patients are adolescents and two thirds of them are females.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Ethical approval

This research and publication were approved by the Ethical Committee of the University Children's Hospital, Belgrade, Serbia (approval number: 017 16/48).

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FAKTORI RIZIKA ZA POJAVU KAMENA U ŽUČNOJ KESI KOD DECE I ADOLESCENATA: ISKUSTVO JEDNOG CENTRA

Vladimir Radlović[1,2], Branislav Jovanović[1,2], Zoran Leković[1,2], Siniša Dučić[1,2], Spasoje Radulović[1], Goran Đurićić[1,2], Polina Pavićević[1,2], Jovana Janković[1], Dejan Nikolić[1,2], Nedeljko Radlović[3]

Sažetak

Uvod: Holelitijaza je etiopatogenetski veoma heterogena i po učestalosti retka bolest u periodu rasta i razvoja. Cilj rada bio je da se analiziraju faktori rizika za nastanak holelitijaze u tom životnom dobu.

Materijal i metode: Ovom retrospektivnom studijom obuhvaćeno je 80 dece i adolescenata, 50 ženskog i 30 muškog pola, uzrasta 4-18 (14±5,55) godina, sa simptomatskom holelitijazom operisanih u Univerzitetskoj dečjoj klinici u Beogradu u periodu od 2000. do 2016. godine. Dijagnoza bolesti postavljana je na osnovu ultrazvučnog nalaza. Podaci o faktorima rizika za nastanak bilijarne kalkuloze dobijeni su iz istorije bolesti.

Rezultati: Kod 46 (57,5%) bolesnika verifikovani su faktori rizika koji predisponiraju pojavu holelitijaze. Kod 34 pacijenta je identifikovan jedan faktor rizika, kod 10 pacijenata dva faktora rizika, kod dva pacijenta su identifikovana tri faktora rizika, dok kod ostalih nisu pronađeni faktori rizika. Porodična sklonost holelitijazi registrovana je kod 28 (35%) pacijenta, gojaznost kod 10 (12,5%), predgojaznost kod devet, prebrzo mršavljenje kod četiri, nasledna hemolizna bolest kod dva, i prevremeno rođenje kombinovano sa parenteralnom ishranom i sepsom kao komplikacijom kod dva. Osim prevremeno rođenih, još 10 pacijenata je imalo udružene faktore rizika za holelitijazu, šest pacijenata je imalo porodičnu predispoziciju i obezitet, a četiri pacijenta obezitet i samoinicijativno započet program brzog gubitka telesne težine.

Zaključak: Prema našim istraživanjima, najčešći faktori rizika za pojavu holelitijaze kod dece i adolescenata su porodična predispozicija i povišena telesna težina. Većina pacijenata pripadala je adolescentnom uzrastu i bila je ženskog pola.

Ključne reči: holelitijaza, faktori rizika, deca, adolescenti

Primljen: 28.11.2023. I **Revizija:** 04.01.2024. I **Prihvaćen:** 10.04. 2024

Medicinska istaživanja 2024; 57(2):1-5

Medicinska istaživanja 2024; 57(2):7-12 | DOI: 10.5937/medi57-45261

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Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Esophagogastroduodenoscopy findings in patients with dyspepsia

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Recived: 30 June 2023
Revised: 03 January 2024
Accepted: 01 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction/Aim: Dyspepsia is a set of symptoms of the gastrointestinal tract that includes epigastric pain or burning, postprandial fullness or early satiety. These symptoms may or may not be associated with pathological changes in the mucosa. Esophagogastroduodenoscopy (EGD) is an invasive diagnostic tool for detecting pathological changes in the mucosa of the upper digestive tract. The aim of this study was to detect how many patients with dyspepsia older than 55 years who had undergone esophagogastroduodenoscopy had a pathological finding and determine the validity of invasive diagnostics in patients with dyspepsia.

Material and methods: This cross-sectional study included 148 patients who underwent EGD in the period from June to December 2021 due to various indications (dyspepsia, vomiting, anemia, positive fecal occult blood test (FOBT), suspected portal hypertension, body weight loss, reflux disorders and melena) of which we considered only dyspepsia in patients older than 55 years.

Results: In the group of patients over 55 years with dyspepsia (n = 61), 8 patients (13.1%) had a normal finding of EGD, defined as the absence of endoscopic and pathohistological changes in the mucosa. One patient (1.6%) had a normal EGD finding, with the presence of Helicobacter pylori. As for pathological endoscopic findings, 24 (39.3%) patients had chronic gastritis and 13 (21.3%) had chronic gastritis in the presence of H. pylori, 19 (31.1%) had signs of gastroesophageal reflux disease (GERD), and 3 (4.9%) had gastric ulcer. The presence of alarming symptoms was detected in 22.9% of patients, however, the findings of upper endoscopy ruled out the existence of gastric cancer.

Conclusion: Based on our results, we can conclude that in patients with dyspepsia older than 55 years, pathological findings in EGD are very frequent, which proves the benefit of using this diagnostic method is great, but the possibility of a successful empirical therapy should be also taken into account.

Key words: esophagogastroduodenoscopy, dyspepsia, functional dyspepsia

Cite this article as: Vasiljević I, Mijač D, Filipović B, Arsenović M, Bunijevac S, Lukić S. Esophagogastroduodenoscopy findings in patients with dyspepsia; Medicinska istaživanja 2024; 57(2):7-12 DOI: 10.5937/medi57-47911

INTRODUCTION

Dyspepsia is a set of symptoms of the gastrointestinal tract that includes epigastric pain or burning, postprandial fullness or early satiety. It is a disorder with a high prevalence of approximately 20% in the general population (1). Dyspepsia can occur without an underlying organic disease that can cause the symptoms, in which case it can be labelled as functional dyspepsia (FD) (2). The pathophysiology of FD is not completely clear, but it could be related to changes in gut motility, immune changes of the mucosa, visceral hypersensitivity, alterations in gastrointestinal microbiota and altered central nervous system processing (3,4). According to Rome IV criteria functional disorders of the digestive system are now called disorders of the gut-brain interaction (DGBI) (5, 10). Rome IV criteria also divide functional dyspepsia into two subgroups: epigastric pain syndrome and postprandial distress syndrome in which symptoms are triggered by a meal (2). The two subgroups can overlap, but following the criteria, in order to diagnose functional dyspepsia, an organic disease must be excluded by upper gastrointestinal endoscopy (1). Organic dyspepsia is most commonly caused by peptic ulcer, gastritis or esophageal and gastric malignancy (3, 10). Gastric cancer is the fourth most common incident cancer and the second most common cause of cancer-related death, with Serbia being a country with a high prevalence of gastric cancer (6, 7). Esophagogastroduodenoscopy (EGDS) is a basic diagnostic procedure for detecting pathological changes in the mucosa of the upper part of the digestive tract. EGDS is an invasive diagnostic procedure, which in a certain, small percentage, carries the risk of perforation or death. In order to ensure greater patient comfort during EGDS, in patients who cannot tolerate the examination, short-term intravenous analgosedation is applied, which carries its own risks for the patient (8). Esophagogastroduodenoscopy (EGDS) is a diagnostic procedure that is indicated in the presence of dyspeptic complaints in patients older than 55 years, patients refractory to previously introduced antacid and proton pump inhibitor therapy, as well as in the presence of alarming symptoms such as weight loss, vomiting, signs of gastrointestinal bleeding and iron deficiency anemia (9-11). Dyspeptic symptoms are among the most common indications for EGDS.

In our cross-sectional study patients underwent EGDS for various indications (dyspeptic symptoms, vomiting, anemia, positive fecal occult blood test (FOBT), suspected portal hypertension, weight loss, reflux complaints and melena) and the aim was to determine how many patients over 55 years of age with dyspeptic symptoms had a pathological finding in EGDS, as well as to determine validity of subjecting patients with dyspepsia to invasive diagnostics.

MATERIAL AND METHODS

Subjects and methods

This is a cross-sectional study, which was conducted using the information from the database of the Clinic for Gastroenterohepatology of the University Clinical Center of Serbia. The study included 148 patients who, in the period from June to December 2021, underwent esophagogastroduodenoscopy with a routine biopsy of the gastric mucosa taken for pathohistological analysis. Demographic data (age and gender), indications (dyspeptic symptoms, vomiting, anemia, positive stool occult blood test (FOBT), suspected portal hypertension, weight loss, reflux complaints and melena) and endoscopic findings were collected on the basis of these patients' medical records. Endoscopic findings were labelled as normal, normal finding with the presence of Helicobacter pylori, H. pylori gastritis, chronic gastritis without H. pylori infection, esophageal varices, gastroesophageal reflux disease (GERD), ulcer disease, and cancer. Normal finding was defined as mucosa with no endoscopic and pathohistological changes in terms of gastritis, ulcers or cancer. The existence of H. Pylori infection and the definitive confirmation of cancer were obtained on the basis of pathohistological findings. When considering the frequency of certain endoscopic findings in patients who had dyspeptic symptoms as an indication, only patients older than 55 years were considered, because most clinical practice guidelines find their subjection to EGDS justified, given that they belong to the greater risk group for gastric cancer, which is a life-threatening condition.

Statistical analysis

The data were entered into the database and processed using the IBM SPSS 25 program. The methods of descriptive and analytical statistics were used. From the methods of descriptive statistics, the following were used: measures of central tendency, measures of variability, and relative numbers, and from the methods of analytical statistics, the methods for assessing the significance of the difference were used: Chi-square test for categorical data and Student's t-test for numerical data. $p<0.05$ was considered statistically significant.

RESULTS

The study included 148 patients, predominantly female (62.2%). The average age was 58.9±15.5 years, where 44 (29.7%) were in the category of patients under 55 years of age, and 104 (70.3%) in the category of patients over 55 years of age **(Figure 1)**.

Figure 1. Demographic characteristics of patients

Variable	n (%)
Gender	
Male	56 (37,8)
Female	92 (62,2)
Age, mean±sd	58,9±15,5
<55 years	44 (29,7)
≥55 years	104 (70,3)

In the group of patients over 55 years of age, 61 (58.7%) patients had dyspeptic symptoms. A statistically significant difference between gender and dyspeptic symptoms was observed, whereby women had dyspeptic complaints significantly more often than men, which could be expected, given that most respondents were female (p=0.008). Statistically significant difference in age was not found between patients with and without dyspeptic symptoms (p>0.05) **(Figure 2)**.

Figure 2. Dyspeptic symptoms and demographic characteristics of patients

Variable	Dyspeptic symptoms		p
	No (n=43)	Yes (n=61)	
Gender, n%			
Male	23 (53,5)	17 (27,9)	
Female	20 (46,5)	44 (72,1)	**0,008**
Age, mean±sd	68,6±6,7	66,7±6,6	0,149

In the group of patients over 55 years of age with dyspeptic symptoms as an indication for EGDS (n=61), 8 patients (13.1%) had a normal EGDS finding, and 1 patient (1.6%) had a normal finding with the presence of H. pylori detected during the pathohistological examination of the biopsy sample of the gastric mucosa. From other pathological endoscopic findings, chronic gastritis was detected in 24 (39.3%) patients, and chronic gastritis with the presence of H. pylori was found in 13 (21.3%) patients. Changes corresponding to gastroesophageal reflux disease (GERD) were found in 19 (31.1%) patients, and gastric or duodenal ulcer in 3 (4.9%). None of the patients who had dyspeptic symptoms as an indication had esophageal varices or gastric cancer on EGDS. In patients without dyspeptic complaints, who underwent EGDS, esophageal varices and chronic gastritis were diagnosed more often than in patients with dyspeptic complaints, which was statistically significant (p=0.004 for esophageal varices, p=0.019 for chronic gastritis) **(Figure 3)**.

Figure 3. Frequency of endoscopic findings in patients with dyspepsia

Variable	Dyspeptic symptoms		p
	No (n=43)	Yes (n=61)	
Normal, n%	1 (2,3)	8 (13,1)	0,077
Normal with H. pylori, n%	1 (2,3)	1 (1,6)	1,000
Esophageal varices, n%	6 (14,0)	0 (0,0)	**0,004**
Chronic gastritis, n%	27 (62,8)	24 (39,3)	**0,019**
H. pylori gastritis, n%	8 (18,6)	13 (21,3)	0,735
GERD, n%	13 (30,2)	19 (31,1)	0,921
Gastric or duodenal ulcer, n%	2 (4,7)	3 (4,9)	1,000
Gastric cancer, n%	2 (4,7)	0 (0,0)	0,169

We also examined the presence of alarming symptoms and signs that could indicate gastric cancer, which were listed as a simultaneously present indication with dyspeptic symptoms in patients over 55 years old. Vomiting was present in 2 (3.3%) patients, weight loss in 3 (4.9%), iron deficiency anemia in 6 (9.8%) patients, and signs of gastrointestinal bleeding in the form of melena in 2 patients (3 .3%) and in the form of a positive FOBT in 1 (1.6%) patient. However, despite the presence of alarming symptoms, none of the patients had gastric cancer **(Figures 3 and 4)**.

Figure 4. Frequency of other indications of importance with dyspeptic symptoms

Variable	Dyspeptic symptoms (n=61)
Vomiting, n%	2 (3,3)
Iron deficiency anemia, n%	6 (9,8)
FOBT, n%	1 (1,6)
Weight loss, n%	3 (4,9)
Melena, n%	2 (3,3)
Total	14 (22,9)

DISCUSSION

In our study organic dyspepsia in the group of patients over 55 showed a high prevalence of 96.7% including: chronic gastritis (39.3%), H. pylori gastritis (21.3%), signs of GERD (31.1%), and ulcer disease (4.9%) (Figure 3). EGDS is an important diagnostic tool for detection of organic diseases that can cause dyspeptic symptoms, among which is gastric cancer that represents a life-threatening condition (6, 9). That is the reason why many guidelines suggest that subjecting patients over 55 years of age to EGDS is appropriate since that is a group in risk of developing gastric cancer (9, 19). Our study included 104 patients over 55 years of age and 61 of them had dyspeptic symptoms but none of them had cancer, which corresponds to a low prevalence of upper gastrointestinal malignancy in dyspeptic patients found in other studies (10). Besides years of age, the presence of so-called alarm symptoms such as weight loss, vomiting, signs of gastrointestinal bleeding or iron deficiency anemia in patients with dyspepsia may indicate an underlying gastric cancer, so these patients should undergo EGDS according to guidelines such as ASGE (American Society for Gastroenterology) or NICE (The National Institute for Health and Care Excellence) guidelines (8, 9, 14). In our study, 22.4% of patients in addition to dyspeptic symptoms as an indication for EGDS had one of the alarm symptoms, but cancer was not diagnosed in any of these patients (Figures 3 and 4). The revised contemporary guidelines suggest that the need for endoscopy should be determined for each patient depending on the patient's physical findings, history (family, disease, tests), and other relevant factors which could eliminate unnecessary EGDS (10, 12). Gastric cancer can often be asymptomatic in the early stages

of the disease, so alarming symptoms as well as dyspeptic complaints appear only in advanced disease (13). In the results of our study, the only case of gastric cancer was detected in the group of patients older than 55 years without dyspeptic complaints (Figure 3). The evidence of the effectiveness and cost-effectiveness of mass endoscopic screening remains controversial in the countries with the population at average risk of gastric cancer, but we should have in mind that around 60% of patients, due to the late presentation of non-specific symptoms and comorbidities, have an extremely poor prognosis (14, 15). However, research shows that globally there is a decline in the incidence and mortality of stomach cancer, and this can also be seen in the data for Central Serbia in the period from 2000 to 2015 (7, 16). This trend could be related to an effective approach in the eradication of H. pylori infection (test and treat strategy), which is an important risk factor for the development of gastric cancer (17, 20). There is a significant decline in the prevalence of this infection globally, especially in the period from 2011 to 2022 (18). The results of our studies correlate with these data because the prevalence of H. pylori infection in patients with dyspepsia was 22.9%, of which 21.3% patients had chronic gastritis with H. pylori infection and 1.6% had normal EGDS findings with H. pylori infection, while the prevalence of H. pylori negative chronic gastritis was 39.3%. However, the presentation of H. pylori negative chronic gastritis was higher (62.8%) in the group of patients without dyspepsia complaints compared to the one with dyspepsia (Figure 3). Considering that around 65% patients with dyspepsia had functional dyspepsia, that could be an explanation for such results of our studies (3, 19). The results of EGDS were normal in 9 (14.7%) patients with dyspepsia, and in one patient H. pylori infection was detected from a routine biopsy sample of the mucosa taken during EGDS (Figure 3). According to this, functional dyspepsia was found in 14.7% of patients. The etiology of FD is not fully understood, but some of the risk factors are known to be acute gastroenteritis, the use of non-steroidal anti-inflammatory drugs, psychological comorbidities such as anxiety; smoking, female gender, and H. pylori infection (1). In our research, the total number of female respondents was higher, and consequently, dyspeptic complaints occurred statistically significantly more often in women than in men (p=0.008). However, if we consider the results of other studies, in which the female gender is one of the risk factors for FD, as well as the fact that the male gender is two times more susceptible to the development of gastric cancer, it could be taken into consideration to perform endoscopy in men older than 55 years more often than in women (1, 6). Of course, this assumption would have to be tested on a larger number of patients.

The Kyoto consensus suggests that dyspepsia with H. pylori infection should be taken as a separate entity distinct from functional dyspepsia (20). That is of great importance because in these patients using "test and treat" strategy has proven to be most effective (20, 21). This strategy includes therapy with proton pump inhibitors, prokinetic agents and eradication therapy for H. pylori, whereby infection with this bacterium can also be detected by one of the non-invasive methods such as the 13C-urea breath test or the detection of bacterial antigens in feces (2, 20). In our study there was a total of 23 patients with H. pylori infection, and 14 of them had dyspepsia (60,8%) (Figure 3). Every patient with dyspeptic symptoms should be tested for H. pylori (non-invasively or by gastroscopy) because H. pylori causes approximately 90% of all gastric cancer cases worldwide, excluding those located at the gastroesophageal junction, and the eradication therapy can be very effective (10, 21).

CONCLUSIONS

In this study, we showed the frequency of different findings in EGDS in patients who had dyspepsia complaints as an indication for undergoing this invasive diagnostic procedure. Based on our results, we can conclude that in patients with dyspepsia older than 55 years, pathological findings in EGDS occur with a high frequency (96.7%). Arguably, this proves that the benefit of subjecting patients to this diagnostic procedure is great. However, the possibility of successful empirical therapy within the "test and treat" strategy should also be taken into account, since it has been proven to control symptoms in most patients with dyspepsia.

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EZOFAGOGASTRODUODENOSKOPSKI NALAZ KOD PACIJENATA SA DISPEPSIJOM

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Sažetak

Uvod/cilj: Dispepsija predstavlja skup simptoma gastrointestinalnog trakta koji obuhvata bol ili osećaj žarenja u epigastrijumu, postprandijalnu nadutost i osećaj ranog zasićenja prilikom obroka. Ovi simptomi mogu i ne moraju biti povezani sa patološkim promenama na sluznici. Ezofagogastroduodenoskopija (EGDS) je invazivna dijagnostička metoda za otkrivanje patoloških promena sluznice gornjih partija digestivnog trakta. Cilj rada je da se kroz studiju preseka koja uključuje ispitanike sa dispeptičnim tegobama starije od 55 godina podvrgnute ezofagogastroduodenoskopiji detektuje učestalost patološkog nalaza i utvrdi opravdanost podvrgavanja ovih pacijenata invazivnoj dijagnostici.

Materijal i metode: Ovom studijom preseka obuhvaćeno je 148 pacijenata koji su u periodu od juna do decembra 2021. godine bili podvrgnuti EGDS zbog različitih indikacija (dispeptične tegobe, povraćanje, anemija, pozitivan test na okultno krvarenje u stolici (FOBT), suspektna portna hipertenzija, gubitak telesne mase, refluksne tegobe i melena), a od kojih smo u razmatranje uzeli samo dispeptične tegobe kod pacijenata starijih od 55 godina i sagledali njihove endoskopske nalaze.

Rezultati: U grupi pacijenata preko 55 godina sa dispeptičnim tegobama kao indikacijom (n=61), 8 pacijenata (13,1%) je imalo normalan nalaz na EGDS, koji je definisan kao odsustvo endoskopskih i patohistoloških promena na sluznici. Jedan pacijent (1,6%) je imao normalan nalaz na EGDS, uz prisustvo Helicobacter pylori tokom patohistološkog pregleda. Od patoloških endoskopskih nalaza kod 24 (39,3%) pacijenta otkriven je hronični gastritis, kod 13 (21,3%) hronični gastritis uz prisustvo H. pylori, kod 19 (31,1%) znaci gastroezofagealne refluksne bolesti (GERB) i kod 3 (4,9%) ulkus želuca. Kod 22,9% pacijenata detektovano je prisustvo alarmnih simptoma, međutim nalaz gornje endoskopije isključio je postojanje karcinoma želuca, što je i histopatološki potvrđeno.

Zaključak: Na osnovu naših rezultata možemo zaključiti da se kod pacijenta sa dispepsijom starijih od 55 godina patološki nalazi na EGDS javljaju sa velikom učestalošću, što dokazuje da je korist od podvrgavanja pacijenata ovoj dijagnostičkoj proceduri velika, ali u obzir treba uzeti i mogućnost uspešnosti empirijske terapije.

Ključne reči: ezofagogastroduodenoskopija, dispeptične tegobe, funkcionalna dispepsija

Primljen: 30.06.2023. I **Revizija:** 03.01.2024. I **Prihvaćen:** 01.04. 2024

Medicinska istaživanja 2024; 57(2):7-12

ORIGINAL ARTICLE

Methodological diversity in micro-CT evaluation of bone micro-architecture: importance for inter-study comparability

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Recived: 29 August 2023

Revised: 10 December 2023

Accepted: 10 December 2023

Check for updates

Funding information:
This study received financial support from the Science Fund of the Republic of Serbia (IDEAS program, grant no. 7749444, BoFraM project) and the Ministry of Science of the Republic of Serbia (grant no. 200110 and 451-03-1524/2023-04/18).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Micro-computed tomography (micro-CT) is a standard 3D technique for non-destructive analysis of bone micro-architecture. Although there have been many micro-CT studies in contemporary literature, inter-study comparability is often challenging due to a lack of methodological standardization, particularly regarding human bone analyses.

Aim: This study aimed to assess the consistency of micro-CT generated micro-architectural parameters obtained by two researchers (inter-observer reliability), by one researcher in two attempts (intra-observer reliability), as well as between manual and semi-automatic determination of the region of interest (ROI).

Material and methods: Superolateral femoral neck samples (n=8) were scanned with Bruker 1172 micro-CT system with a voxel size of 10 μm. We manually determined cortical and trabecular ROI (two authors, two attempts with a 45-day span). Also, trabecular ROI was determined using a semi-automatic method (round-shaped ROI with 6.5 mm diameter).

Results: The intraclass correlation coefficient (ICC) showed a high degree of consistency in the measurement of micro-architectural parameters of the superolateral femoral neck using the micro-CT (ICC range: 0.721-0.998; $p<0.05$). However, a detailed analysis revealed significant inter-observer and intra-observer differences, predominantly reflected in cortical porosity parameters (Student's *t*-test for dependent samples, $p<0.05$). On the other hand, the choice of ROI did not significantly affect trabecular micro-architectural parameters among researchers and between manual and semi-automatic demarcation methods (Student's *t*-test for dependent samples, $p>0.05$).

Conclusion: Our study emphasizes the importance of standardizing the methodology used in micro-CT evaluations of human bone samples, which could facilitate reliable inter-study comparison and ensure an adequate interpretation of results.

Keywords: Micro-CT, femoral neck, region of interest, bone micro-architecture, human

Cite this article as: Andjelic U, Djuric M, Jadzic J. Methodological diversity in micro-CT evaluation of bone micro-architecture: importance for inter-study comparability; Medicinska istaživanja 2024; 57(2):13-21 DOI: 10.5937/medi57-46221

INTRODUCTION

Micro-computed tomography (micro-CT) is based on sequential X-ray scanning of limited-size samples, usually resulting in images of transversal sections that can detect many small details [1]. As such, micro-CT opened up new possibilities for advanced analysis of mineralized and non-mineralized tissues in a non-destructive manner [1,2]. To analyze bone tissue, researchers predominantly used histomorphometric analysis of trabecular bone samples obtained by transiliac biopsy, which still represents the "gold standard" for clinical evaluation of bone status [3]. However, the preparation and procession of bone samples for histomorphometry has some disadvantages (for example, two-dimensionality, dependence on researchers' experience, and tissue destruction that makes it impossible to evaluate bone tissue using different methods) [4]. For all thise reasons, micro-CT has been increasingly described in contemporary literature as the method of choice for animal and human bone analyses [5].

Initially, micro-CT was used to calculate the mineralized bone ratio in an examined sample [6]. After that, the consistency of trabecular micro-architectural results obtained by histomorphometry and micro-CT was shown [1,7]. Soon enough, the importance of using micro-CT for the analysis of cortical bone was noted (which is of great significance in overall bone fragility), confirming comparability of the results obtained with micro-CT and histomorphometry [5,8]. Being a promising method, micro-CT has been applied in numerous human [9,10] or animal bone studies [11–14]. Considering the abundance of previous animal micro-CT studies, there was a need to compare the obtained results. This raised concerns about the validity of conclusions derived from these comparisons, considering different sample processing methodologies used in these studies. Therefore, protocols for semi-automatic differentiation of animal cortical and trabecular compartments were developed (with some persistent shortcomings), enabling a more reliable inter-study comparison using uniform methodological approaches [15–17]. On the other hand, studies conducted on human bone samples show significantly less uniformity in the micro-CT methodology of trabecular and cortical micro-architecture analyses. This indicates the necessity for standardization of micro-CT methodology to achieve well-grounded conclusions derived from comparing numerous human studies, especially in femoral studies, since these fractures are most severe in aged individuals [18].

This study aimed to determine the reliability of micro-CT derived micro-architectural femoral parameters obtained by two researchers (inter-observer reliability), by one researcher in two attempts (intra-observer reliability) as well as between manual and semi-automatic region of interest (ROI) determination.

MATERIALS AND METHODS

Material sampling for microstructural analysis

It has been shown that the trabeculae of the superolateral femoral neck represent the fracture-initiating site [19], while its cortex suffers the most significant forces during a side fall [20], so the reproducibility of the analysis of this part of the skeleton is of great importance. For this reason, we analyzed eight proximal femora collected from institutional osteological collection. The criteria for including samples in the study were as follows: fully preserved dry proximal left femora of an adult individual, without visible changes (e.g., cortical surface erosion, tumor-like masses, osteolytic changes, etc.) that would suggest the existence of structural bone damage.

Given that the micro-CT method cannot scan the entire human femur, samples of the superolateral part of the femoral neck were extracted **(Figure 1)** using Oscillating Autopsy Saw (HB-740-Accu-250, Kugel, Germany). The samples were then cleaned in an ultrasonic bath (SONOCOOL 255, Bandelin, Germany) and air-dried for at least two weeks. Finally, the samples were scanned using micro-CT system (1172 SkyScan, Bruker, Belgium).

Scanning of samples using the micro-CT method

To scan the transcervical region of the superolateral femoral neck, the samples were placed in the scanning chamber using identical sample orientation (basicervical parts facing the holder, cortical surface set perpendicular to the imaging camera). Orthodontic wax was used to attach the samples to the holder and prevent sample movement. The samples were scanned in dry conditions, using the following parameters: 80 kV, 124 μA, 10W, exposure time 1220 μs, voxel size of 10 μm, and Aluminium-Copper filter [10]. After scanning, the reconstruction of the projection images was made using NRecon software (version 1.6.9.8, Bruker, Belgium) accelerated with InstaRecon CBR software (2.0.2.1 version, InstaRecon, Illinois, USA). The reconstruction parameters were as follows: beam hardening correction of 25%, ring artefact correction of 5, smoothing correction of 2, and autogenerated compensation for thermal drift and misalignment.

Using the updated version of the microstructural analysis software (CT.An 2020, Bruker, Belgium), all samples were standardized and marked so that the central 60% of the superolateral femoral neck's length (transcervical region of the neck) was analyzed **(Figure 1)**. The total length of the analyzed volume of interest (VOI) of trabecular and cortical bone was 1101 sections (central section ± 550). Using manually adjusted two-dimensional regions of interest (ROIs), two investigators independently marked trabecular and cortical VOIs. After 45 days, the two researchers repeated the manual ROI determination. Researchers consistently followed the rule that margin-

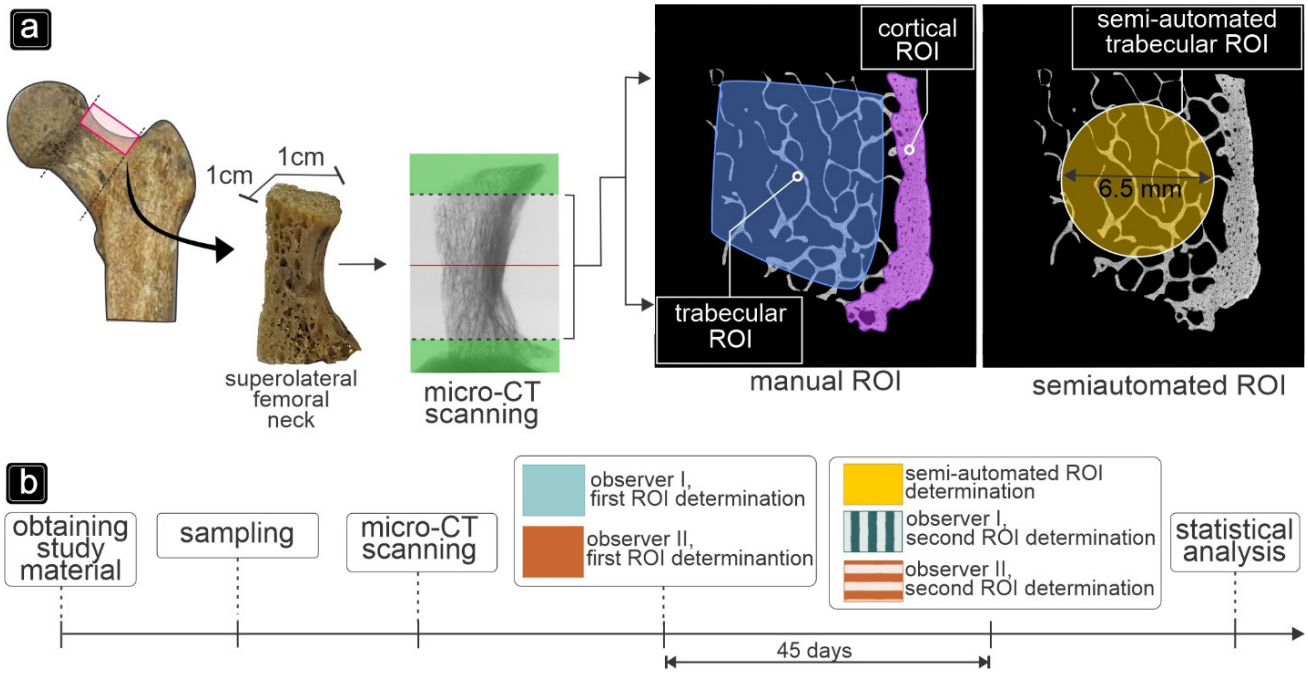

Figure 1. The schematic representation of the used methodology (a) and flow chart of the present study (b)
Abbreviations: Micro-CT – micro-computed tomography; observer I – early-career researcher; observer II – experienced researcher; ROI – region of interest.

alized damaged bone tissue was excluded from manually adjusted ROIs. For semi-automatic trabecular ROI determination, a centrally positioned round-shaped ROI was used (diameter: 6.5mm; **Figure 1**). Using CT.An 2020 software, the analysis of trabecular and cortical micro-architecture parameters was performed after segmenting the mineralized (greyscale value levels ranging from 95 to 255) and non-mineralized parts of the sample (greyscale value levels below 95), which was consistently used for all analyzed samples. The following parameters were generated and analyzed: cortical tissue volume (Ct.TV), cortical tissue surface (Ct.TS), cortical thickness (Ct.Th), total cortical porosity (Po.tot), closed cortical porosity (Po.cl), open cortical porosity (Po.op), total cortical porosity volume (Po.V.tot), closed cortical porosity volume (Po.V.cl), open cortical porosity volume (Po.V.op), cortical pore diameter (Po.Dm), cortical pore separation (Po.Sp), trabecular tissue surface (Tb.TS), trabecular tissue volume (Tb.TV), trabecular bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), connectivity density (Conn.Dn), degree of anisotropy (DA), fractal dimension (FD) and structural model index (SMI).

Statistical data analyses

The Kolmogorov-Smirnov test was used to assess the data distribution normality. The intraclass correlation coefficient (ICC) was used to evaluate the consistency of the results obtained by two researchers, as well as the results of semi-automatic and manual trabecular ROI determination [21]. Student *t*-test for dependent samples was used to evaluate the significance of the difference between researcher results (inter-observer differences), the difference of the results obtained during two ROI determinations by the same researcher (intra-observer differences), and the difference of results obtained through manual and semi-automatic ROI determination. Open-source statistical software (Easy R on R Commander, EZR) was used for statistical analyses with a significance level of 5% and a confidence interval of 95%.

RESULTS

Intra-observer differences in ROI determination

By comparing two manual ROIs marked in 45-day span, observer I (early-career researcher) marked a larger ROI during the second attempt, which was manifested by a larger cortical volume (Ct.TV, p=0.047; **Table 1**). As presented in Table 1, this ROI choice resulted in a statistically significantly higher Po.tot, Po.V.tot, Po.op, Po.V.op, Po.cl and Po.V.cl values (p=0.005, p=0.017, p =0.005, p=0.017, p=0.017, p=0.051, respectively). In contrast, the results of the second ROI determination by observer II (experienced researcher) only showed a trend towards the selection of a smaller volume of the cortex (Ct.TV, p=0.047), which resulted in a marginal decrease in the cortical pore separation (Po.Sp, p=0.041) **(Table 1)**. Unlike cortical bone, trabecular micro-architecture did not show a statistically significant difference between the two measurements by both observers (p>0.05, **Table 1**).

Table 1. Intraobserver differences in trabecular and cortical micro-architectural parameters obtained by micro-CT

Parameters	Observer I 1st attempt (mean± SD)	Observer I 2nd attempt (mean± SD)	(*p*)	Observer II 1st attempt (mean± SD)	Observer II 2nd attempt (mean ± SD)	(*p*)
Ct.TS (mm^2)	339.73 ± 61.24	329.34 ± 52.99	0.401	355.94 ± 47.17	365.23 ± 51.42	**0.050**
Ct.TV (mm^3)	95.86 ± 32.46	109.13 ± 24.33	**0.047**	107.42 ± 27.68	106.93 ± 27.28	**0.047**
Ct.Th (mm)	0.29 ± 0.12	0.29 ± 0.13	0.158	0.30 ± 0.14	0.30 ± 0.14	0.302
Po.Dm (mm)	0.35 ± 0.13	0.39 ± 0.15	0.067	0.19 ± 0.06	0.20 ± 0.06	0.466
Po.Sp (mm)	0.27 ±0.08	0.27 ± 0.07	0.238	0.27 ± 0.06	0.28 ± 0.08	**0.041**
Po.tot (%)	21.95 ± 11.60	26.23 ± 11.62	**0.005**	18.99 ± 10.43	18.78 ± 10.36	0.450
Po.cl (%)	0.34 ± 0.23	0.36 ± 0.24	**0.017**	0.37 ± 0.24	0.37 ± 0.24	0.525
Po.op (%)	21.67 ± 11.67	25.96 ± 11.68	**0.005**	18.69 ± 10.48	18.49 ± 10.41	0.451
Po.V.tot (mm^3)	19.11 ± 7.99	27.03 ± 9.26	**0.017**	18.44 ± 6.99	18.11 ± 6.85	0.209
Po.V.cl (mm^3)	0.26 ± 0.18	0.30 ± 0.22	0.051	0.33 ± 0.23	0.33 ± 0.23	0.251
Po.V.op (mm^3)	18.85 ± 8.08	26.72 ± 9.32	**0.017**	18.11 ± 7.08	17.78 ± 6.94	0.212
Tb.TS (mm^2)	465.17 ± 97.08	461.15 ± 76.45	0.712	533.81 ± 93.49	540.15 ± 96.44	0.411
Tb.TV (mm^3)	500.18 ± 170.23	505.27 ± 162.67	0.758	630.14 ± 168.19	633.00 ± 182.12	0.878
BV/TV (%)	22.88 ± 7.29	22.72 ± 7.40	0.431	22.38 ± 7.25	22.37 ± 7.14	0.964
Tb.N (1/mm)	1.28 ± 0.41	1.26 ± 0.37	0.475	1.26 ± 0.39	1.27 ± 0.40	0.418
Tb.Th (mm)	0.19 ± 0.07	0.18 ± 0.07	0.371	0.18 ± 0.06	0.18 ± 0.06	0.205
Tb.Sp (mm)	0.88 ± 0.18	0.88 ± 0.16	0.993	0.88 ± 0.17	0.87 ± 0.16	0.150
Conn.Dn (1/mm)	22.051 ± 31.50	21.56 ± 28.98	0.698	21.64 ± 29.81	22.64 ± 32.38	0.347
DA	2.13 ± 0.36	2.13 ± 0.39	0.872	2.11 ± 0.37	2.11 ± 0.38	0.934
FD	2.42 ± 0.07	2.42 ± 0.06	0.912	2.44 ± 0.07	2.44 ± 0.06	0.865
SMI	-1.00 ± 2.58	-0.84 ± 2.24	0.292	-0.88 ± 2.38	-0.79 ± 2.23	0.323

Student's t-test for two dependent samples was used to assess microstructural differences between the two attempts in the region of interest determination in two independent investigators (the significant difference is presented in bold).
Abbreviations: SD – Standard deviation; observer I – early-career researcher; observer II – experienced researcher; Ct.TS – Cortical tissue surface; Ct.TV – Cortical tissue volume; Ct.Th – Cortical thickness; Po.Dm – Cortical pore diameter; Po.Sp – Cortical pore separation; Po.tot – Total cortical porosity; Po.cl – Closed cortical porosity; Po.op – Open cortical porosity; Po.V.tot – Total volume of cortical porosity; Po.V.cl – Volume of closed cortical porosity; Po.V.op – Volume of open cortical porosity; Tb.TS – Trabecular tissue surface; Tb.TV – Trabecular tissue volume; BV/TV – Trabecular bone volume fraction; Tb.N – Trabecular number; Tb.Th – Trabecular thickness; Tb.Sp – Trabecular separation; Conn.Dn – Connectivity density; DA – Degree of anisotropy; FD – Fractal dimension; SMI – Structural model index.

Inter-observer differences in ROI determination

Although ICC showed a high degree of consistency of data on cortical and trabecular micro-architecture of the femoral neck between two researchers (ICC range: 0.721-0.998; p<0.05), suggesting a high degree of reliability in measuring micro-architectural parameters by micro-CT, a significant inter-observer inconsistency was shown in the values of the diameter of the cortical pores (ICC value: 0.026; p=0.565). To determine the cause of these inter-observer inconsistencies in the microstructural parameter values, the Student's *t*-test was used to compare the second ROI determination of the younger researcher and the experienced researcher. It was shown

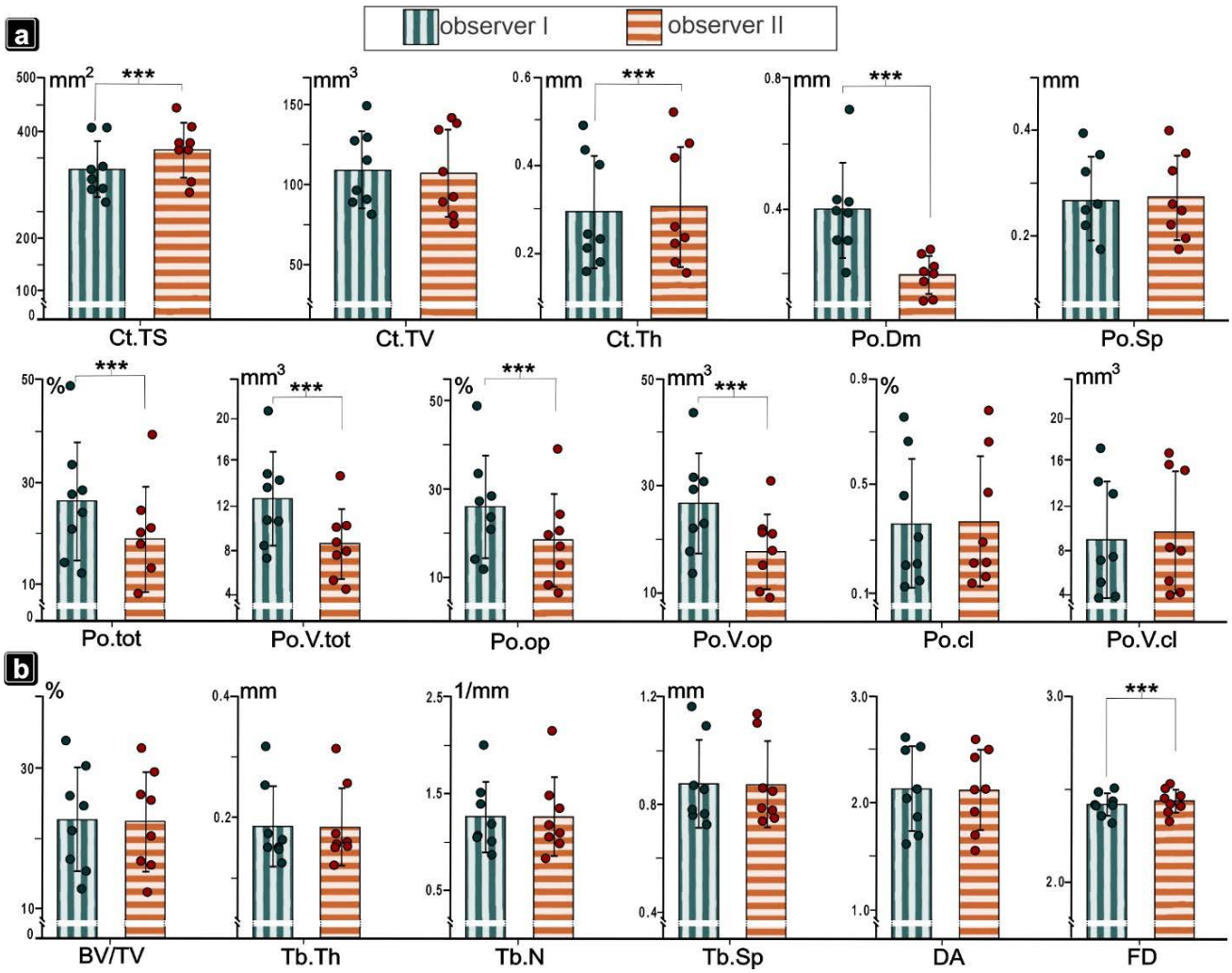

Figure 2. The inter-observer reliability for micro-CT assessment of femoral cortical and trabecular bone

Student's t-test for two dependent samples was used to assess the difference in microstructural parameters obtained by two independent investigators (*** p<0.05). Bar graphs represent the data as mean ± standard deviation, including the individual data points.

Abbreviations: Observer I – early-career researcher; Observer II – experienced researcher; Ct.TS – Cortical tissue surface; Ct.TV – Cortical tissue volume; Ct.Th – Cortical thickness; Po.Dm – Cortical pore diameter; Po.Sp – Cortical pore separation; Po.tot – Total cortical porosity; Po.V.tot – Total volume of cortical porosity; Po.op – Open cortical porosity; Po.V.op – Volume of open cortical porosity; Po.cl – Closed cortical porosity; Po.V.cl – Volume of closed cortical porosity; BV/TV – Trabecular bone volume fraction; Tb.Th – Trabecular thickness; Tb.N – Trabecular number; Tb.Sp – Trabecular separation; DA – Degree of anisotropy; FD – Fractal dimension;

that the early-career researcher chose a smaller Ct.TS (p=0.005), which resulted in a significantly thinner cortex (p=0.021) that was substantially more porous (Po.tot, p<0.001; Po.V.tot p=0.001; **Figure 2**). Increased Po.tot when marking was done by the early-career researcher was conditioned by increased Po.op and Po.V.op (p<0.001, p=0.001, respectively). Pore diameter was significantly greater (p=0.011), while cortical Po.Sp was lower in the cortex marked by the observer I (p=0.005, **Figure 2**). On the other hand, the experienced researcher analyzed a statistically significantly larger Tb.TS and Tb.TV (p<0.001, p<0.001, respectively) and obtained higher FD values (p=0.013, **Figure 2**).

Differences between manual and semi-automatic ROI determination

The ICC analysis showed a high degree of consistency of femoral neck trabecular micro-architecture data between manual and semi-automatic ROI determination (ICC range: 0.658-0.994; p<0.05), suggesting that the micro-CT method is highly reliable in evaluation of trabecular micro-architecture in superolateral femoral neck. However, it was shown that there was also a significant inconsistency in the DA values (ICC value: 0.165; p=0.739).

The possibility of selecting an irregularly shaped ROI that includes a larger part of the trabecular zone is reflected by a significantly larger Tb.TS and Tb.TV obtained by the manual method (p=0.001, p=0.003 respectively), and the selected trabeculae demonstrated higher degree of anisotropy (p=0.002, **Figure 3**).

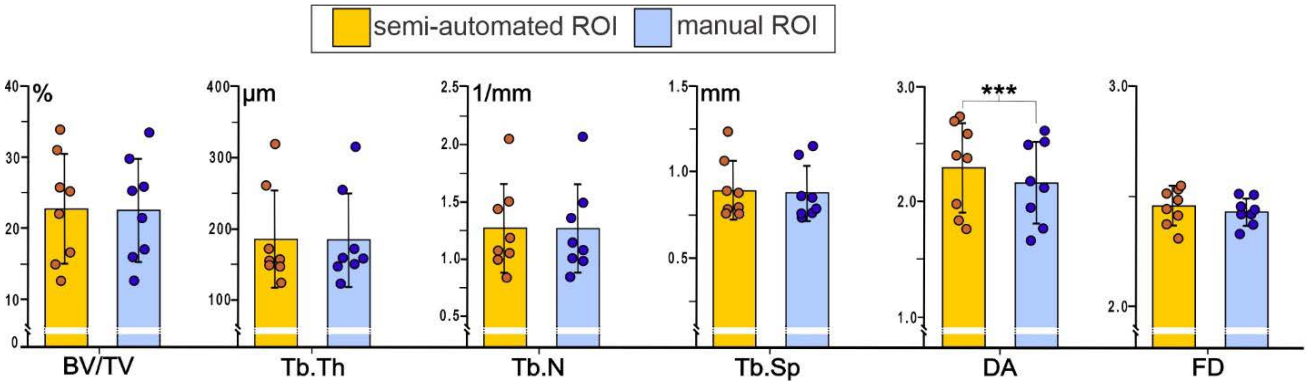

Figure 3. Differences in trabecular micro-architecture parameters due to manual and semi-automatic ROI demarcation methods.
Student's t-test for two dependent samples was used to assess the difference in microstructural parameters between manual and semi-automatic demarcation methods (*** $p<0.05$). Bar graphs represent the data as mean ± standard deviation, including the individual data points.
Abbreviations: BV/TV – Trabecular bone volume/total volume; Tb.Th – Trabecular thickness; Tb.N – Trabecular number; Tb.Sp – Trabecular separation; DA – Degree of anisotropy; FD – Fractal dimension

DISCUSSION

To analyze the bone sample with micro-CT, it is necessary to select an adequate VOI, which illustrates the importance of adequately standardized segmentation of different tissue compartments [22]. Analyzing various human and animal skeletal sites, numerous techniques for selecting bone tissue of interest have been applied (semi-automatic segmentation by determining the threshold of mineralized tissue [5,23], circular or rectangular ROIs of constant area and manual demarcation of the cortex and trabeculae [22]). Although the micro-CT methodology has been increasingly present in the human bone research practice, clear guidelines for manual ROI determination have not been agreed upon yet [22–24]. There are numerous dilemmas about manual ROI determination in the bone research field, which compromises the validity of the conclusion derived from inter-study comparison. It may arise due to inter- and intra-group, as well as inter- and intra-observer differences in research practice. It is important to emphasize that previous studies on human bone tissue used either a unique ROI selection methodology (methodology mentioned once in the work of one research group) or a methodology that was not fully described to enable exact reproducibility [23,24]. In addition, the methodology of including the transitional cortico-trabecular zone is rarely described (given that it is susceptible to subjectivity), which can have a significant impact on the data interpretation and interstudy comparison [1,10]. On the other hand, due to often conflicting results and a complex understanding of the effects of various conditions on animal models [25–31], guidelines for standardized micro-CT analyses of rodent bone tissue were recommended [32].

To our knowledge, this is the first study that investigated the inter-observer and intra-observer consistency of micro-CT derived trabecular and cortical micro-architectural parameters in the human superolateral femoral neck. In addition, the variability of the results obtained by manual and semi-automatic demarcation methods was investigated. Our study demonstrated a high degree of consistency in the results of trabecular micro-architectural parameters, except for a statistically significant difference in ROI size. A potential explanation may lie in the distance of the demarcation edge from the tissue contaminated with fragments created during sampling **(Figure 4, Figure 5)**. Given that manual marking is time-consuming and susceptible to subjective evaluation, there have been attempts to develop a protocol for a semi-automatic method of segmenting cortical from trabecular bone [11,16,17]. The protocols were based on different greyscale thresholds, and they had shortcomings that were primarily based on the conditions of sample size and sample surroundings, which is the rationale for the absence of consensus regarding methodology standards. Nevertheless, an unambiguous description of semi-automatic demarcation methodology in future studies may contribute to more reliable inter-study comparisons [11,16,17].

Although the effect of choosing the cortical ROI has not been investigated, the deterioration of cortical micro-architecture conditioned by various factors can be of great importance in the occurrence of increased bone fragility [33,34]. It is important to emphasize that cortical micro-architecture was more susceptible to inter-observer and intra-observer variability in our study **(Figure 4)**. One of the primary causes of this finding may arise from different approaches in ROI inclusion of transitional cortico-trabecular zone **(Figure 5)**. If the transitional cortico-trabecular zone (in some studies reported as "cortical trabecularization") is considered part of the cortical compartment, there is a possibility of overestimating cortical porosity [35]. An additional cause of cortical variability may be transcortical vascular pores **(Figure 5)** [36,37]. Although bone vascularization is increasingly analyzed in animal and human bone [36–38], it is not clear whether and to what extent vascular pores can influence bone fragility. This suggests that a clear explanation of the manual demarcation protocol in cortical tissue (with particular reference to the transitional corti-

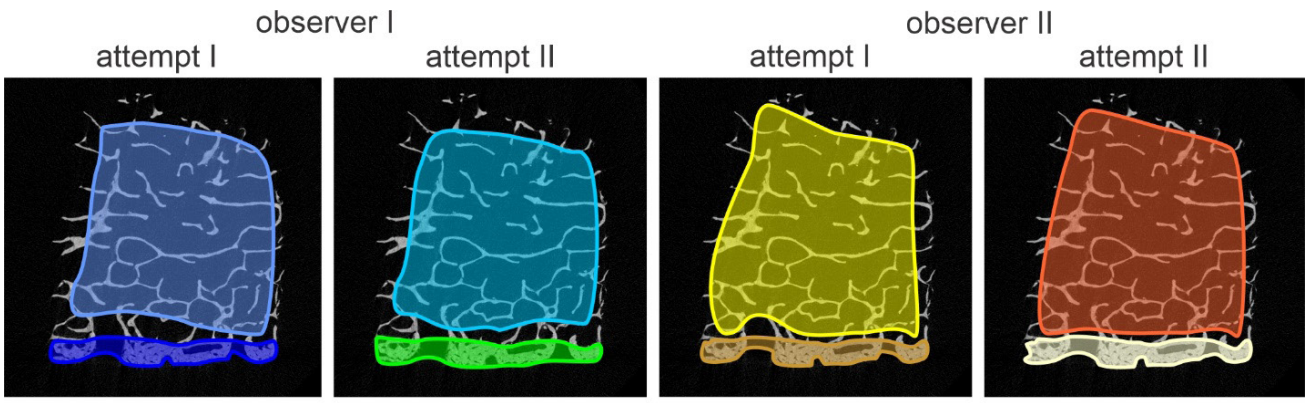

Figure 4. Graphic representation of diversity of manual ROI demarcation methods used in our study.

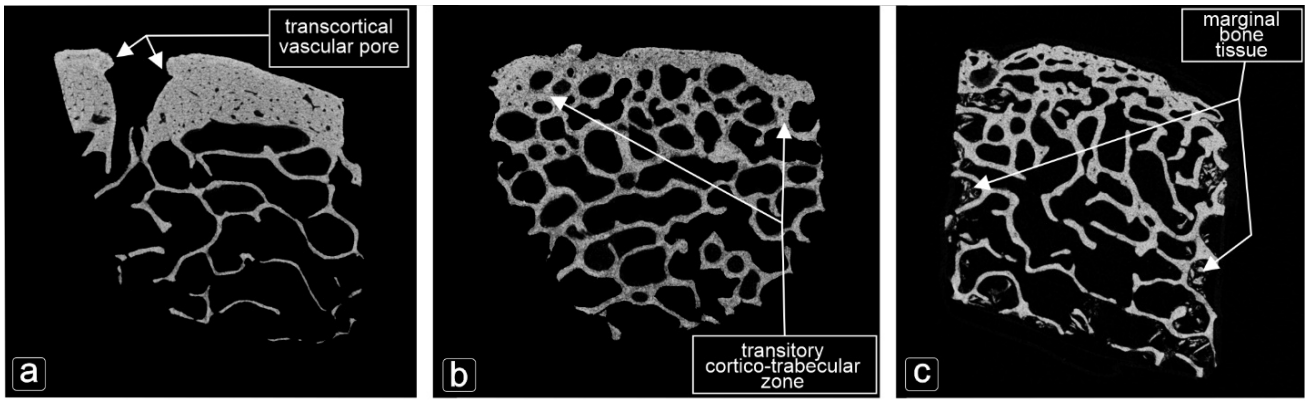

Figure 5. Representative findings that can significantly influence the values of bone micro-architectural parameters obtained by micro-CT analysis.

Transcortical vascular pores (a), transitional cortico-trabecular zone (b), and marginally damaged trabecular tissue (c) should be taken into account and clearly reported in the manuscript methodology section to ensure proper data interpretation and reproducibility.

co-trabecular zone and transcortical vascular pores) could contribute to greater reliability of the interpretation of the findings of different studies.

It is necessary to mention our study limitations. Although a statistically significant difference in specific parameters of bone micro-architecture was shown, and the number of samples is comparable to previous research on human material [39], the interpretation of our results could be complemented by including a larger number of bone samples derived from various skeletal sites. The correctness of mineralized and non-mineralized tissue differentiation was done using fixed greyscale intensity values by comparing to the original scanning image, which may result in the subjective estimation of bone fraction, especially when analyzing limited-volume samples with a small amount of bone tissue (such as in postmenopausal osteoporosis [40]). Lastly, our data could be subject to bias because included investigators (both from medical backgrounds) belong to the same research group and had similar training for micro-CT application in bone research.

CONCLUSION

Our study demonstrated that trabecular micro-architectural parameters were not significantly sensitive to various approaches in ROI demarcation methodology, given that a high degree of consistency was noted between two observers, between two attempts of the same observer and between manual and semi-automatic ROI-determination methods. In contrast, ROI determination had a significant effect on cortical microstructural parameters. As a result of the non-standardized ROI determination methodology, inter-observer and intra-observer differences were mainly reflected in cortical porosity parameters. Thus, our data highlight a need to standardize the methodology used in micro-CT evaluations of human bone samples in order to facilitate reliable inter-study comparison and ensure adequate result interpretation.

Ethical approval and patient consent: All procedures performed in this study were under the institutional ethics committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Author contribution statement: Conceptualization: JJ; Data acquisition: UA and JJ; Data interpretation: all authors; Data visualization: JJ and UA; Writing – original draft: UA; Writing – Review and Editing: JJ and MD; Project administration and funding: MD; Approval of the submitted manuscript version: all authors.

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RAZLIČITI METODOLOŠKI PRISTUPI PROCENI MIKROARHITEKTURE KOSTI POMOĆU MIKRO-KOMPJUTERIZOVANE TOMOGRAFIJE: ZNAČAJ ZA POREĐENJE REZULTATA RAZLIČITIH STUDIJA

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Sažetak

Uvod: Mikro-kompjuterizovana tomografija (mikro-CT) je često korišćen 3D metod za nedestruktivnu analizu mikro-arhitekture koštanog tkiva. Iako su u savremenoj literaturi dostupne brojne mikro-CT studije, poređenje nalaza ovih studija je otežano izostankom standardizacije metodologije, posebno tokom analize humanog materijala.

Cilj: Ova studija je imala za cilj da utvrdi konzistentnost mikro-arhitekturnih parametara dobijenih mikro-CT analizom, između dva istraživača (međuposmatračka pouzdanost), jednog istraživača u dva pokušaja (unutarposmatračka pouzdanost), kao i između manuelnog i poluautomatskog metoda određivanja regiona od interesa (ROI).

Materijal i metode: Uzorci superolateralnog dela vrata humanih butnih kostiju (n=8) skenirani su Bruker 1172 mikro-CT sistemom koristeći veličinu voksela od 10 µm. Određivanje ROI-a kortikalne i trabekularne kosti je vršeno manuelno (dva istraživača, u dva vremena sa razmakom od 45 dana). Takođe, određivanje ROI-a trabekularne kosti vršeno je i poluautomatski pomoću ROI-a okruglog oblika (prečnik 6,5 mm).

Rezultati: Intraklasni koeficijent korelacije (*intraclass correlation coefficient* - ICC) pokazao je visok stepen konzistentnosti mikro-CT metodologije u merenju mikro-arhitekturnih parametara superolateralnog dela vrata femura (opseg ICC vrednosti: 0,721-0,998; p<0,05). Međutim, detaljnija analiza je pokazala značajne razlike među istraživačima, kao i između načina obeležavanja jednog istraživača, koje se pretežno ogledaju u parametrima kortikalne poroznosti (Studentov t-test za zavisne uzorke, p<0,05). S druge strane, izbor ROI-a nije značajno uticao na parametre trabekularne mikro-arhitekture, kako među istraživačima, tako i između manuelnog i poluautomatskog metoda obeležavanja (Studentov t-test za zavisne uzorke, p>0,05).

Zaključak: Rezultati ove studije ukazuju na to da postoji potreba za standardizacijom metodologije mikro-CT analize koštanog tkiva humanog porekla kako bi se olakšalo poređenje i omogućila adekvatna interpretacija rezultata različitih studija.

Ključne reči: mikro-CT, vrat butne kosti, region od interesa, mikro-arhitektura kosti, humani materijal

Primljen: 29.08.2023. I **Revizija:** 10.12.2024. I **Prihvaćen:** 10.12.2024.

Medicinska istaživanja 2024; 57(2):13-21

ORIGINAL ARTICLE

What influences pensioners to use social protection services?

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Recived: 25 October 2023

Revised: 23 February 2024

Accepted: 23 February 2024

Check for updates

Funding information:
The study was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project No. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction/Aim. Ageing is a natural phase in the life of every individual, and it is correlated to life changes like retirement when the active working life of a person is ending and is compensated for with sources of income that do not have to be earned by working, which is called pension. The aim of this study was to analyze the use of social services among the retired population and examine the factors associated with the use of social care.

Material and Methods. This cross-sectional study is a secondary analysis of the data collected in a study conducted by the Union of pension holders of Serbia which was done on a convenient sample of retirees. An anonymous written questionnaire was designed for this specific research. Univariate and multivariate statistic regression were used to analyze the factors associated with the use of social services.

Results. Only 6.5% of retirees use some social protection services. Older age, living alone, lower level of education, and not having a mobile phone or computer significantly decrease the probability of using social protection services. Besides, spending time with friends and family significantly decreases the likelihood of using some social protection services.

Conclusion. Work in the field of health education is of utmost importance, especially spreading the knowledge about health-related, psychological, and social aspects of ageing in order to overcome stereotypes, prejudices and misunderstandings of this stage of life.

Keywords: older population, pensioners, social services

Cite this article as: Lekić I, Stamenković Ž, Matejić B, Maksimović N. What influences pensioners to use social protection services? Medicinska istaživanja 2024; 57(2):23-32 DOI: 10.5937/medi57-47332

INTRODUCTION

Ageing is a biological process that has its dynamics and is a natural phase in the life of every individual; it manifests itself in a broad spectrum of changes that over a period of time lead to gradual physical and cognitive decline, higher risk of disease and eventually death. The United Nations and the World Health Organization state that older people are defined as individuals who are 65 years old or older (1, 2). The share of the older population in the world is constantly getting higher due to different factors like declining birth rates, an increase in life expectancy and intense migrations. In addition to biological changes, ageing is also associated with other life changes such as retirement, relocation, or the partner's death. Retirement represents the age when a person ceases to be active, and at the same time has a certain income or pension.

In the Republic of Serbia, the number of pensioners has been progressively increasing since the beginning of the last decade of the 20th century, which is partly a consequence of demographic changes, and largely a consequence of liberal conditions for acquiring pension insurance rights (3, 4). The pension system on the territory of the Republic of Serbia, as well as in many other countries, is based on the principle of intergenerational solidarity. It is a pay-as-you-go model of the pension system within which the funds for the payment of current pensions are provided by the income generated from current payments of contributions for pension and disability insurance. It is a system of financing that is sustainable if there are conditions of favorable demographic parameters that provide a satisfactory proportion between the insured and those who exercise their rights related to social insurance (5). Based on the data of the Republic Pension and Disability Insurance Fund of the Republic of Serbia, as of June 2023, the total number of pension beneficiaries was 1.646.171, 1.082.185 of which were old-age pension beneficiaries (65.7%), 235.770 were disability pension beneficiaries (14.4%) and 328.216 were beneficiaries of survivors' pensions (19.9%). During the last decade, there was an increase in the number of old-age pension beneficiaries, while the number of beneficiaries of survivors' pensions did not change significantly (3).

The population of societies around the world, including Serbia, is ageing and the need for social and health services is increasing. Older people are the majority among beneficiaries of the social and healthcare systems, and this is a great challenge in planning services for older people (6). Traditionally, health and social service sectors operate separately. A growing demand for integrated services and rising costs lead to more intensive cooperation between healthcare institutions and social service providers (7, 8). Social protection services include activities that provide support and assistance to individuals and families in order to improve or preserve their quality of life, eliminate or mitigate the risk of adverse life circumstances, as well as create opportunities to live independently in society. The Law on Social Protection of the Republic of Serbia clearly defines a set of social protection services related to older people. Social protection services are divided into the following groups: assessment and planning services, daily services in the community, support services for independent living, counselling-therapeutic and social-educational services, and accommodation services (9).

The use of social protection services is related to individual characteristics such as age, gender, and level of education, but also to characteristics at the environmental level such as place of residence (10-12). On the other hand, the use of social protection services has proved to be one of the factors influencing better self-assessment of health status in the way that users of these services assessed their health status better than older people who were not users of social services (13, 14).

The social support provided to the older population is extremely important because it acts as a protective factor against problems such as loneliness, stress, and depression (15). A study conducted in Poland showed that the health condition of the older population was different in relation to where the care was provided, whether it was an institutionalized environment or home environment. The authors pointed out that older people living in their homes indicated significant limitations in performing basic daily activities and instrumental daily activities, a higher prevalence of depression and cognitive impairment, and consequently a greater need for social protection services (16).

When it comes to social support, a study conducted in Spain showed that 40% of older respondents had three to five close people at their disposal in case of a serious problem, while only 7% used social protection services (17). A study conducted in Estonia analyzed two groups of women who used social services – an elderly person who lived in an old people's home in Tallinn and an elderly person who received home care. The results showed that 65% of users of old people's homes were satisfied with the treatment and life in old age, while 40% of home care users were satisfied, which indicates the importance and positive impact of living in old people's homes in relation to living at home (18).

Unfavorable social and economic situation increases the risk of deteriorating health outcomes (19, 20). In this regard, it is important to have information about the type of care that an elderly person needs and the reasons why they need it, all in order to establish their independence and maximize their quality of life (21, 22). Although there are no official data in the Republic of Serbia regarding the number of informal caregivers, the assumption is that the number of people in need of someone else's care and assistance is far higher than the number receiving this material compensation and that it is around 200,000 inhabitants. i.e., 195,000 individuals, which makes up approximately 2.6% of the population in the country (23).

According to the 2011 Census, 93,000 older people (65+) needed support in performing basic daily activities, noting that due to the negative natural increase and ageing of the population in future, this percentage can be expected to be significantly higher (11).

Although there are numerous studies on the use of social services in the world, a study of this type has not been conducted in Serbia among the population of pensioners so far, so this study aims to examine the frequency of using these services in the population of pensioners, as well as to investigate the factors related to the use of social protection services.

METHOD

Data source and the type of study. A cross-sectional study was conducted as a secondary analysis of data obtained from the study of the Association of Pensioners' Unions of Serbia (APUS) conducted on a sample of pensioners in the period from April to June 2019.

Sample. During the research, individuals who received a pension (pensioners) were interviewed, and those who were available to the interviewers - family members, neighbors, friends and members of various organizations or associations. In June 2019, there were 1,707,592 pensioners in Serbia, of which 368,884 lived in Belgrade, according to the data from the Statistical Bulletin of the Republic Fund for Pension and Disability Insurance. A total of 625 retirees were surveyed, i.e., 0.17% of the retired population of Belgrade, which makes this sample suitable.

In preparation for the interviews, a short training of APUS activists was conducted, and they had face-to-face interviews in their communities, mainly in central and suburban municipalities of Belgrade, but also in some areas of Serbia.

Research instrument. An anonymous questionnaire in paper form was used as a research instrument, constructed for the needs of this research, the previous research in this field (14, 24, 25), expert opinions, and the consent of the USPS presidency. The questionnaire had a total of 37 questions.

The variables used in research analysis were selected based on evidence from the literature, as well as on the opinions of researchers on their significance. The variables used in the research were related to (1) socio-demographic characteristics of the respondents, (2) income and quality of life, (3) use of health care services, and (4) social inclusion/support.

Statistical methods of data processing. The obtained data were analyzed using methods of descriptive and inferential statistics. Comparisons were made about the use of social protection services. Using univariate and multivariate logistic regression, the connection of predictive-independent variables for dependent, i.e., variables of interest, were analyzed. The variable of interest was the use of social protection services.

All independent variables, whose p-value in univariate logistic regression was less than 0.05 were included in multivariate logistic regression models. Results (odds ratio with 95% confidence interval) in univariate as well as in multivariate logistic regression were considered significant if the p-value was less than 0.05 in the final model. All statistical analyzes are based on the probability of a null hypothesis of 0.05. The IBM SPSS Statistics for Windows software package, version 26 (IBM Corp., Armonk, N.Y., USA) was used for database preparation as well as for statistical processing.

RESULTS

A small number of surveyed pensioners use social protection services, only 39 (6.5%). When asked which services they used, the most common were geronto-housewife services, then material assistance, and only in one case meals in the soup kitchen and staying at a nursing home (data not shown in the table).

Demographic, socio-economic characteristics and the use of social protection services

Demographic and socio-economic characteristics of respondents in relation to the use of social protection services are shown in **Table 1**. The average age of respondents who use social protection services is 75 years. Males are more likely to use social protection services compared to females (61.5% vs. 38.5%). Social protection services are mostly used by people living with a partner (42.1%), followed by people living alone (36.8%), people living only with children (18.4%) and people living with their spouses and children (2.6%). When it comes to the jobs the users did before retirement, the largest percentage of the users of social protection services is among those who were engaged in creative occupations before retirement (41.7%), while the least percentage is among housewives (8.3%).

Univariate logistic regression showed a connection between the use of social protection services and the following independent variables: age, who the person lives with, level of education, and possession of a mobile phone and computer. After inserting all the socio-economic characteristics that proved significant in the univariate model into the multivariate model of logistic regression, the same pattern of connectivity was confirmed. Age is significantly associated with the use of social protection services (OR 1.06, CI95% 1.0-1.11). Also, people living with a partner and children are less likely to use some of the social protection services than those living alone (OR 0.08, CI95% 0.01-0.6). With a higher level of education, the chance that a person will use one of the social protection services decreases (OR 0.8, CI95% 0.6-1.1). Having

Table 1. Demographic, socio-economic characteristics of respondents and the use of social protection services

Sociodemographic characteristics of the respondents	Total	They use social protection services n (%)	They do not use social protection services n (%)	Univariant logistic regression OR (CI 95%)	Multivariant logistic regression OR (CI 95%)
Age (as±sd)	71,3±6,5	75,2±8,6	71,0±6,2	1,09 (1,04-1,14)	1,06 (1,0-1,11)
Gender					
Male	290 (48,0%)	24 (61,5%)	266 (47,1%)		
Female	314 (52,0%)	15 (38,5%)	299 (52,9%)	0,6 (0,3-1,1)	
Who they live with					
Alone	138 (23,1%)	14 (36,8%)	124 (22,1%)	Referency category	Referency category
With spouse	258 (43,1%)	16 (42,1%)	242 (43,2%)	0,6 (0,3-1,2)	0,7 (0,3-1,5)
With spouse and children	114 (19,1%)	1 (2,6%)	113 (20,2%)	0,1 (0,01-0,6)	0,08 (0,01-0,6)
Only with children	68 (11,4%)	7 (18,4%)	61 (10,9%)	1,0 (0,4-2,7)	0,8 (0,3-2,2)
With somebody else	20 (3,3%)	0 (0,0%)	20 (3,6%)	-	-
Accommodation					
Own apartment	434 (72,8%)	26 (66,7%)	408 (73,2%)	Reference category	
House	138 (23,2%)	11 (28,2%)	127 (22,8%)	1,4 (0,7-2,8)	
Tenant	12 (2,0%)	1 (2,6%)	11 (2,0%)	1,4 (0,2-11,5)	
Other	12 (2,0%)	1 (2,6%)	11 (2,0%)	1,4 (0,2-11,5)	
Level of education					
Primary school	29 (4,8%)	7 (17,9%)	22 (3,9%)		
Three-year school/ Occupation	55 (9,2%)	5 (12,8%)	50 (8,9%)		
High school	177 (29,5%)	10 (25,6%)	167 (29,8%)	0,7 (0,5-0,9)	0,8 (0,5-1,1)
College	122 (20,3%)	6 (15,4%)	116 (20,7%)		
Faculty	217 (36,2%)	11 (28,2%)	206 (36,7%)		
Occupation before retirement					
Housewife	18 (3,1%)	3 (8,3%)	15 (2,8%)	3,6 (0,9-13,9)	
Worker	112 (19,3%)	12 (33,3%)	100 (18,3%)	2,2 (0,9-4,9)	
Officer	164 (28,2%)	6 (16,7%)	158 (29,0%)	0,7 (0,3-1,8)	
Creative occupations	287 (49,4%)	15 (41,7%)	272 (49,9%)	Reference category	
Do you own a mobile phone?					
Yes	558 (95,1%)	32 (82,1%)	526 (96,0%)	0,2 (0,1-0,5)	0,5 (0,2-1,4)
No	29 (4,9%)	7 (17,9%)	22 (4,0%)		
Do you own a computer?					
Yes	357 (61,3%)	12 (31,6%)	345 (63,4%)	0,3 (0,1-0,6)	0,6 (0,2-1,3)
No	225 (38,7%)	26 (68,4%)	199 (36,6%)		

a mobile phone and a computer reduces the likelihood that a person will use social protection services (mobile phone: OR 0.5, CI95% 0.1-1.4; computer: OR 0.5, CI95% 0.2-1.01).

Revenues and the use of social protection services

Table 2 refers to income and the use of social protection services in the population of surveyed pensioners. The majority of the users of social protection services are beneficiaries of old-age pensions (60.5%), and the smallest number of these individuals use retirement pensions (5.3%). In both categories of respondents, there is the highest percentage of those who support their children from using their owns pensions, while the lowest percentage is of those who use their pensions to support their spouses (6.3%). When it comes to additional sources of income, there is a significantly lower percentage of individuals without additional income among the users of social protection services compared to those who are not (2.6% vs. 18.2%).

The analysis of univariate logistic regression showed that the type of pension and the existence of an additional source of income are significantly related to the use of social protection services. After including the variables that proved significant in the univariate model in the multivariate logistic regression model, the correlation remained unchanged. Beneficiaries of survivors' and disability pensions are multiple times more likely to use some of the social protection services compared to beneficiaries of old-age pension (beneficiaries of survivors' pensions: OR 3.8, CI 95% 1.4-10.2; beneficiaries of disability pensions: OR 2, 3, CI95% 0.9-5.7), while the existence of an additional source of income significantly reduces the chance of using social protection services (OR 0.1, CI95% 0.02-1.01).

Table 2. Revenues and the use of social protection services

Sociodemographic characteristics of the respondents	Total n (%)	They use social protection services n (%)	They don't use social protection services n(%)	Univariant logistic regression OR (CI 95%)	Multivariant logistic regression OR (CI 95%)
Type of pension					
Old age	460 (77,8%)	23 (60,5%)	437 (79,0%)	Reference category	Reference category
Early	36 (6,1%)	2 (5,3%)	34 (6,1%)	1,1 (0,3-10,4)	1,0 (0,2-4,6)
Survivors'	35 (5,9%)	6 (15,8%)	29 (5,2%)	3,9 (1,5-10,4)	3,8 (1,4-10,2)
Disability	60 (10,2%)	7 (18,4%)	53 (9,6%)	2,5 (1,0-6,1)	2,3 (0,9-5,7)
Supporting someone else using their pension					
Yes	221 (37,3%)	19 (50,0%)	202 (36,5%)	1,7 (0,9-3,4)	
No	371 (62,7%)	19 (50,0%)	352 (63,5%)		
Who else the respondent supports using their pension *					
Spouse	24 (12,3%)	1 (6,3%)	23 (12,8%)	0,5 (0,1-3,6)	
Children	152 (77,9%)	12 (75,0%)	140 (78,2%)	0,8 (0,3-2,7)	
Grandchildren	23 (11,8%)	2 (12,5%)	21 (11,7%)	1,0 (0,2-5,1)	
Someone else	6 (3,5%)	1 (7,1%)	5 (3,2%)	2,4 (0,3-21,7)	
Do they have an additional source of income in addition to their pension?					
Yes	102 (17,2%)	1 (2,6%)	101 (18,2%)	0,1 (0,02-0,9)	0,1 (0,02-1,01)
No	492 (82,8%)	37 (97,4%)	455 (81,8%)		
Were you among those whose pensions were reduced?					
Yes	472 (80,8%)	31 (83,8%)	441 (80,6%)	1,3 (0,5-3,1)	
No	112 (19,2%)	6 (16,2%)	106 (19,4%)		
What was affected by the reduction of pensions **					
Food quality	101 (29,7%)	6 (28,6%)	95 (29,8%)	0,9 (0,4-2,5)	
Purchase of medicines, aid	146 (42,9%)	10 (47,6%)	136 (42,6%)	1,2 (0,5-3,0)	
Going to spa, trips, excursions	189 (55,6%)	6 (28,6%)	183 (57,4%)	0,3 (0,1-0,8)	
Cultural activities	133 (39,1%)	3 (14,3%)	130 (40,8%)	0,3 (0,1-0,8)	
Socializing, relatives, friends	73 (21,5%)	3 (14,3%)	70 (21,9%)	0,6 (0,2-2,1)	
Something else	77 (22,6%)	12 (57,1%)	65 (20,4%)	5,2 (2,1-12,9)	
Do you live better today than a few years ago?					
Yes	24 (4,3%)	0 (0%)	24 (4,5%)		
Nothing significant has changed	184 (32,7%)	10 (33,3%)	174 (32,6%)	1,3 (0,9-2,0)	
Lives a little worse	195 (34,6%)	8 (26,7%)	187 (35,1%)		
Lives a lot worse	160 (28,4%)	12 (40%)	148 (27,8%)		

** Applies only to respondents who answered that they are supporting someone else from their pension*
*** Applied only to respondents whose pension has been reduced*

Health care, satisfaction with health care services and the use of social protection services

Table 3 refers to health care, satisfaction with health care services and the use of social protection services. When it comes to the users of social protection services, the largest percentage were those who were dissatisfied with health care services (33.3%), while none of the respondents stated that they were very satisfied. More than half of the users of social protection services used the services of both a private doctor (57.9%) and a private dentist (52.6%) in the past year. Slightly more than two-thirds of the users of social protection services (68.8%) have a chronic illness, while almost all of them (97.4%) have to buy medication to treat their illness. When it comes to self-assessment of their health status, among the users of social protection services, there is the highest percentage of those who assess their condition as neither good nor bad (37.8%), and the lowest percentage of those who depend on someone else's care and assistance (13.5%).

In the examined population of pensioners, there is a significant correlation between the self-assessment of health status and the use of social protection services in the following way: the worse the self-assessment of the

Table 3. Health care, satisfaction with health care services and the use of social protection services

	Total	They use social protection services n (%)	They don't use social protection services n (%)	Univariant logistic regression OR (CI 95%)
Satisfaction with health care				
Very satisfied	13 (2,2%)	0 (0,0%)	13 (2,4%)	1,3 (0,9-1,8)
Satisfied	183 (31,0%)	10 (25,6%)	173 (31,4%)	
Neither satisfied nor dissatisfied	187 (31,7%)	11 (28,2%)	176 (31,9%)	
Dissatisfied	159 (26,9%)	13 (33,3%)	146 (26,5%)	
Very dissatisfied	48 (8,1%)	5 (12,8%)	43 (7,8%)	
Have you used the services of a private doctor in the past year?				
Yes	341 (57,8%)	22 (57,9%)	319 (57,8%)	1,0 (0,5-2,0)
No	249 (42,2%)	16 (42,1%)	233 (42,2%)	
Have you used the services of a private dentist in the past year?				
Yes	315 (53,6%)	20 (52,6%)	295 (53,6%)	1,0 (0,5-1,9)
No	273 (46,4%)	18 (47,4%)	255 (46,4%)	
Do you have a chronic illness?				
Yes	390 (68,4%)	22 (68,8%)	368 (68,4%)	1,0 (0,5-2,2)
No	180 (31,6%)	10 (31,3%)	170 (31,6%)	
Do you have to buy medicines to treat your illness?	483 (84,9%)	36 (94,7%)	447 (84,2%)	3,4 (0,8-14,3)
Self-assessment of health status				
Good	228 (39,2%)	8 (21,6%)	220 (40,4%)	2,0 (1,4-3,1)
Neither good nor bad	221 (38,0%)	14 (37,8%)	207 (38,0%)	
Bad but takes care of himself	122 (21,0%)	10 (27,0%)	112 (20,6%)	
Depends on someone else's care and help	11 (1,9%)	5 (13,5%)	6 (1,1%)	

health condition, the higher the probability that the person will use one of the social protection services (OR 2.0, CI95% 1.4-3.1).

Social inclusion and the use of social protection services

Table 4 shows data related to social inclusion and the use of social protection services. Among the users of social protection services, 85.7% hang out and see their family, relatives and friends, while 13.9% are socially engaged. While slightly more than a third of social protection service users read the daily press (38.5%), two-thirds watch TV (66.7%).

Combining variables into a multivariate logistic regression model confirmed that socializing with family and friends significantly reduced the likelihood of using one of the social protection services (OR 0.1, CI95% 0.04-0.4). Also, respondents who knew how to use a computer were less likely to be users of these services (OR 0.3, 95% 0.2-0.8).

DISCUSSION

A cross-sectional study conducted on a sample of pensioners provided an answer to the question concerning the perception of the position of pensioners in the Republic of Serbia, primarily in the field of using social services and the factors related to them.

Apart from life expectancy, the number of older people who need health and social services is on the increase as well. Although informal support systems for the older population have been present for much longer than the formal ones, especially those provided by close relatives such as spouses, children and siblings, formalization of care through the social protection system plays a significant role in providing care and support to pensioners. The results of the conducted research show that even among older people, with the increase in the number of years, the use of social protection services is increasing as well. This is in line with the results of other studies that have also shown that ageing enhances the need to use social protection services, which is expected since ageing reduces functional capacity and increases the risk of developing chronic diseases (11, 12, 19, 26-30). Given that older individuals have greater needs of social services and are more often represented among service users, the importance of age as a determinant of social service use was not unexpected (31).

The study conducted on the territory of the Republic of Serbia has shown that social protection institutions have an insufficient role in the network of support and assistance to poor older people. Those who need help to function daily rely minimally on all institutions that can potentially support them, such as healthcare institutions and centers for social work (32).

Pensioners living in a household with a partner and children use social protection services significantly less than pensioners living alone or with a partner only. These results are not surprising given that direct assistance from

Table 4. Social support and use of social protection services

	Total	They use social protection services n (%)	They don't use social protection services n (%)	Univariant logistic regression OR (CI 95%)	Multivariant logistic regression OR (CI 95%)
Do you socialize and see family, friends, and relatives?					
Yes	557 (97,5%)	30 (85,7%)	527 (98,3%)	0,1 (0,03-0,3)	0,1 (0,04-0,4)
No	14 (2,5%)	5 (14,3%)	9 (1,7%)		
Are you socially engaged?					
Yes	106 (19,3%)	5 (13,9%)	101 (19,7%)	0,7 (0,3-1,7)	
No	443 (80,7%)	31 (86,1%)	412 (80,3%)		
Do you read daily press?					
Yes	276 (47,3%)	15 (38,5%)	261 (48,0%)	0,7 (0,3-1,3)	
No	307 (52,7%)	24 (61,5%)	283 (52,0%)		
Do you watch TV?					
Yes	392 (64,6%)	26 (66,7%)	366 (64,4%)	1,1 (0,6-2,2)	
No	215 (35,4)	13 (33,3%)	202 (35,6%)		
Do you use a computer?					
Yes	357 (61,3%)	12 (31,6%)	345 (63,4%)	0,3 (0,1-0,5)	0,3 (0,2-0,8)
No	225 (38,7%)	26 (68,4%)	199 (36,6%)		

family members is available in a multi-member household. This is in line with the results of another study highlighting that the increased tangible support was associated with greater satisfaction with family relationships and a greater total number of close friends and family (31). These results indicate good targeting of users in terms of family status. In their research, Matković and Stanić also stated that the largest percentage of social protection beneficiaries lived alone and did not have direct support of their closest ones, which indicated a fair distribution of social services in the older population (11).

The probability that retirees will be beneficiaries of social protection services decreases significantly with the increase in the level of education. These results are significant among pensioners. The analysis of service users in Belgrade confirms the findings of some previous studies that services are more directed towards more educated and wealthier sections of the population (27), but in terms of education, the higher one's education, the lower the chance that a person will use one of the social protection services. It is assumed that a higher level of education significantly contributes to easier access to information on social services and thus to the use of these services. However, access to information alone does not guarantee their use, as shown by the analysis. The analysis of service users in a previous period in Serbia confirmed the findings of some earlier studies that services were more directed towards more educated and well-off people (27).

The results of the conducted research show that having a mobile phone and a computer significantly reduces the probability that a person will use social protection services. It is explained by the fact that in that way, older people can connect and communicate with family and friends, who they can turn to in need. These results are in line with the results of a study conducted in America on the use of computers, the Internet and email among the older population, which states that the benefits of using a computer are a sense of connectedness, satisfaction, usefulness, and positive learning experiences (33, 34).

The data related to the type of pension speak in favor of the fact that the largest number of users of social services are users of old-age pensions, as expected. However, it is important to emphasize that family pension beneficiaries were 3.8 times more likely to use one of the social protection services than old-age pension beneficiaries. As the conditions for acquiring the right to an old-age and survivor's pension differ, it is clear that in addition to age, the amount of income can significantly affect the use of these services. Baronijan points out that the differences in the poverty of pensioners in the Republic of Serbia according to age can be mostly attributed to the type of pension that pensioners receive. Namely, pensioners over the age of 75 mostly belong to agricultural and family pensioners and have the highest poverty index (35). Regarding the amount of pension, Stanić points out that over half of pensioners receive pensions that are below average, emphasizing that the situation with agricultural pensioners and beneficiaries of disability pensions is somewhat specific, given that the average pension of these two groups of pensioners is lower than the minimum pension, so that a small number of pensioners receive a pension below the average pension, but almost all - over 90% of pensioners - receive a pension up to the minimum and the minimum amount (36).

Thus, the results of the research show that beneficiaries of survivors' and disability pensions are many times more likely to use one of the social protection services than beneficiaries of old-age pensions, which is in line with the fact that beneficiaries of these two pension groups receive low pension amounts resulting in the need to use social protection services. This is supported by the fact that the existence of an additional source of income reduces the chance of using social protection services by 10 times. The findings obtained by this research corre-

spond to the results of previous studies which showed that people with lower incomes, i.e., with lower socio-economic status, used social protection services to a greater extent, in relation to persons with higher incomes, i.e., better socioeconomic status (12, 27-30, 32, 37-39).

Spending time with family and friends reduces the probability that a person will use one of the social protection services by ten times. In their research on the position of older people in the Republic of Serbia, Matković and Stanić also state that those older individuals who hang out with family and friends feel less lonely and rely on them for help (informal care), which reduces their chances of using some social protection services. (11). Retirees who know how to use a computer and use it properly are less likely to be users of social security services, which corresponds to the results of research on computer use among the older population in America, which shows that computer and Internet use is extremely important in the lives of these older people considering that older people remain connected to family and friends through the use of computers (34).

Advantages and limitations of the study. As with all research, this study has some limitations to consider when looking into its findings. The cross-sectional study design prevents the drawing of causal conclusions from the relationships indicated between independent variables (predictors) and the outcome (the use of social protection services and purchase of medicines). Furthermore, the research was conducted pro bono, which had an impact on the sample of pensioners included in the research. Also, the questionnaire as a research instrument has not been officially validated.

However, an important advantage of this study is that, to date, the first study of this type examines the factors associated with the use of social and healthcare services in the retiree population. Also, the study covered four groups of indicators, so we were able to check which of the given categories most influenced the outcome variables. Precisely owing to this, the results can be used in the development of future programs and interventions aimed at improving the health of retirees.

CONCLUSION

In this research, only 6,5% of retirees use some of the social protection services. Older age, living alone, lower level of education, and not having a mobile phone or a computer significantly decrease the probability of using social protection services. On the other hand, spending time with friends and family significantly decreases the probability that a person will use some social protection services.

Based on the results of this research, it is indicated that activities in the field of population education are of great importance and the focus is mainly on spreading knowledge about health, psychological and social aspects of ageing and old age in order to change stereotypes, prejudices and misunderstandings of this age, support for intergenerational and intragenerational solidarity and the development of personal responsibility for life in old age. In this process, which should be long-lasting and systematic, it is necessary to include all known formal and informal channels. The promotion of a healthy lifestyle and individual behavior, including old age, should be one of the regular activities, not only of the Ministry of Health and health institutions but also of the education system, social protection institutions, local governments, civil society organizations and the media. As we are facing the decade of healthy ageing 2020 - 2030 (Decade of Healthy Aging), it is important to monitor and get involved in global initiatives in the field of active ageing because only in this way the socio-economic position of older people and their quality of life can be improved.

Acknowledgement

The authors would like to thank the Association of Pensioners' Unions of Serbia for the support and access to the participants of the study.

Financial Disclosure

The funders had no role in study design, data collection and analysis, the decision to publish, or the preparation of the manuscript.

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ŠTA UTIČE NA PENZIONERE DA KORISTE USLUGE SOCIJALNE ZAŠTITE?

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Sažetak

Uvod/Cilj. Starenje, kao prirodna faza u životu svakog pojedinca, je u korelaciji sa životnim promenama poput odlaska u penziju kada se završava aktivni radni vek čoveka, a nadoknađuje se izvorima prihoda koji ne moraju da se ostvaruju radom i koji se nazivaju penzija. Cilj ove studije bio je da se analizira korišćenje usluga socijalne zaštite u penzionerskoj populaciji i da se ispitaju faktori u vezi sa korišćenjem usluga socijalne zaštite.

Materijal i metode. Istraživanje predstavlja sekundarnu analizu podataka prikupljenih istraživanjem Unije penzija Srbije, koje je urađeno na prigodnom uzorku penzionera. Kao instrument istraživanja korišćen je upitnik osmišljen za potrebe istraživanja. Univarijantna i multivarijantna regresiona analiza je korišćena za identifikaciju faktora povezanih sa korišćenjem usluga socijalne zaštite.

Rezultati. Svega 6,5% penzionera koristi neke od usluga socijalne zaštite. Starija životna dob, osoba koja živi sama, niži stepen obrazovanja i neposedovanje mobilnog telefona ili računara značajno smanjuju verovatnoću korišćenja usluga socijalne zaštite. Osim toga, druženje sa prijateljima i porodicom značajno smanjuje verovatnoću da će osoba koristiti neke od usluga socijalne zaštite.

Zaključak. Rad u domenu zdravstvenog vaspitanja je od ključnog značaja, sa akcentom na znanju o zdravstvenim, psihološkim i socijalnim aspektima procesa starenja u cilju prevazilaženja stereotipa, predrasuda i nerazumevanja ove faze života.

Ključne reči: populacija starih, penzioneri, usluge socijalne zaštite

Primljen: 25.10.2023. I **Revizija:** 23.02.2024. I **Prihvaćen:** 23.02.2024.

Medicinska istaživanja 2024; 57(2):23-32

Medicinska istaživanja 2024; 57(2):33-40 | DOI 10.5937/medi57-48031

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Preemptive administration of oral, fast-acting tapentadol compared to tramadol/ketoprofen i.m. to reduce acute pain during and after ESWL procedure in renal stone disease

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Recived: 05 December 2023
Revised: 31 January 2024
Accepted: 29 February 2024

Check for updates

Funding information:
The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Extracorporeal shock wave lithotripsy (ESWL) used to be performed under general anesthesia. Today, although it is a painful procedure, it is performed under analgosedation. The aim of the study was to determine the severity of acute pain associated with ESWL using two comparative protocols for preemptive analgesics: tramadol vs. ketoprofen plus tapentadol.

Methods: A clinical prospective randomized cohort study included 200 patients of both sexes aged 18-80 years who were divided into two groups: group 1 received a combination of ketoprofen 100 mg/ tramadol 50 mg i.m. 30 minutes before surgery; group 2 received tapentadol IR 50 mg orally, 1 hour before surgery. Pain intensity (NRS) and complications were recorded before, during and at the end of the procedure, respectively.

Results: No difference was found in the preoperative characteristics of patient population, size and localization of the stone. Dimensions of kidney-localized stones were significantly higher in group 2 compared to group 1 (T test .000). There was a statistically significant increase in pain intensity before and during the procedure as well as pain intensity decrease during and after the procedure in each group (T test .000). In group 2, 10% of patients experienced severe pain during the procedure, compared to 3%of patients in group 1 (Mann-Witney 0.005). In the severe pain subgroups of each patient group, drowsiness occurred in 5% of patients in group 2, which was significantly more than the 1% in group 1 (overall incidence in both groups was 25%).

Conclusion: Although both protocols offered average moderate pain intensity during the procedure, severe pain and nausea were observed more frequently in the tapentadol group, which was related to stone size and JJ stent insertion.

Keywords: preemptive analgesia, tapentadol, ESWL procedure

Cite this article as: Aleksandar V, Lađević N, Jovičić J, Petrović N, Jovanović V, Likić Lađević I, Lađević N, Lazić M. Preemptive administration of oral, fast-ascting tapentadol compared to tramadol/ketoprofen i.m. to reduce acute pain during and after eswl procedure in renal stone disease; Medicinska istaživanja 2024; 57(2):33-40 DOI: 10.5937/medi57-48031

INTRODUCTION

After the introduction of extracorporeal shock wave lithotripsy (ESWL), the procedure was performed under general anesthesia. Technical improvement of the ESWL device made it possible to perform the treatment without general anesthesia, although less energy is used to break up the stone. Nevertheless, ESWL is still generally considered a painful procedure. This may be because shock waves reach superficial (skin and muscles) and deeper structures (ribs, nerves and kidney capsule) (1,2).

Pain is generally believed to affect the outcome of ESWL, as involuntary pain is caused by movements and excessive breathing excursions during the procedure, which interferes with the surgeon's efforts to focus on the stone. A high pain sensation may also limit the ability to apply the appropriate dose of energy (3,4). In addition, pain that limits the patient's cooperation may limit the energy and number of shock waves and lead to more complications, such as a higher rate of renal hematoma due to increased blood pressure (5).

To date, there are no guidelines for pain management during ESWL treatment, and different treatment protocols and different medications are used. Traditionally, non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ketorolac and piroxicam are used, but sometimes opioids such as morphine, pethidine and fentanyl are also used (6,7).

Pain caused by shock waves is usually described as burning and stabbing (8,9).

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely used analgesics in the world, mainly because of their analgesic and anti-inflammatory properties (10,11). In 1991, molecular biologists discovered that there are two different gene codes for cyclooxygenases - COX1 and COX2 (12). Both enzymes are distributed differently in the organism and are regulated differently. COX 1 is a constitutively present enzyme in most tissues of the body. They most likely influence the production of a constant amount of eicosanoids (prostaglandins and similar substances) to maintain physiological homeostasis in many organs such as the kidneys, lungs, stomach, etc. COX2 is found in macrophages and other cells of inflamed tissue (13). The expression of this enzyme is suppressed by glucocorticoids (14, 15). Opioids are substances with morphine-like effects, including both agonists and antagonists as well as natural and synthetic opioid peptides.

Opioids exert their pharmacological effects by binding to the opioid receptors MOP(μ), KOP(κ), DOP(δ), NOP - nociceptive orphanin FQ receptors, also known as ORL (opioid receptor-like). The receptors achieve their efficacy by binding to the inhibitory G protein. When using opioids, precautions are necessary in cases of liver and kidney disease, emphysema, asthma, pneumonia, head injuries, allergic reactions, interactions with antihistamines, sedatives, antiemetics and MAO inhibitors (16,17).

The group of weak opioid drugs is named this way because they have an upper limit of effectiveness, meaning there is a maximum effective daily dose that can be used in the treatment of moderate pain intensity score. Weak opioids are most commonly combined with non-opioids, such as: acetylsalicylic acid, acetaminophen and other NSAIDs. Weak opioids include: tramadol, tapentadol, codeine, dihydrocodeine, dextropropoxyphene (18).

THE AIM

The aim of the study was to evaluate the severity of acute pain related to ESWL procedure of kidney stones under preemptive analgesia with tramadol/ketoprofen combination intramuscular and tapentadol tablets orally as well as to compare analgesic effects of these two protocols in acute pain control for ESWL procedure.

METHODOLOGY

The research was conducted at the University Clinical Center of Serbia in accordance with the Helsinki Declaration and approved by the Ethics Committee (decision number 57/13).The clinical prospective cohort study included 200 consecutive patients of both genders, aged 18-80 years, ASA I-III status, who underwent an elective ESWL (extracorporeal shock wave lithotripsy) procedure for the treatment of kidney stones. The study lasted for 6 months.

Preoperative demographic data, gender, age, body mass index (BMI), place of residence (urban/rural), education, occupation, smoking habit, comorbidities, previous surgeries were collected from all patients. Regarding the procedure, the characteristics of the kidney stone (size, localization) and the presence of a JJ urethral stent were recorded.

The following patients were excluded from the study: those with advanced chronic renal insufficiency, chronic hepatic insufficiency, psychiatric comorbidity, dizzy spells, ASA status IV group, asthma, active peptic ulcer, and previous allergies to administered drugs.

The subjects were divided into two study groups according to the received pain therapy protocols both used as the standard procedure in everyday practice. Group 1 received the combination of ketoprofen 100mg plus tramadol 50mg intramuscularly, 30 minutes before the start of the ESWL procedure. Group 2 received tapentadol IR 50mg orally, 1 hour before the start of the ESWL procedure. Stone-related pain intensity score was recorded before the administration of the medication, during the ESWL procedure and at the end of the ESWL procedure using the NRS scale (numerical rating scale).

Parametric and non-parametric tests were used in the statistical analysis of the data, and the statistical differ-

Table 1. Distribution of preoperative patient characteristics in both study groups

Patient characteristics	Group 1 (n=100) n (%)	Group 2 (n=100) n (%)	Test value/ p(probability)
Gender (male/female)	42 / 58	39 / 61	0.666**/p ≥0.05
Age (years)	51.98±13.18	51.70±13.72	0.884*/ p≥ 0.05
BMI (kg/m2)	24.66±2.85	24.34±3.16	0.453*/ p ≥0.05
Smoking habit (Y/N)	48 / 52	59 / 41	0.120**/ p ≥0.05
Occupation (Y/N)	66 / 34	72 / 28	0.360**/ p ≥0.05
Place of residence (urban/rural)	73 / 27	66 / 34	0.284**/ p ≥0.05
Education (basic/ middle/ faculty)	30 / 40 / 30	33 / 48 / 19	0.199**/ p ≥0.05
Comorbidities (Y/N)	51 / 49	57 / 43	0.396**/ p ≥0.05
Previous surgeries (Y/N)	53 / 47	51 / 49	0.778**/ p ≥0.05

*Student T- test, ** Mann-Witney test; Y- yes, N-no

ence was expressed by two levels of significance (p≥0.05, p<0.05) using SPSS 21 statistic software. The variables were categorized according to the median value.

RESULTS

Two hundred patients were included in the study and classified into two groups according to the study protocol. There was no statistically significant difference between groups in preoperative patient characteristics and stone characteristics (stone size and localization).

In both groups of patients, the majority of respondents were from urban areas (66% vs. 73%), with secondary school degree (48% vs. 40%). In both groups the majority of patients had jobs (72% vs. 66%) but there was no statistically significant difference between the groups related to occupational status. Also, there were no statistically significant differences between groups in the prevalence of tobacco smoking (59% vs. 48%). Patient characteristics in two groups are shown in **Table 1**.

Stone localization was divided into five groups (left kidney, right kidney, left UP segment, right UP segment, ureter) with no statistically significant difference among the groups (X^2 0.856, p≥0.05). The most frequent localization in both study groups was the kidney **(Chart 1)**. The average stone size was 13.24±2.803 mm in group 1 and 13.14±2.370 mm in group 2, also with no statistically significant difference (T test 0.786, p≥0.05). No correlation was found between stone size and localization in group 1 (Pearson X^2 0.150, p≥0.05). A significant correlation was found in group 2 patients between stone size and stone localization (Pearson X^2 .000, p≥ 0.01) related to greater dimensions of kidney-localized stones.

Overall, 33% of patients in group 1 and 25% of patients in group 2 had a protectively placed JJ stent after the procedure. The presence of a JJ stent did not influence pain intensity after the procedure in patients from group 1 (Pearson X^2 0.486, p≥0.05). JJ stent insertion had influence on pain intensity after the procedure in group 2 (tapentadol group) with 21% of patients suffering from mild pain and 4% of patients suffering from moderate pain intensity related to JJ stent insertion (Pearson X^2 0.015, p<0.05).

Pain intensity was measured before, during and immediately after ESWL procedure, and the distribution of mean pain scores is shown in Chart 2.

Chart 1. The distribution of stone position by group (UP segment- ureteropelvic segment)

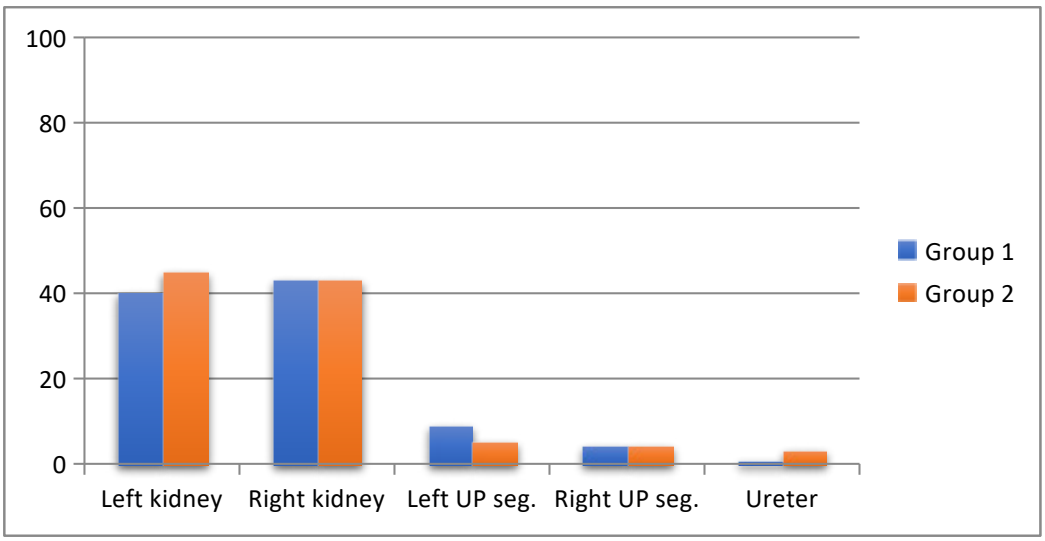
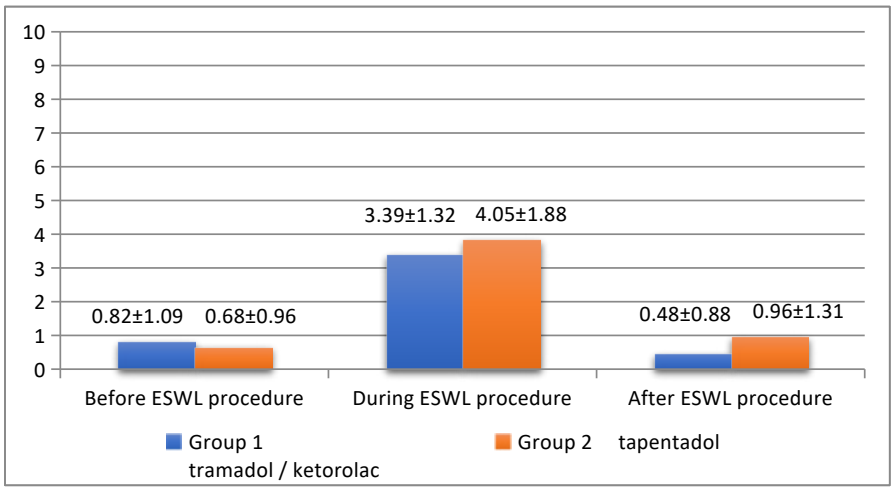


Chart 2. Average pain values measured by the NRS scale before, during and after the ESWL procedure



During the procedure, pain intensity was statistically significantly higher in both groups of patients (T test .000, CI 0.95, p<0.05) compared to pain before and after the procedure. No statistically significant difference was found in pain intensity before –after measurements in group 1 and group 2 patients respectively (T test 0.737 and T test 0.320 CI 0.95, p≥0.05). Before ESWL procedure no statistically significant difference was found in NRS between the groups (T test 0.338 CI 0.95, p≥0.05). Statistical difference was found in NRS during the procedure (T test 0.039 CI 0.95, p≥0.05) and after the procedure close to the level of significance (T test 0.055 CI 0.95, p≥0.05)

Chart 3. Distribution of patients related to pain intensity score during the ESWL procedure (Group 1)

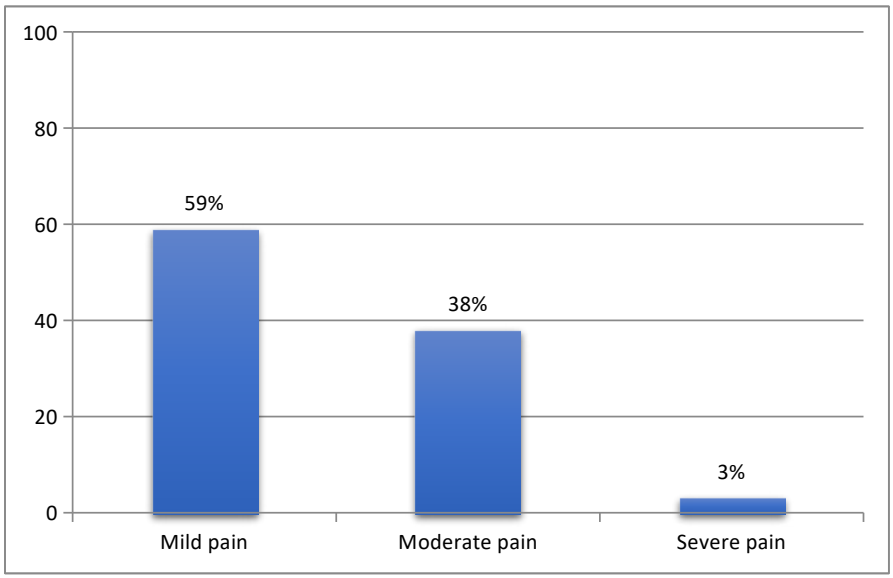


Chart 4. Distribution of patients related to pain intensity score during the ESWL procedure (Group 2)

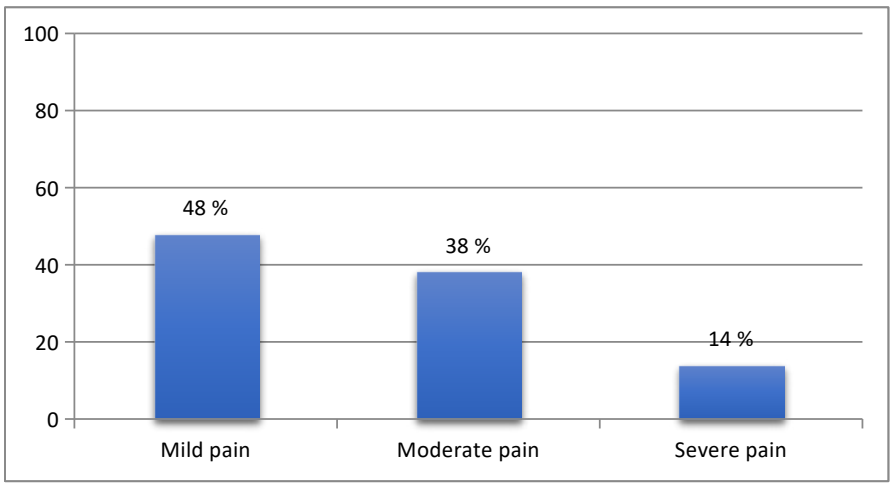
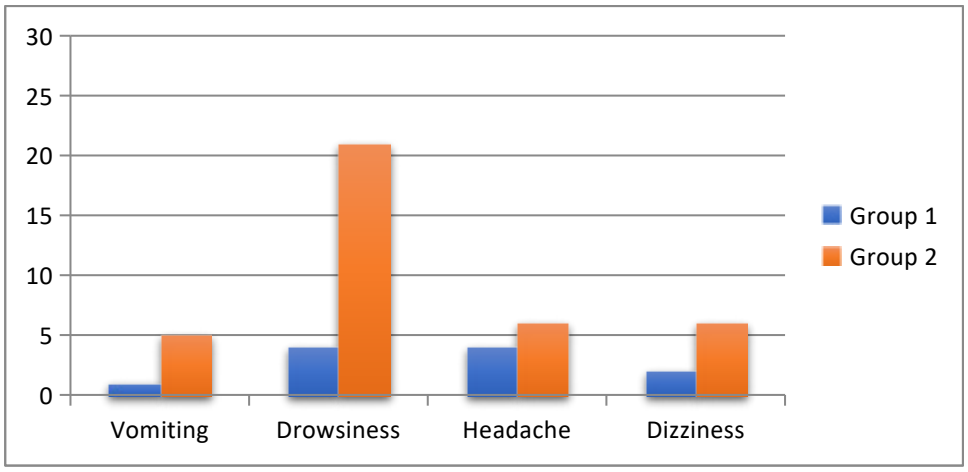


Chart 5. Frequency distribution of vomiting, drowsiness, headache and dizziness (both groups)



between the study groups. In group 1 patients, statistically significant difference was found between NRSs before - during procedure and NRSs during-after procedure (T test .000 CI 0.95, p<0.05). In group 2, a statistically significant difference was found between all three follow-up periods respectively (T test .000 and T test .000 and T test 0.058 CI 0.95, p<0.05). Compared to group1 patients, in group 2 patients average mild pain score recorded before the procedure remained mild pain score after the procedure although statistically significant.

The distribution of pain score within the individual groups is shown in **Chart 3** and **Chart 4**.

Drowsiness was present in 25% of patient population: 21% of patients in group 2 and 4% of patients in group 1 with a statistically significant difference between the groups (X^2 .000, p<0.05). Compared to drowsiness, there was no statistically significant difference among groups in occurrence of vomiting, headache, dizziness respectively (X^2 0.212 and X^2 0.748 and X^2 0.279, p≥0.05), **Chart 5**. In the entire patient population, 12,5% of patients with kidney-localized stones felt drowsiness without statistical difference between groups (X^2 0.229,p ≥0.05). Drowsiness was not reported by the patients with UP segment or ureter stone localizations.

Only during the procedure, severe pain intensity score was recorded in both study groups. Severe pain during the procedure was reported by 10% of patients from tapentadol group and by 3% of patients from tramadol/ketoprofen group. Severe pain was reported with statistical difference in distribution between the groups (X^2 0.005, p<0.05), but it was reported only by 7,6% of those with kidney-localized stones. In tapentadol group (group 2) 5% of patients reported severe pain with drowsiness compared to 1% of patients in tramadol/ketoprofen group (X^2 0.021, p<0.05). Moderate pain intensity after the procedure was reported by 6% of patients in group 2 and 1% of patients in Group1 (X^2 0.059, p ≥0.05). We found weak statistical correlation between drowsiness (with kidney stone localization) and NRS during the procedure (in group 1 Spearman $^{0.020}$, p<0.05 and in group 2 Spearman 0. 028, p<0.05). No correlation was found swithin study groups between drowsiness and NRS after the procedure in both groups of patients (group 1 Spearman $^{0.113}$, p≥0.05 and group 2 Spearman 0.113, p≥0.05). Multivariate regression analysis did not find any correlation between NRS after the procedure and the examined variables except for drowsiness with the statistical level close to significant (Sig. 0.072, CI 0.95, t -1.772).

DISCUSSION

The introduction of ESWL was revolutionary for the treatment of urolithiasis. However, ESWL causes shock wave pain during the treatment. The right dose of analgesics is mandatory to maintain the patient's comfort and can improve the result of the treatment (19). The pathogenesis of pain related to ESWL procedure has not been fully understood yet, but cavitations seem to play a key role rather than direct mechanical effects on the nociceptive nerve endings. The formation, movement and implosion of the resulting shock wave, microbubbles in body fluids or tissues lead to the stimulation of superficial nociceptors in the skin as well as deeper visceral nociceptors in the renal capsule, periosteum, pleura, peritoneum and muscles. Another component of pain associated with shock waves is the movement of the stone caused by the impact of the shock waves (6).

It has been found that several physical variables influence the treatment of pain: the type of shock wave source, the size and location of the stone load (e.g., an upper pole stone near the ribs), the peak pressure of shock waves, the diameter of the focal zone, and the size of the shock wave source orifice, which reflects an important role of the surface area of the shock wave entering the skin. In addition, patient-related factors such as age, gender and habitus are responsible for the sensation of pain during ESWL (7,8).

In our study, the mean pain score during the procedure ranged from NRS 3.39± 1.32 to NRS 4.05± 1.88 (moderate pain) depending on the subject group. Even though statistical significance in pain intensity was recorded between follow-up periods in tapentadol group, a

clinical significance was not found (average pain intensity scores < 1/10 NRS). Also, in tramadol/ketoprofen group there was no clinical and statistical significance in perioperative pain scores (before and after the procedure). Only 6,5% of the patient population reported severe pain during procedure (NRS 7/10) with statistical significance between the groups. After completing the procedure, average mild pain intensity was reported in both groups of patients. Both results indicate good pain control after the procedure related both applied analgesic regimens. Bovelander E. et al. reported the mean pain score described as "5", which is a relatively high pain score, considering that analgesics are administered (20). In addition, one third of the patients had severe pain (pain score 7–10). These data show that high pain scores are associated with lower intensity during ESWL. This suggests that pain avoidance protocol is not sufficient and should be revised. Additional analgesics (and a combination of paracetamol, NSAIDs and opioids) reduced the mean pain score and improved the patients' well-being. Also, they reported no significant difference in pain scores between the patients who received additional opioids (n = 46) and those who did not. This is likely due to the small group of patients who received opioids. The study by Tokgoz et al. which analyzed pain perception during ESWL supports these findings by describing the mean pain score for the first ESWL session as 4.67 on the NRS scale (1).

Even with the latest generation of lithotripters, ESWL is still a potentially painful procedure and adequate analgesia is essential for good treatment outcomes. General anesthesia should be reserved for selected cases and the treatment of children; the same applies to spinal anesthesia. Both guarantee optimal pain control but have high personnel, resource and management requirements leading to a longer recovery time, making them less suitable for ESWL as an outpatient procedure.

In this context, inhalation anesthesia with nitrous oxide is another very interesting option as it provides good analgesia, is easy to use and does not lead to a prolonged recovery period. Subcutaneous infiltration with local anesthetics has also proven to be effective in terms of pain control and safety, as it avoids the side effects of opioids. The concept of dermal anesthesia is not new, but remains an interesting option due to the ease of application and convenience for the patient, but it has not shown the best results.

Opioids, sometimes in combination with sedatives or NSAIDs, are classic pain control agents for ESWL. They have a very good analgesic effect, but sometimes unpleasant side effects as well, they require monitoring the patient and lead to a delayed discharge of the patient. Analgosedation and patient-controlled analgesia lead to good pain relief and patient satisfaction, but are expensive and also limited in the outpatient setting.

NSAIDs are very convenient for both surgeons and patients. They are easy to use, do not require patient monitoring and patients can be discharged immediately after surgery. NSAIDs mainly act in the area of pain transmission and modulation. Peripheral prostaglandins influence the development of hyperalgesia, and for many years it was thought that inhibition at the site of inflammation was the main mechanism of action of NSAIDs. Prostaglandins stimulate a certain number of primary afferent nerve fibers, the so-called "silent" nociceptors, and thus cause primary hyperalgesia via tetrodoxin-resistant Na+ channels. Prostaglandins act on nociceptors in the periphery, also produced in the dorsal horns of the spinal cord in response to peripheral inflammation (16,17).

Substances such as paracetamol and tamsulosin are often used for other indications and have recently come into focus, but do not have a significant place in ESWL analgesia. Their very favorable side effect profile makes them very interesting, although the analgesic component, especially of tamsulosin, is not convincing (21).

A study by Hashem A et al. compared safety and efficacy of xylocaine gel and ketorolac as opioid-sparing analgesics versus pethidine for shock wave lithotripsy (ESWL) pain (22). A single-blind, randomized, controlled trial (RCT) was conducted in 132 patients with renal and upper ureteral stones which were eligible for treatment with the ESWL procedure.

The first group of patients received intravenous pethidine and placebo gel; the second group received IV ketorolac plus placebo gel; the third group received topical lidocaine gel plus IV normal saline. Dissolution of the stone was classified as none (no change from baseline by kidney, ureter, X-ray, or ultrasound), partial (fragmented and residual fragments > 4 mm), and complete (≤4 mm residual fragments).

The disintegration of the stone was assessed by X-ray of the bladder and ultrasound. Pain was assessed using the Numerical Pain Rating Scale (NRS). NRS scores were highest in the xylocaine group at 10, 20 and 30 minutes (p=0.0001), with no significant difference between the ketorolac and pethidine groups except at 10 minutes (p=0.03) and almost significant difference at 30 minutes (p=0.054) in favor of ketorolac. The results for stone dissolution (no, partial or complete dissolution) were as follows: 25 (50.0%), 23 (46.0%), and 2 (4.0%) for pethidine; 19 (35.8%), 23 (43.4%), and 11 (20.8%) for ketorolac; and 26 (89.7%), 3 (10.3%), and 0 (0.0%) for lidocaine (p=0.008). The authors concluded that the use of ketorolac was a safer and more effective alternative to morphine derivatives for ESWL analgesia. Lidocaine gel should not be used as monoanalgesia for ESWL (23).

Bovelander E. and colleagues showed in their study that there was a correlation between the severity of pain and the success of the ESWL procedure (24,25). Non-steroidal anti-inflammatory drugs such as diclofenac or ketoprofen and opioids such as tramadol are most commonly used to prevent and treat pain during and immediately after the ESWL procedure (26). Tramadol is a synthetic analgesic. It acts via NOP, KOP and DOP receptors and

then unfolds its effect as an opioid analgesic, i.e. it inhibits its nociception. Another mode of action of tramadol is to block the uptake of serotonin and noradrenaline and then act as a non-opioid analgesic. The properties of tramadol are reflected in the rapid absorption, the effect after 30 minutes, the manifestation of the maximum effect after 1-2 hours and the application intervals of 5-6 hours (27).

Today, oral pain therapy is increasingly used for acute pain. Tapentadol is a new central analgesic with a dual mechanism of action in a single molecule: μ-opioid receptor agonist and noradrenaline reuptake inhibitor (MOR-NRI) (19,20). Moderate affinity for the μ-opioid receptor and the opioid-sparing effect of noradrenaline reuptake inhibition allow for the occurrence of fewer side effects. Effects are compared to other μ-agonists (28,29). The most recent recommendations of the European Association of Palliative Care (from 2012) did not include this new drug as it was not available until after they were produced (30).

Viscisi ER et al. showed good analgesic properties of tapentadol and its excellent tolerability in the treatment of acute postoperative pain (31).

All these studies have shown so far that there is no universal combination of drugs that prevents the occurrence of pain 100% and that this depends on a number of factors. However, it is also clear that our respondent groups were satisfied with the therapy used and that analgesics should be used,even per os in the form of the opioid tapentadol if administered at the right time.

CONCLUSION

Severe pain was reported only during the ESWL procedure in both study groups with no statistical difference between the groups. In tapentadol group of patients, statistically significant difference was also found in pain intensity scores before and after the procedure compared to tramadol/ketoprofen group. Severe pain intensity score during the procedure was reported only by the patients with kidney stone localization. The size of the stone had no effect on the intensity of pain during ESWL but it influenced drowsiness whose occurrence was related to stone localization. Although statistical difference was noted, the clinical significance in terms of pain intensity scores before and after the procedure was not found. Both protocols ensured safety and low pain intensity scores after the procedure.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation and data collection were performed by Aleksandar Vuksanović and Nikola Lađević. The first draft of the manuscript was written by Aleksandar Vuksanović, Nikola Lađević, Jelena Jovičić, Vesna Jovanović, Nataša Petrović and Miloš Lazić. Nebojša Lađević and Ivana Likić Lađević edited the manuscript. All authors read and approved the final manuscript.

Consent to participate

Written informed consent was obtained from the patient.

Consent to publish

The authors affirm that the patient provided informed consent for publication.

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PREVENTIVNA PRIMENA ORALNOG TAPENTADOLA SA BRZIM DEJSTVOM NASPRAM PRIMENE INTRAMUSKULARNOG TRAMADOLA/KETOPROFENA SA CILJEM SMANJENJA BOLA TOKOM I NAKON EKSTRAKORPORALNE LITOTRIPSIJE UDARNIM TALASIMA KOD BUBREŽNOG KAMENA

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Sažetak

Uvod: Ekstrakorporalna litotripsija udarnim talasom (ESWL) ranije je rađena u opštoj anesteziji, ali se danas, iako bolna procedura, radi u analgosedaciji. Cilj studije je da se utvrdi jačina akutnog bola povezanog sa ESWL-om korišćenjem dva uporedna protokola preeemptivnih analgetika: tramadol plus ketoprofen nasuprot tapentadolu.

Metode: Klinička, prospektivna, randomizovana kohortna studija obuhvatila je 200 pacijenata oba pola starosti od 18-80 godina koji su podeljeni u dve grupe: grupa 1 – primila kombinaciju ketoprofena 100 mg/tramadola 50 mg i.m. 30 minuta pre procedure; grupa 2 – primala tapentadol IR 50 mg oralno 1 sat pre procedure. Intenzitet bola (NRS) i komplikacije su evidentirani pre, tokom i na kraju zahvata.

Rezultati: Nije nađena razlika u preoperativnim karakteristikama populacije pacijenata i veličini i lokalizaciji kamena. Veličina kamena lociranog u bubregu u grupi 2 je bila statistički značajno veća (T- test .000). U svakoj grupi došlo je do statistički značajnog povećanja intenziteta bola pre i tokom operacije (T-test .000) kao i do smanjenja intenziteta bola tokom i nakon procedure bez razlike među grupama. U grupi 2, 10% pacijenata je imalo jake bolove tokom postupka u poređenju sa 3% u grupi 1 (Mann-Whitney 0.005). U podgrupama sa jakim bolom svake grupe pacijenata, mučnina se javila kod 5% pacijenata u grupi 2, što je značajno više od 1% u grupi 1(ukupna incidenca u obe grupe je 25%). **Zaključak:** Iako oba protokola obezbeđuju prosečno umeren intenzitet bola tokom procedure, jak bol i mučnina su češće primećeni u grupi koja je primala tapentadol, što je povezano sa renalnom veličinom kamena i plasiranjem JJ stenta.

Ključne reči: preemptivna analgezija, tapentadol, ESWL procedura

Primljen: 05.12.2023. I **Revizija:** 31.01.2024. I **Prihvaćen:** 29.02.2024.

Medicinska istaživanja 2024; 57(2):33-40

Medicinska istaživanja 2024; 57(2):41-47 | DOI 10.5937/medi57-47995

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

The prognostic significance of the age-adjusted Charlson comorbidity index in the prediction of postoperative outcome after liver resection for colorectal cancer metastases

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Recived: 11 December 2023

Revised: 29 January 2024

Accepted: 01 April 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: The prognostic value of the age-adjusted Charlson comorbidity index (ACCI) for patients with colorectal liver metastases (CRLM) undergoing liver resection is still unclear. The aim of this study is to analyze the impact of ACCI in the prediction of short- and long-term outcomes after liver resection in patients with CRLM.

Material and methods: Data related to 101 patients who underwent liver resection for CRLM at the University Clinic for Digestive Surgery between October 2019 and October 2022 were analyzed in this cohort retrospective study. ACCI was determined according to an established point scale. Patients were further divided into two groups: group 1, ACCI <7, and group 2, ACCI ≥7.

Results: There was no statistically significant difference in the length of postoperative ICU stay (p=0.9670), semi-ICU stay (p=0.627), and hospital stay (p=0.243). Overall morbidity was higher in group 1 (60%) than in group 2 (39.3), p=0.042, while major morbidity (grade ≥3) was similar between groups, p=0.127. Biliary fistula was more common in group 1 compared to group 2 (12.5% vs 1.6%), p=0.035. In-hospital mortality, 30-day, and 90-day mortality were similar between the study groups (p=1; p=0.517 and p=0.517). During the follow-up period, recurrence was registered in 48.5% of patients. There was no difference in recurrence-free survival between groups, p=0.430. The overall survival was similar between the groups, p=0.141.

Conclusion: ACCI can be used to predict postoperative morbidity after liver resection for CRLM. The postoperative mortality and recurrence-free survival are similar regardless of age and comorbidity.

Keywords: colorectal cancer, liver metastases, Charlson comorbidity index

Cite this article as: Đorđić A, Bogdanović A, Zdujić P, Đinđić U, Basarić D, Dugalić V. The prognostic significance of the age-adjusted charlson comorbidity index in the prediction of postoperative outcome after liver resection for colorectal cancer metastases; Medicinska istaživanja 2024; 57(2):41-47 DOI: 10.5937/medi57-47995

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the second leading cause of cancer-related death worldwide (1). In 2020, the prevalence of CRC was 1.9 million, while 0.9 million people died of this disease.

Colorectal liver metastases (CRLM) are present in about 17% of patients at the time of diagnosis of CRC (synchronous metastases) (2). An additional third of patients with CRC will develop CRLM during their disease (metachronous metastases) (3) Liver resection is currently the only potentially curative treatment option.

Comorbidities are chronic diseases affecting the patient's quality of life. The impact of comorbidities on the treatment plan and prognosis was previously confirmed. In 1987, anesthesiologist Mary Charlson introduced a new prognostic parameter – the Charlson comorbidity index (CCI) (4). The authors performed a longitudinal study, analyzing the impact of 17 most common comorbidities on one-year survival. A certain point was assigned to each disease according to the relative risk of dying from the disease under investigation, whereby a higher number of points meant a more severe degree of comorbidity. A few years later, the age-adjusted Charlson Comorbidity Index (ACCI) was introduced as a clinical predictor of the short-and long-term survival (5). The modified CCI considers age as an additional risk of postoperative complications.

Traditionally, ACCI was demonstrated as an optimal predictor of treatment outcomes of benign diseases including renal, heart, and pulmonary diseases (6-8). The prognostic impact of ACCI was also confirmed in patients with malignant conditions (9-10). Previous studies analyzed the predictive value of ACCI in terms of short- and long-term outcomes after surgery for primary cancers of the digestive system (11-12). There is a lack of literature data about the predictive value of ACCI in patients with CRLM treated by liver resection. However, patients suffering from CRLM were at an advanced age with one or more coexisting comorbidities, which may affect treatment outcomes and long-term prognosis. Furthermore, patients with CRLM are usually presented with a more advanced stage of disease at the initial diagnosis, requiring perioperative chemotherapy. Aggressive onco-surgical management combined with a high level of comorbidities may lead to a poorer prognosis.

The aim of this study was to analyze the significance of ACCI in the prediction of short- and long-term postoperative outcomes after liver resection in patients with CRLM.

MATERIAL AND METHODS

Between October 2019 and October 2022, 101 patients who had undergone liver resection for CRLM at the University Clinic for Digestive Surgery, were included in this study. Patients with extrahepatic metastatic disease were excluded. All data were collected retrospectively from medical histories, and analyzed within this cohort retrospective study. The present research was approved by the Ethics Committee of the University Clinical Center of Serbia (N° 87/13). Informed consent was obtained prior to surgical treatment.

Preoperative patient preparation included a detailed physical examination, laboratory analyses, transabdominal ultrasound, and computed tomography or magnetic resonance imaging. All patients were examined by cardiologists and anesthesiologists. Demographic and clinical-laboratory parameters included sex, age, body mass index (BMI), ASA score, localization of the primary tumor, involvement of regional lymph nodes, preoperative chemotherapy (HT), total bilirubin, aspartate aminotransferase values (AST) and alanine aminotransferase (AST), prothrombin time (PT), number of tumors and their size were recorded.

Table 1. Comorbidities included in the Charlson Comorbidity Index (CCI)

Points	Comorbidity	n (%)
1	Myocardial infarction	3 (2.9)
	CHF	
	CVA or TIA	
	Peripheral arterial disease	3 (2.9)
	Dementia	
	Chronic obstructive lung disease	
	Connective tissue disease	
	Peptic ulcer	
	Liver disease	
	Age	
2	Diabetes mellitus	2 (1.9)
	Hemiplegia	
	Leukemia	
	Lymphoma	
	Moderate to severe chronic kidney disease/ Solid tumor	4 (3.9)
3	Moderate to severe liver disease	
6	Metastatic disease	101 (100)
	AIDS	

*for every decade > 40 years, 1 point is assigned to each patient (up to 4 points)
CHF, congestive heart failure, CVA, cerebral vascular accident, TIA, transient ischemic attack, AIDS, acquired immunodeficiency syndrome

Operative details included the type and extent of resection, the duration of surgery, cumulative liver ischemia time, blood loss, and the need for intraoperative transfusion. Postoperative transfusion requirements, intensive and semi-intensive care unit stay, postoperative hospital stay, postoperative morbidity, and mortality were registered. The Clavien-Dindo classification was

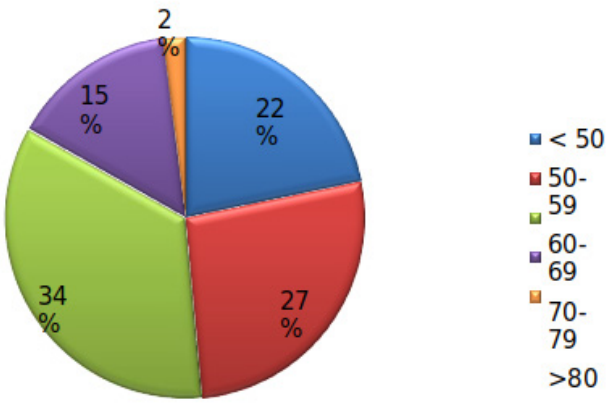

Figure 1. Age distribution

used for grading adverse events after surgery (13). Severe complications were defined as a score of 3 or more. Liver-specific complications including posthepatectomy liver failure, hemorrhage, and bile leakage were defined by the International Study Group of Liver Surgery (14-16). Mortality was defined as any death in the hospital, or within 30 and 90 days after surgery (hospital, 30-days, and 90-days mortality rate).

ACCI was calculated according to the established scale (**Table 1**). Age distribution is presented in **Figure 1**. Based on ACCI score, the patients were divided into two groups: group 1 (ACCI <7) and group 2 (ACCI ≥7). The two groups were compared according to preoperative, intraoperative, and postoperative data.

Patients were followed up every three months for two years after surgery. Abdominal CT or MRI was performed during the visit, while thoracic CT was performed annualy. Any appearance of metastatic disease during follow-up was defined as recurrence.

STATISTICAL ANALYSIS

Continuous variables are presented using median values (range) and were compared using Student's t-test. Nominal variables are presented as percentages and they were compared using the Pearson chi-squared test or Fisher's exact test, as appropriate. Two-tailed $P < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS for Windows, version 19.0. (SPSS Inc, Chicago, IL).

RESULTS

All 101 patients who underwent liver resection for CRLM at the University Clinic for Digestive Surgery were included in this study. Patients in group 2 were older than those in group 1 (66.7 years (range, 50-84) vs 49 years (range, 33-59), respectively), ($p<0.001$). The number of metastases was higher in group 1 than in group 2, $p=0.02$. Other demographic and clinical laboratory parameters are summarized in **Table 2**.

Table 2. Demographic and clinical characteristics

	Total (n=101)	Group 1 (n=40)	Group 2 (n=61)	p
Age	60 (33-84)	49 (33-59)	66.7 (50-84)	**<0.001**
Sex, n (%)				
Male	62 (61.4)	23 (57.5)	39 (63.9)	0.516
Female	39 (38.6)	17 (42.5)	22 (36.1)	
ASA score, n (%)				
I/II	99 (98)	40 (100)	59 (96.7)	0.247
III/IV	2 (2)	0	2 (3.3)	
BMI (kg/m2)	24.5 (18.7-167.7)	24.4 (18.7-167.7)	24.8 (18.7-34.2)	0.489
Tumor localization				
Colon	52 (51.5)	20 (50)	32 (52.5)	0,809
Rectum	49 (48.5)	20 (50)	29 (47.5)	
T stadium				
T1/2	12 (11.9)	2 (5)	10 (16.4)	0.118
T3/4	89 (88.1)	38 (95)	51 (83.6)	
N stadium				
N0	22 (21.8)	6 (15)	16 (26.2)	0.181
N1	79 (78,2)	34 (85)	45 (73.8)	
Preoperative CHT, n (%)	41 (40.59)	18 (45)	23 (37.7)	0.465
Total bilirubin (mmol/L)	15 (4.2-35.4)	14.9 (4.2-35.4)	15.1 (4.9-25)	0.295
AST (IU/L)	21 (11-67)	20.5 (12-67)	22.2 (11-65)	0.574
ALT (IU/L)	22 (10-187)	24,8 (10-187)	20 (10-115)	0.158
PT (INR)	0.9 (0.8-1.2)	0.9 (0.8-1.2)	0.9 (0.8-1.1)	0.289
Tumor size (mm)	30 (7-190)	30 (12-190)	30 (7-150)	0.511
Number of tumors	2 (1-21)	2 (1-21)	2 (1-10)	**0.020**

Bolded values are statistically significant. Data are presented as median (range) unless indicated otherwise.
BMI, body mass index; PT, prothrombin time, CHT, chemotherapy, ASA score, American Society of Anesthesiologists, AST, aspartate aminotransferase, ALT, alanine transaminas

Table 3. Operative details

	Total (n=101)	Group 1 (n=40)	Group 2 (n=61)	p
Extent of resection, n (%)				
Minor	79 (78.2)	28 (70)	51 (83.6)	0.105
Major	22 (21.8)	12 (30)	10 (16.4)	
Resection type, n (%)				
Atypical resection	70 (69.3)	29 (72.5)	41 (67.2)	
Segmentectomy	17 (16.8)	7 (17.5)	10 (16.4)	0.843
Sectorectomy	7 (6.9)	2 (5)	5 (8.2)	
Hepatectomy	7 (6.9)	2 (5)	5 (8.2)	
Type of resection, n (%)				
Non-anatomical	88 (87.1)	36 (90)	52 (85.2)	0.485
Anatomical	13 (12.9)	4 (10)	9 (14.8)	
Surgery duration (min)	290 (30-780)	300 (60-780)	270 (30-650)	**0.035**
Total ischemia time (min)	45 (10-240)	49 (10-240)	45 (10-175)	0.155
Blood loss (ml)	200 (0-4000)	275 (0-2000)	200 (40-4000)	0.447
Intraoperative transfusion, n (%)	9 (8.91)	6 (15)	3 (4.91)	0.150

Bolded values are statistically significant. Data are presented as median (range) unless indicated otherwise.

The operative time was longer in group 1 (median 300 min, range 60-780) compared to group 2 (median 270 min, range 30-650), p=0.035. All other operative data are listed in **Table 3**.

Postoperative transfusion rate was higher in group 1 (27.5%) compared to group 2 (9.8%). Overall perioperative transfusion rate was higher in group 1 (32.5%) than in group 2 (13.1%). There was no statistically significant difference in the length of postoperative ICU stay (p=0.9670), semi-ICU stay (p=0.627), and hospital stay (p=0.243).

Overall morbidity was higher in group 1 (60%) than in group 2 (39.3), p=0.042. Major morbidity (grade ≥3) was similar between the groups, p=0.127. Biliary fistula was more common in group 1 compared to group 2 (12.5% vs 1.6%), p=0.035. No statistical difference was observed in the incidence of liver failure and postoperative bleeding between the two groups: 10% vs 4.9% and 20% vs 6.6 %, respectively, p=0.430 and p=0.059, respectively.

Of the 61 patients with an ACCI score >7 (group 2), one (1.6%) patient died at hospital during postoperative recovery. There was no in-hospital mortality in patients with an ACCI score ≤7. In-hospital mortality was similar between the groups (p=1). No death occurred within 30 and 90 days after surgery in group 1, while two patients died within 90 days after surgery in group 2. The difference in 30-day and 90-day mortality was not statistically significant (p=0.517 and p=0.517, respectively).

The median follow-up was 10 (range, 1-33) months. One- and 2-year survival was 96% and 77% in group 1, respectively, and 81% and 65% in group 2. The overall survival was similar between the groups, p=0.141 **(Figure 2a)**. The median time to recurrence was 7 (range, 1-33) months. During the follow-up period, recurrence was registered in 48.5% of patients. Two-year recurrence-free survival was 31% in group 1 and 28% in group 2. There was no difference in recurrence-free survival between groups, p=0.430 **(Figure 2b)**.

DISCUSSION

The prognosis of various benign and malignant diseases is affected by age and comorbidities. This fact is particularly important for public health systems because the

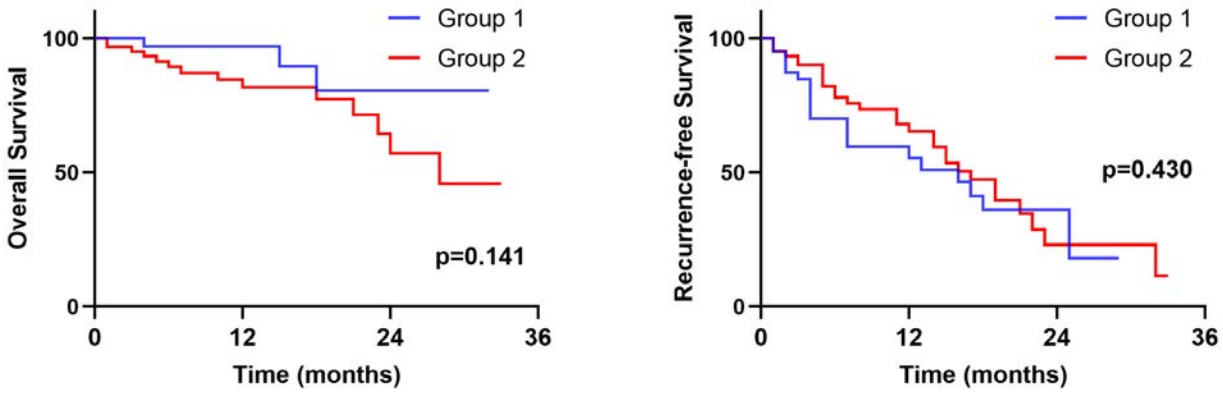

Figure 2. Overall (a) and recurrence-free survival (b)

proportion of the elderly population is gradually increasing. The present study is one of the rare reports of correlation analysis between ACCI score and postoperative outcomes in patients undergoing liver resection for metastatic disease.

Table 4. Postoperative parameters

	Total (n=101)	Group 1 (n=40)	Group 2 (n=61)	p
Postoperative transfusion, n (%)	17 (16.8)	11 (27.5)	6 (9.8)	**0.020**
Perioperative transfusion, n (%)	21 (20.8)	13 (32.5)	8 (13.1)	**0.019**
ICU stay, (days)	1 (0-11)	1 (0-3)	1 (0-11)	0.967
Semi-ICU stay, (days)	5 (2-28)	5 (3-26)	5 (2-28)	0.627
Postoperative hospitalization, (days)	7 (4-42)	8 (5-40)	7 (4-42)	0.243

Bolded values are statistically significant. Data are presented as median (range) unless indicated otherwise.
ICU, intensive care unit

The optimal cut-off value was determined using various methods in some previous studies designed to assess the association between ACCI scores and surgical outcomes after cancer treatment, including ROC curve analysis, X-tile software, or the value chosen from the literature (17-19). The threshold used in the current study was 7. Since all study participants had metastatic liver tumors, the minimal ACCI score was six in the entire cohort. Therefore, patients with additional points from comorbidity or age were classified in group 1, while those with more than one added point were classified in group 2.

Table 5. Postoperative morbidity and mortality

	Total (n=101)	Group 1 (n=40)	Group 2 (n=61)	p
Overall morbidity, n (%)	48 (47.5)	24 (60)	24 (39.3)	**0.042**
Major morbidity, n (%)	18 (17.8)	10 (25)	8 (13.1)	0.127
Liver-specific complications, n (%)				
Post-hepatectomy liver failure	7 (6.9)	4 (10)	3 (4.9)	0.430
Bile leakage	6 (5.9)	5 (12.5)	1 (1.6)	**0.035**
Postoperative bleeding	12 (12.9)	8 (20)	4 (6.6)	0.059
General complications, n (%)	10 (9.9)	4 (10)	6 (9.8)	1
In hospital mortality, n (%)	1 (1)	0 (0)	1 (1.6)	1
30-day mortality, n (%)	2 (2)	0 (0)	2 (3.3)	0.517
90-day mortality, n (%)	2 (2)	0 (0)	2 (3.3)	0.517

Bolded values are statistically significant. Data are presented as median (range) unless indicated otherwise

The present study showed that ACCI can predict postoperative overall morbidity and bile leakage. However, major morbidity and mortality were similar between the groups. Interestingly, patients with a lower ACCI score experienced higher overall morbidity and a higher percentage of bile fistula compared to those with a higher ACCI score. The result might be explained by the fact that younger patients with fewer comorbidities were selected for more extensive surgeries. Moreover, patients with lower ACCI scores had a higher number of tumor nodules in the liver, requiring more radical and more complex surgery related to a higher rate of postoperative complications. Different perioperative transfusion requirements between the groups were also the consequence of technically demanding liver resection in optimal surgical candidates.

The incidence of biliary fistula as a complication varies between 5.8 and 11% in different studies (20, 21). A study by Sadamori et al. showed that the duration of surgery ≥ 300 min is an independent risk factor for biliary fistula occurrence (22). In our study, patients who suffered from bile leakage also had prolonged operative time and were more often exposed to more complex resections where a higher percentage of bile leakage was expected.

The perioperative transfusion rate in the entire cohort was approximately 21%, while transfusion requirement was more common in patients with lower ACCI scores. According to the data of the National Surgical Quality Improvement Program of the American College of Surgeons, transfusion rates showed no statistically significant decreasing trends from 2014 to 2020 (18.13%-16.71%) (23). Prolonged operative time was associated with increased transfusion odds. Intraoperative transfusion was confirmed as an independent risk factor associated with a worse long-term prognosis (24). Furthermore, the restrictive policy of allogenic blood transfusion is recommended.

Similar major morbidity and postoperative mortality between the groups were highlights of the present study. Based on these findings, liver resection is an equally safe procedure in the elderly population weighted by comorbidity. Di Martino et al. reported similar major morbidity, liver-specific complications, and mortality among patients aged 75 and older characterized by higher ASA scores and patients younger than 75 (25). However, younger patients were presented with more intraoperative blood loss.

One- and 2-year overall survival and 2-year recurrence-free survival were similar between the groups. Despite higher overall morbidity, the two study groups showed no differences in long-term prognosis. In the study by Watanabe et al., early recurrence within 6 months after the initial hepatectomy was developed in 20.7% of patients undergoing resection for CRLM (26). In our cohort, approximately 49% of patients developed recurrence after the median follow-up of 10 months.

This study has several limitations. The strength of conclusions is decreased by the retrospective method and

the relatively small sample size. A larger study is needed to improve the predictive potential of ACCI. Additionally, a relatively short follow-up period should be replaced in future by five-year follow-up data, as it is a better indicator of long-term prognosis.

CONCLUSION

ACCI can be used to predict postoperative morbidity after liver resection for CRLM. Postoperative mortality and recurrence-free survival are similar regardless of age and comorbidity.

Conflict of interest

None to declare

Autor contribution

AD, the conception or design of the work, the acquisition, preparing of the draft of the manuscript; AB, the conception or design of the work, analysis, or interpretation of data, preparing the draft of the manuscript; PZ, the acquisition, preparing of the draft of the manuscript, analysis; UD, the acquisition, preparing of the draft of the manuscript, interpretation of data; DB, the conception or design of the work, the acquisition, analysis, or interpretation of data; VD, the conception or design of the work, interpretation of data, interpretation of revised version of manuscript.

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PROGNOSTIČKI ZNAČAJ ČARLSONOVOG INDEKSA KOMORBIDITETA PRILAGOĐENOG STAROSTI U PREDVIĐANJU POSTOPERATIVNOG ISHODA NAKON RESEKCIJE JETRE USLED METASTAZA KOLOREKTALNOG KARCINOMA

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Sažetak

Uvod: Prognostički značaj Čarlsonovog indeksa komorbiditeta (ČIK) prilagođenog starosti za bolesnike sa metastazama kolorektalnog karcinoma koji su podvrgnuti resekciji jetre je još uvek nerazjašnjen. Cilj ove studije je da analizira uticaj ovog indeksa u predviđanju kratkoročnog i dugoročnog ishoda nakon resekcije jetre kod bolesnika sa metastazama kolorektalnog karcinoma u jetri.

Materijal i metode: U kohortnu retrospektivnu studiju je uključen 101 bolesnik koji je podvrgnut resekciji jetre zbog metastaza kolorektalnog karcinoma, na Klinici za digestivnu hirurgiju u periodu od oktobra 2019. do oktobra 2022. godine. ČIK prilagođen starosti je određen prema bodovnoj skali. Bolesnici su prema broju bodova podeljeni u dve grupe: grupa 1 (ČIK <7), i grupa 2 (ČIK ≥7).

Rezultati: Ne postoji statistički značajna razlika u vremenu provedenom u jedinici intenzivnog lečenja (p=0,9670), jedinici poluintenzivnog lečenja (p=0,627) i vremenu provedenom u bolnici (p=0,243) nakon operacije. Ukupni morbiditet je veći u grupi 1 (60%) u odnosu na grupu 2 (39,3), p=0,042, dok su teške komplikacije (stepen ≥3) bile slične učestalosti kod upoređenih grupa p=0,127. Bilijarna fistula je češća u grupi 1 u poredjenju sa grupom 2 (12,5% vs 1,6%), p=0,035. Mortalitet u bolnici, tridesetodnevni i devedesetodnevni mortaliteti su slični (p=1; p=0,517 and p=0,517). Tokom perioda praćenja, recidiv bolesti je utvrđen kod 48,5% bolesnika. Nema značajne razlike u periodu bez recidiva bolesti, p=0,430. Ukupno preživljavanje je slično između grupa, p=0,141.

Zaključak: ČIK prilagođen starosti se može koristiti kao prediktor postoperativnog morbiditeta nakon resekcije jetre zbog metastaza kolorektalnog karcinoma. Postoperativni mortalitet i period bez recidiva bolesti su slični bez obzira na starost i komorbiditete.

Ključne reči: kolorektalni karcinom, metastaze u jetri, Čarlsonov indeks komorbiditeta

Primljen: 11.12.2023. I **Revizija:** 29.01.2024. I **Prihvaćen:** 01.04.2024.

Medicinska istaživanja 2024; 57(2):41-47

Medicinska istaživanja 2024; 57(2):49-55 | DOI 10.5937/medi57-48273

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Clinical characteristics and short-term outcomes of neonates with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia – a single-center experience from Serbia

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Recived: 18 December 2023

Revised: 08 February 2024

Accepted: 01 April 2024

Check for updates

Funding information:
The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Aim: This study aimed to report clinical characteristics and short-term outcomes of neonates with hypoxic-ischemic encephalopathy (HIE) treated with hypothermia. Furthermore, we analyzed the factors associated with short-term outcomes.

Material and Methods: We conducted a single-center retrospective study including neonates with HIE treated with hypothermia who survived hospital discharge. The short-term outcomes included normal or abnormal neurological examination findings on discharge.

Results: Forty-five neonates with HIE treated with hypothermia were identified. Median Apgar score at 1 minute was 3 (range 0-7), at 5 minutes it was 5 (range 1-10), while the median blood pH in the first postnatal hour was 6.94 (range 6.8-7.2). On admission, there were more neonates with moderate encephalopathy than those with severe encephalopathy according to the modified Sarnat classification (77.8% vs. 22.2%, p<0.001). Twelve (26.7%) neonates presented with complications during hypothermia. The most common complications were coagulopathy presented in 33.3% of cases and arterial hypotension presented in 25% of cases. Twenty-nine (64.44%) neonates had normal neurological examination, whereas 16 (35.56%) presented with abnormal neurological examination findings at discharge (0.006). Twenty-four (53.3%) neonates were discharged from hospital without any therapy. According to univariate analysis, abnormal neurological examination findings on discharge were significantly associated with pH < 7 (p=0.009), 5-minute Apgar score ≤ 5 (p=0.010), and the presence of complications during hypothermia (p=0.013). According to multivariate analysis, abnormal neurological examination findings on discharge were significantly associated with pH < 7 (p=0.030) and the presence of complications (p=0.035).

Conclusion: Our results during the first five years of experience with hypothermia support the beneficial effect of hypothermia in neonates with HIE.

Keywords: neonates, hypoxic-ischemic encephalopathy, therapeutic hypothermia

Cite this article as: Međo B, Karličić M, Z. Jovandarić M, Atanasković-Marković M, Raus M, M. Nikolić D, P. Nikolić D. Clinical characteristics and short-term outcomes of neonates with hypoxic-ischemic encephalopathy treated with therapeutic hypothermia – A single-center experience from Serbia; Medicinska istaživanja 2024; 57(2):49-55 DOI: 10.5937/medi57-48273

INTRODUCTION

Neonatal hypoxic-ischemic encephalopathy (HIE) is one of the leading causes of mortality and neurodevelopmental disability (1-3). Hypothermia has been an important intervention used in the treatment of neonates with HIE in developed and developing countries for many years. Multiple studies have confirmed beneficial effects of hypothermia in term neonates with HIE (4-6). A recently published systematic review evaluated the effects of hypothermia on clinical outcomes and found that hypothermia reduced neurologic disability and cerebral palsy in neonates with HIE. Another important finding of this review was the observation that the setting in which therapeutic hypothermia was implemented affected the outcomes (7). There are no published studies regarding hypothermia treatment in neonatology in Serbia. Therefore, we conducted a study to assess clinical characteristics and short-term outcomes of neonates with HIE managed with hypothermia in a tertiary level hospital in Serbia and to analyze the factors associated with short-term outcomes.

MATERIAL AND METHODS

The inpatient electronic medical records of neonates with HIE admitted to University Children's Hospital in Belgrade between February 2018 and October 2023 who were treated with therapeutic hypothermia and survived to hospital discharge were reviewed retrospectively. Hypothermia was introduced to the Neonatal Intensive Care Unit (NICU) of the University Children's Hospital in Belgrade therapeutic in February 2018.

Data extracted from the electronic medical records were demographic data, prenatal and perinatal events, the mode of delivery and the following neonatal characteristics: birth weight, restitution at birth, Apgar score, blood gases within the first postnatal hour, severity of encephalopathy on admission according to Sarnat criteria (8), the presence of early seizures, the use of inotropes, electroencephalography (EEG) and brain imaging reports, complications during hypothermia, complications during hospital stay, the duration of mechanical ventilation, NICU and hospital length of stay, and neurological examination findings on discharge. All extracted data were summarized and analyzed. Five neonates who had died were excluded from our study. The study was approved by the Ethics Committee of the University Children's Hospital in Belgrade (number 017 16/108).

Hypothermia method

Whole-body hypothermia therapy was initiated in all neonates admitted in the first 6 postnatal hours if they met the physiological and neurological criteria (5). The physiological criteria included a pH < 7.0, or base deficit ≥16 mmol/l in a sample of blood during the first hour after birth. If pH was 7.01-7.15, the base deficit 10-15.9 mmol/l, or blood gas was not available, additional criteria were required. These were acute perinatal events and either a 10-min Apgar score < 5 or assisted ventilation at birth for at least 10 min. The neurological criteria were the presence of seizures or confirmation of moderate or severe encephalopathy based on the modified Sarnat classification (8).

All neonates received whole-body cooling using the cooling system (Criticool–MTRE, Israel)

Core temperature was monitored via a rectal probe attached to the monitor. The target temperature was 33.5°C. Surface temperature was measured via a skin probe. Rewarming started after 72 h, and the temperature was increased gradually at a rate of 0.5 °C/h.

Clinical data

During hypothermia therapy, all vital signs, blood gases, EEG reports, serum electrolytes, and laboratory analyses were monitored and collected. All complications that occurred during hypothermia therapy were recorded. These included thrombocytopenia (<100,000 per μl), coagulopathy requiring treatment, persistent metabolic acidosis (persistent after 24 postnatal hours), arterial hypotension requiring treatment, intracranial hemorrhage, and pulmonary hypertension requiring treatment. Furthermore, complications that occurred during hospital stay, the duration of mechanical ventilation, NICU, and hospital length of stay were collected. All these data were summarized and analyzed.

Short-term outcomes

Short-term outcomes were defined as normal or abnormal neurological examination findings on discharge, assessed by attending neonatologist and pediatric neurologist.

Statistical analysis

The results were presented as whole numbers (N) and percentages (%), while continuous values were presented as mean values (MV) with standard deviation (SD) and medians with ranges. Student's t-test and Mann- Whitney U test were applied to compare continuous variables depending on the normality of distribution, and Chi-square test or Fishers exact test were used for categorical variables. The degree of association of different neonatal data with short-term outcome were expressed using odds ratio and 95% confidence interval. Neonatal data with $p<0.05$ using univariate analysis were included in the multivariate logistic regression model. A value of $p<0.05$ was considered as statistically significant.

RESULTS

The current study included 45 neonates with HIE treated with therapeutic hypothermia who survived hospital discharge. The maternal characteristics of these neonates are presented in **Table 1**. The type of delivery was vaginal in 48.89% of cases and cesarean section in 51.11% of cases (p=0.833).

Table 1. Maternal characteristics (n = 45)

Complications during pregnancy - n (%)	
Diabetes	4 (8.89%)
Hypertension	6 (13.33%)
Chorioamnionitis	3 (6.67%)
Hemorrhage	1 (2.22%)
Pre-labor rupture of membranes	1 (2.22%)
None	23 (51.11%)
Data were not available	7 (15.6%)
Complications during labor or delivery - n (%)	
Meconium-stained amniotic fluid	8 (17.78%)
Abnormal fetal heart rate	5 (11.11%)
Placental abruption	3 (6.67%)
Uterine rupture	1 (2.22^)
Cord prolapse	1 (2.22^)
Placenta previa	1 (2.22^)
None	21 (46.67%)
Data were not available	5 (11.11%)
Mode of delivery - n (%)	
Cesarean section	23 (51.11%)
Vaginal	22 (48.89%)

Characteristics of neonates are presented in **Table 2**. Among them 28 (62.2%) were males and 17 (37.8%) were females (p=0.020). Median Apgar score at 1 minute was 3 (range 0-7) and Apgar scored at 5 min. was 5 (range 1-10). Almost 90% of neonates were intubated and over 90% of them had already been intubated in the delivery room. More than half of the neonates (64.4%, p=0.006) did not require resuscitation at birth, whereas more than half of those who did (62.5%, p<0.001) required only chest compressions. The median blood pH in the first postnatal hour was 6.94 (range 6.8-7.2), and the median base deficit was 19.82 (range 32-12) mmol/l. We found it statistically significant (<0.001) that more neonates on admission had moderate than severe encephalopathy according to the modified Sarnat classification (77.8% vs. 22.2%). Twenty-one of 45 (46.7%) neonates had early clinical seizures. When we analyzed EEG data during cooling, we found that in 40 (88.9%) neonates, EEG recordings were abnormal and that in 22 (55%) of these neonates EEG recordings revealed non-specific encephalopathy as a predominant pattern. Furthermore, we found that 10 (25%) neonates had seizures present on EEG.

Twelve (26.7%) neonates presented with complications during hypothermia therapy. Eleven neonates had one complication, whereas one neonate developed coagulopathy with thrombocytopenia and intracranial (intraparenchymal) hemorrhage and in this case, cooling was discontinued due to complications. The most common complications were coagulopathy presented in 4 (33.3%) cases and arterial hypotension presented in 3 (25%) cases, followed by persistent metabolic acidosis in 2 (16.7%), thrombocytopenia in 2 (16.7%), intracranial hemorrhage in 2 (16.7%), and pulmonary hypertension in 1 (8.3%) case.

Table 2. Neonatal characteristics (n = 45)

Male - n (%)	28 (62.2%)
GA (weeks) - mean ±SD	38.71±1.93
Birth weight (g) - mean ±SD	3233.82±477.53
Apgar score at 1 min - median (range)	3 (range 0-7)
Apgar score at 5 min - median (range)	5 (range 1-10)
Intubations - n (%)	40 (88.89%)
Intubation in delivery room - n (%)	37 (82.22%)
Resuscitation at birth - n (%)	16 (35.56%)
Chest compressions only - n (%)	10 (22.22%)
First blood pH - median (range)	6.94 (range 6.8-7.2)
First base deficit (mmol/l) - median (range)	19.82 (range 32-12)
Fist lactate level (mmol/l) - median (range)	13.29 (range 6-24)
Moderate encephalopathy - n (%)	35 (77.78%)
Severe encephalopathy - n (%)	10 (22.22%)
Early clinical seizures - n (%)	21 (46.67%)
Inotropic support - n (%)	7 (15.56%)
Abnormal EEG during hypothermia - n (%)	40 (88.89%)
Seizures on EEG - n (%)	10 (22.22%)

Moreover, we analyzed the complications that occurred during hospital stay and found that 16 (35.6%) neonates developed some complications. Four (25%) neonates developed culture-proven early-onset sepsis, 3 (18.7%) developed hyperglycemia, 2 (12.5%) of each developed hyperbilirubinemia and hypoglycemia, and 2 (12.5%) of each presented with respiratory distress and symptomatic patent ductus arteriosus. One (6.2%) neonate developed necrotizing enterocolitis.

The mean duration of mechanical ventilation was 4.14±2.98 days. Most neonates (88.9%) required respiratory support > 48 hours.

Unfortunately, brain magnetic resonance imaging (MRI) was performed on only 6 (13.3%) neonates before discharge, and 4 (66.7%) of these had signs suggestive of HIE, while a CT scan was performed on 2 (4.4%) neonates before discharge. In neonates with serious complications in whom hypothermia was discontinued, both MRI and CT scans of the brain were performed during hospital stay.

The mean NICU length of stay was 6.84±2.82 days while the mean hospital length of stay was 17.69±8.99 days. Out of 45 neonates with HIE treated with hypothermia, 29 (64.44%) had normal neurological examination findings, whereas 16 (35.56%) neonates presented with abnormal neurological examination findings on discharge and the difference was significant (p=0.006). Moreover, 24 (53.3%) neonates were discharged from hospital without therapy, whereas 21 (46.7%) neonates were discharged with anticonvulsant therapy.

Table 3. Associations between abnormal neurological examination findings on discharge and selected neonatal parameters.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	OR (95% CI)	p
Sex				
female	1		-	-
male	2.438 (0.633-9.380)	0.195		
Gestational age				
< 37 GN	1		-	-
≥ 37 GN	1.458 (0.249-8.539)	0.676		
Birth weight				
< 2500g	1		-	-
≥ 2500g	1.458 (0.249-8.539)	0.667		
Blood pH				
pH ≥ 7	1			
pH < 7	7.091 (1.643-30.607	*0.009*	6.656 (1.200-36.917)	0.030
Base deficit				
≤ 16 mmol/l	1		-	-
> 16 mmol/l	3.684 (0.695-19.528)	0.125		
Apgar score at 5 min				
>5	1			
≤ 5	5.775 (1.521-21.932	*0.010*	4.642 (0.995-21.655)	0.051
Early seizures				
no	1		-	-
yes	1.231 (0.362-4.182)	0.739		
Sarnat classification				
severe encephalopathy	1		-	-
moderate encephalopathy	0.458 (0.110-1.916)	0.285		
Use of inotropes				
no	1		-	-
yes	0.256 (0.028-2.341)	0.227		
Seizures present on EEG				
no	1		-	-
yes	2.182 (0.522-9.120)	0.285		
Complications during hypothermia				
no				
yes	1			
	6.250 (1.480-26.387)	0.013	5.897 (1.134-30.662)	0.035

Furthermore, using univariate analysis **(Table 3)**, we found that abnormal neurological examination findings on discharge were significantly associated with pH < 7 in first postnatal hour (p=0.009), 5-min Apgar score ≤ 5 (p=0.010) and the presence of complications during hypothermia (p=0.013). According to multivariate analysis, abnormal neurological examination findings on discharge were significantly associated only with pH < 7 (p=0.030) and the presence of complications during hypothermia (p=0.035). For Apgar score ≤ 5 as a risk factor, the significance was borderline (p=0.051).

DISCUSSION

In this study, we reported clinical characteristics and short-term outcomes of neonates with HIE treated with therapeutic hypothermia. Furthermore, among different neonatal data, we assessed which of them could be associated with abnormal neurological examination findings on discharge.

We found 45 neonates with HIE treated with therapeutic hypothermia who survived hospital discharge during the period of 5 years. Although our institution is a tertiary level hospital, hypothermia was only introduced to our center in February 2018. The fact that in our center, neonates with HIE received hypothermia only if they were admitted to the NICU in the first six postnatal hours, could influence the number of neonates in our study. Possibly, some neonates with HIE who met the criteria for hypothermia were not treated if they were admitted after the sixth hour, especially given the fact that many neonates were born in hospitals located far from our hospital and that there were many problems with transporting these children. Limited transport services with trained medical staff for neonatal care and insufficient equipment are the most frequent reasons for delayed transfer to the NICU.

We used the six-hour limit according to the recommendations (3, 5, 9-11) and data that hypothermia should be initiated within the first six hours for effective neuroprotection (12), although there are different data in the literature. It was suggested that cooling should be started

in the first three hours, to achieve an optimal neuroprotective effect (13). On the other hand, in some studies, hypothermia therapy was started after the sixth hour of life, within the first eight hours (14).

In this study, hypothermia was applied more often in males than in females. These results are consistent with some of the previously published studies where in the group of newborns treated with hypothermia, the male gender was more common (14-16). Furthermore, the median Apgar score at 1 min, at 5 min, and median blood pH of the neonates was similar to other studies (14, 16-18). In our study group, nearly 90% of neonates were intubated, and over 90% of them were already intubated in the delivery room. A large number of intubated and ventilated neonates on admission to the NICU could be a true reflection of the neonates' degree of illness in this study. However, it is statistically significant that more neonates on admission had moderate (77.8%) than severe encephalopathy (22.2%,). This could explain the good outcome in more than half of neonates (64.44%) in our study bearing in mind that hypothermia is shown to be more beneficial in neonates with moderate encephalopathy compared to those with severe encephalopathy (4,19,20). Other authors studying hypothermia treatment reported more neonates with moderate than severe encephalopathy as well (14-17, 21). In the current study, 25% of neonates had seizures present on EEG, consistent with the rate reported in a large study from the USA involving over 900 children (22).

Approximately one-quarter of neonates (26.7%) presented with complications during hypothermia therapy in this study. The most common complication was coagulopathy requiring treatment presented in 33.3% of cases, which is in accordance with the published data [18–43%] (5,6,17). On the other hand, some authors reported higher frequencies of complications [48-50%] (14,18). In some of the previously published studies, none of the neonates with coagulopathy had significant bleeding (14). In our study, one neonate developed coagulopathy with thrombocytopenia and that was probably the reason for the occurrence of intracranial hemorrhage. This was the only case in our study when cooling was discontinued due to complications. The second most frequent complication was arterial hypotension re+quiring treatment with inotropes. It was confirmed in 25% of cases. In previously published studies these data were variable, reported in 17-40% of cases (14,18).

In addition, we found that hospital length of stay was 17.6 days, which is longer compared to other studies (13-14 days) (17, 22). Severe illness in the newborns on admission and a large number of intubated and ventilated neonates could have affected the length of hospital stay in the current study.

In the current study, 64.44% of neonates had a normal neurological examination, whereas 35.56% presented with abnormal neurological examination findings on discharge, which was a similar finding to a study in India. Catherine et al. studying 78 neonates with HIE treated with hypothermia, reported more normal survivors on discharge than those with neurological abnormality (23). Moreover, 53.3% of neonates in our study, were discharged from hospital without any therapy.

Another important finding in this research is that abnormal neurological examination findings on discharge were significantly associated with pH < 7, 5-min Apgar score ≤ 5, and the presence of complications during hypothermia. Furthermore, in multivariate analysis perinatal and postnatal data significantly associated with abnormal neurological examination findings with pH < 7 and the presence of complications. Recently, Suppiej et al. found that a severe outcome, defined as death or severe neurological sequelae at 12 months of age, was significantly associated with Apgar score, Sarnat score, and cesarean section (24). However, in our study the significance of the Apgar score as a risk factor for abnormal neurological examination was borderline and we did not find a significant association with the Sarnat score. Wayock et al. studying early predictors of neurologic injury in neonates with HIE treated with hypothermia found that initial blood pH predicted a significantly increased risk of severe brain injury such as abnormal brain MRI at 7-10 days or death. These authors did not find that a 5-minute Apgar score <5 was associated with severe brain injury (25). Moreover, Ambalavanan et al. identified metabolic acidosis as a risk factor for brain injury related to HIE and indicated its importance in predicting neonatal outcomes. (26). To our knowledge, this is the only study that showed the presence of complications during hypothermia treatment was significantly associated with abnormal neurological examination findings on discharge.

Our study has several limitations. We collected data retrospectively. A small number of newborns were included and therefore only survivors were studied because the number of neonates who died was too small for statistical analysis. It is partly because this is a single-center experience and hypothermia was introduced quite late to our center. The fact that we did not assess the long-term outcomes, and neurodevelopmental impairments at one and/or two years of age is the most significant limitation of this study. However, it was shown that discharge exams may have predictive value for death and disability, more precisely an abnormal neurological exam at discharge was associated with a greater risk of death or disability (27). Another limitation is a lack of brain imaging data. Magnetic resonance imaging was performed on only 6 (13.3%) neonates before discharge from hospital due to the lack of trained pediatric radiologists and unavailable equipment.

CONCLUSION

The current study has shown that more than half of the neonates with HIE treated with hypothermia had normal neurological examination findings at discharge and were discharged from hospital without any therapy. We found that not only low initial blood pH but also different complications during hypothermia therapy were significantly associated with abnormal neurological examination on discharge. Further larger studies are required to assess mortality and long-term neurodevelopmental outcomes among survivors.

Conflict of interest

None to declare.

Author Contributions

Biljana Medjo, Marija Karlicic, Miljana Z Jovandaric, Marina Atanaskovic-Markovic, Misela Raus, Dimitrije M. Nikolic and Dejan P. Nikolic gave critical contribution in writing the paper and formulating basic idea of this work, acquisition and interpretation of data, drafting the work, as well as defining conclusions.

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KLINIČKE KARAKTERISTIKE I KRATKOROČNI ISHODI NOVOROĐENČADI SA HIPOKSIČNO-ISHEMIJSKOM ENCEFALOPATIJOM LEČENIH PRIMENOM TERAPIJSKE HIPOTERMIJE – ISKUSTVO JEDNOG CENTRA U SRBIJI

Biljana Međo[1,2], Marija Karličić[1], Miljana Z. Jovandarić[3], Marina Atanasković-Marković[1,2], Misela Raus[1,2], Dimitrije M. Nikolić[1,2], Dejan P. Nikolić[1,2]

Sažetak

Cilj rada: Cilj ovog istraživanja bio je da se opišu kliničke karakteristike i kratkoročni ishodi novorođenčadi sa hipoksično-ishemijskom encefalopatijom (HIE) koja su lečena hipotermijom. Porede toga, analizirali smo faktore udružene sa kratkoročnim ishodima.

Metode: Ova retrospektivna studija obuhvatila je preživelu novorođenčad sa HIE, koja su lečena hipotermijom u jednom centru. Kratkoročni ishodi obuhvatili su normalan ili patološki neurološki nalaz na otpustu iz bolnice.

Rezultati: Identifikovano je 45 novorođenčadi sa HIE lečenih hipotermijom. Mediana Apgar skora u 1. minutu iznosila je 3 (opseg 0-7), u 5. minutu 5 (opseg 1-10), dok je medijana pH krvi u prvom satu života bila 6,94 (opseg 6,8-7,2). Na prijemu je bilo više novorođenčadi sa srednjom nego sa teškom encefalopatijom prema Sarnatovoj klasifikaciji (77,8% vs. 22,2%, p<0.001). Dvanaestoro (26,7%) novorođenčadi imalo je komplikacije tokom hipotermije. Najčešće komplikacije bile su koagulopatija u 33,3% slučajeva i arterijska hipotenzija u 25% slučajeva. Bilo je 29 novorođenčadi (64,44%) sa normalnim nalazom, dok je njih 16 (35,56%) imalo patološki nalaz pri neurološkom pregledu na otpustu (0,006). Dvadeset četiri (53,3%) novorođenčeta su otpuštena iz bolnice bez terapije. Univarijantnom analizom utvrđeno je da je patološki nalaz pri neurološkom pregledu na otpustu značajno povezan sa pH < 7 (p=0,009), petominutnim Apgar skorom ≤ 5 (p=0,010) i prisustvom komplikacija tokom hipotermije (p=0,013). Multivarijantnom analizom, utvrđeno je da je patološki nalaz pri neurološkom pregledu na otpustu značajno povezan sa pH < 7 (p=0,030) i prisustvom komplikacija (p=0,035).

Zaključak: Naši rezultati i prvo, petogodišnje iskustvo sa terapijskom hipotermijom ukazuju na povoljan efekat ove terapije kod novorođenčadi sa HIE.

Ključne reči: Novorođenčad, Hipoksično-ishemična encefalopatija, Terapijska hipotermija

Primljen: 18.12.2023. I **Revizija:** 08.02.2024. I **Prihvaćen:** 01.04.2024.

Medicinska istaživanja 2024; 57(2):49-55

Medicinska istaživanja 2024; 57(2):57-64 | DOI 10.5937/medi57-48454

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Comparison of short-term maternal and neonatal outcomes between elective and emergent Cesarean section – a single center experience

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Recived: 28 December 2023
Revised: 22 January 2024
Accepted: 22 January 2024

Check for updates

Funding information:
None

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction/Aim: Increased rates of Cesarean section (CS) and subsequent short-term and long-term maternal complications (MC) and neonatal complications (NC) have been reported. The aim of this study was to compare short-term MC and NC between elective and emergent CS.

Material and Methods: Data from medical records of pregnant women who had undergone CS at Gynecology and Obstetrics Clinic "Narodni front" were retrospectively collected. The inclusion criteria were as follows: low-risk, term monofetal pregnancies with obstetrical CS-indications and other non-life-threatening maternal conditions (ophthalmological, orthopedic, psychiatric, lower genital-tract infections). Short-term MC were the following: surgical complications, inflammatory syndrome, the need for blood transfusion, and hospital stay ≥5 days. NC were as follows: respiratory morbidity, asphyxia, sepsis, injuries/lacerations, admission to neonatal intensive-care-unit, hospital stay >4 days.

Results: We included 1056 singleton pregnancies. Mean age was 32.63±5.38 years, mainly primipara 566 (53.6%). Of all CS, 774 (73%) were performed emergently. Cephalopelvic disproportion/fetal macrosomia and other CS indications carried a significantly high risk for emergent CS (OR=3.943, 95%CI 2.036-6.591; OR=7.560, 95%CI 3.994-8.327, respectively). Regardless of the urgency of CS there were no significant differences in the frequency of MC. Neonatal sepsis was significantly higher after emergent CS (p=0.027), with a two-times greater risk for its development (OR=2.070, 95% CI 1.072-3.997). There were no fatal maternal/neonatal outcomes and no need for additional care.

Conclusion: There were no notable disparities in MC and NC among the individuals who had undergone emergent and elective CS. Neonates born by means of emergent CS had a higher risk of developing neonatal sepsis. Indications for CS had a greater impact on short-term maternal and fetal outcomes than the type of CS.

Key words: elective Cesarean section, emergent Cesarean section, early maternal complications, early neonatal complications

Cite this article as: Lukić R, Lazić Mitrović T, Rovčanin M, Tomić A, Zamurović M. Comparison of short-term maternal and neonatal outcomes between elective and emergenT Cesarean section – A single center experience; Medicinska istaživanja 2024; 57(2):57-64 DOI: 10.5937/medi57-48454

INTRODUCTION

Cesarean section (CS) as a delivery mode is present in 28% to 32% of all births in developed countries (1) and its rate is growing globally, even though efforts are being made in many countries to reduce CS rate (1). There has been an explosive increase in CS rates and thus in some countries current CS rates are higher than 40% (1). Obviously, the CS rate has been increasing, with a parallel increase in costs due to short-term and long-term maternal and neonatal complications (2).

The short-term complications for mothers who have undergone CS include high rates of infection, massive hemorrhage, complications related to surgery, including death, urinary complications during and after CS, reduced likelihood of breastfeeding, as well as complications related to anesthesia (3, 4). The most prominent long-term maternal complication of CS is a great likelihood of subsequent CS complications such as: rupture of uterus or aberrant placentation, specifically placenta previa variations (3).

While CS may offer a high level of safety, short-term neonatal complications are still present in neonates: asphyxia, infections including sepsis, respiratory morbidity, and lacerations as a consequence of surgical procedures (5). Moreover, changes in physiological adaptability could have significant long-term effects on the immune system of newborns (6, 7). The incidence of SC anesthetic complications in mothers and newborns is extremely low, affecting only 0.5% of cases, involving difficulties with intubation, adverse responses to drugs, aspiration pneumonitis, and complications related to regional anesthesia (7).

The rise in CS complication rates can be attributed to various factors, including economic pressures faced by hospitals, the influence of private health care insurance, concerns about potential lawsuits (8). Thus, it is of great importance to develop (inter)national strategies to achieve optimal utilization of healthcare services and the concept of women-centered care. The aim of this study was to compare short-term maternal and neonatal complications between elective and emergent Cesarean Section in Gynecology and Obstetrics Clinic "Narodni Front".

MATERIALS AND METHODS

Study design and participants

The data from medical records of women who had undergone CS at the Clinic for Gynecology and Obstetrics "Narodni front", University teaching hospital, Belgrade, Serbia, from 1st January to 31st December 2018 were retrospectively collected and analyzed.

The study included all patients who had undergone emergent and elective CS with exclusively obstetrical indications or the ones that were not an acute threat to the mother or the fetus. The inclusion criteria were a low-risk, term pregnancy (from 37^{+1} to 41^{+0} gestational weeks) with the following obstetrical CS indications: breech presentation, cephalopelvic disproportion (CPD) with fetal macrosomia, previous CS. We also included patients who had undergone emergent and elective CS due to non-life-threatening maternal conditions (ophthalmological, orthopedic, psychiatric, and lower genital tract infections) that did not pose an immediate danger to the mother or the fetus. This study implemented exclusion criteria that encompassed pregnant women with multiple pregnancies, patients experiencing gestational or fetal complications, and pregnancies involving endangered fetuses during the peripartal period. We collected demographic data, CS urgency (elective or emergent), indication (obstetrical or "other"), and short-term maternal complications (surgical complications, inflammatory syndrome, blood transfusion, and hospital stay longer than five days) or neonatal complications (respiratory morbidity, asphyxia, suspected or proven sepsis, injuries and lacerations, admission to neonatal intensive care unit (NICU), and hospital stay longer than four days) data from patients' medical records.

Ethical consideration

The procedures conducted on human participants adhered to the ethical standards set by the Ethical Committee of the Clinic for Gynecology and Obstetrics "Narodni front" (Ethical Committee number 22008/2023/024435; 5th December 2023) and the 1964 Helsinki Declaration, or equivalent ethical standards.

Statistical analysis

The numerical data were reported as the mean accompanied by a 95% confidence interval or as the median along with the minimum and maximum values. The categorical variables were summarized using absolute numbers accompanied by their corresponding percentages. The Kolmogorov–Smirnov test was used to measure the normality of the data distribution for continuous variables. Categorical variables were subjected to appropriate analysis using the Chi-square test or Fisher's exact test. The Mann–Whitney–Wilcoxon or Kruskal–Wallis tests were utilized to analyze continuous variables that did not follow a normal distribution. Significant variables were further analyzed using univariate logistic regression to investigate the factors influencing the adverse maternal and short-term neonatal outcomes. The significance level for all analyses was established at 0.05. The statistical analysis was conducted using IBM SPSS statistical software (SPSS for Windows, release 25.0, SPSS, Chicago, IL).

Table 1. Maternal and neonatal clinical characteristics in relation to elective and emergent Cesarean section

		Cesarean Section Elective (N=282)		Emergent (N=774)		Total		p^*
		N	(%)	N	(%)	N	(%)	
Age groups (years)	< 35	161	(23.1%)	537	(76.9%)	698	(100%)	**<0.001**
	≥ 35	121	(33.8%)	237	(66.2%)	358	(100%)	
Parity	Primipara	81	(14.3%)	485	(85.7%)	566	(100%)	**<0.001**
	Multipara	201	(41.0%)	289	(59.0%)	490	(100%)	
CS indications								
Breech presentation	No	249	(26.9%)	678	(73.1%)	927	(100%)	0.758
	Yes	33	(25.6%)	96	(74.4%)	129	(100%)	
CPD / Macrosomia	No	259	(28.7%)	643	(71.3%)	902	(100%)	**<0.001**
	Yes	23	(14.9%)	131	(85.1%)	154	(100%)	
Previous CS	No	104	(15.4%)	570	(84.6%)	674	(100%)	**<0.001**
	Yes	178	(46.6%)	204	(53.4%)	382	(100%)	
Other CS indications	No	214	(45.0%)	262	(55.0%)	476	(100%)	**<0.001**
	Yes	70	(12.0%)	512	(88.0%)	582	(100%)	

CS - Cesarean Section; CPD - Cephalopelvic Disproportion; * Significant differences between CS groups were analyzed by Chi-square test ($p < 0.05$)

Table 2. Association between emergent CS and clinical parameters

Method		p - value*	OR	95% CI
Enter	Age over 35 years	**0.002**	0.601	0.438-0.825
	Multiparous	0.135	0.661	0.384-1.138
	CPD/macrosomia	**< 0.001**	3.246	1.923-5.478
	Previous CS	0.569	0.855	0.479-1.525
	Other CS indications	**< 0.001**	5.521	3.700-8.238
Backward	Age over 35 years	**0.002**	0.602	0.439-0.826
	Multiparous	**0.004**	0.592	0.415-0.846
	CPD/macrosomia	**< 0.001**	3.943	2.036-6.5.591
	Other CS indications	**< 0.001**	7.560	3.994-8.327

OR, Odds Ratio; CI, Confidence Interval; CPD, Cephalopelvic Disproportion; CS, Cesarean section;*significant at $p < 0.05$

RESULTS

In our study we included a total of 1056 women with singleton term pregnancies. Mean age of study participants were 32.63 ± 5.38 years, with age range from 16 to 56 years. There were 698 (66.1 %) pregnant women younger than 35 years, mainly primipara 566 (53.6 %). Of all CS, 774 (73 %) were performed emergently. Clinical characteristics of all participants together with indications for CS delivery are presented in **Table 1**.

Pregnant women who had undergone emergent CS were much more frequently presented in the group of participants younger than 35 years ($p < 0.001$), primiparous ($p < 0.001$) and with following indications: CPD/fetal macrosomia, previous CS along with all other CS indications stated in the methodology chapter ($p < 0.001$). Logistic regression modeling was used to analyze the relationship between dependent parameters (i.e., emergent CS) with only statistically significant clinical characteristics **(Table 2)**.

This analysis showed that age, CPD/fetal macrosomia, and all other CS indications highly correlated with urgent CS. Multiparous women, who were 35 old and older had a reduced risk for emergent CS ($p = 0.004$, $p = 0.002$, respectively). In contrast, CPD and fetal macrosomia, with other CS indications carried a significantly high risk for emergent CS (OR = 3.943, 95% CI 2.036 - 6.5.591; OR = 7.560, 95% CI 3.994 - 8.327).

As shown in **Table 3**, there were no significant differences in the frequency of maternal complications following CS, regardless of the urgency of the procedure. There were no fatal maternal outcomes and no need for transportation to other institutions for additional care.

Among the investigated parameters of neonatal complications following Cesarean Section **(Table 4)**, the occurrence of sepsis (either suspected or confirmed) was significantly higher following emergent cesarean section ($p = 0.027$).

However, there were no fatal neonatal outcomes and no need for transportation to other institutions for additional care. Regression analysis **(Table 5.)** confirmed this association ($p = 0.03$), where newborns had two times higher risk for developing sepsis after emergent CS (OR = 2.070, 95% CI 1.072 - 3.997).

Table 3. Maternal complications in relation to the emergent Cesarean section

		Cesarean section						*p* - value*
		Elective (N=282)		Emergent (N=774)		Total		
		N	(%)	N	(%)	N	(%)	
Surgical Complications	No	279	(26.7%)	765	(73.3%)	1044	(100%)	0.893
	Yes	3	(25.0%)	9	(75.0%)	12	(100%)	
Inflammatory syndrome	No	263	(27.0%)	711	(73.0%)	974	(100%)	0.451
	Yes	19	(23.2%)	63	(76.8%)	82	(100%)	
Transfusion after CS	No	269	(26.9%)	730	(73.1%)	999	(100%)	0.494
	Yes	13	(22.8%)	44	(77.2%)	57	(100%)	
Hospitalization (days)	≤5	217	(27.2%)	581	(72.8%)	798	(100%)	0.528
	>5	65	(25.2%)	193	(74.8%)	258	(100%)	

CS, Cesarean Section
* Significant differences between CS groups were analyzed by chi-square test ($p < 0.05$)

Table 4. Neonatal complications in relation to the urgency of CS

		Cesarean section						*p* *
		Elective (N=282)		Emergent (N=774)		Total		
		N	(%)	N	(%)	N	(%)	
Respiratory morbidity (RDS, TTN)	No	266	(26.4%)	741	(73.6%)	1007	(100%)	0.335
	Yes	16	(32.7%)	33	(67.3%)	49	(100%)	
Asphyxia	No	273	(26.6%)	754	(73.4%)	1027	(100%)	0.593
	Yes	9	(31.0%)	20	(69.0%)	29	(100%)	
Suspected or proven sepsis	No	271	(27.5%)	714	(72.5%)	985	(100%)	**0.027**
	Yes	11	(15.5%)	60	(84.5%)	71	(100%)	
Intracranial hemorrhage	No	274	(27.1%)	736	(72.9%)	1010	(100%)	0.144
	Yes	8	(17.4%)	38	(82.6%)	46	(100%)	
Neonatal injuries and lacerations	No	281	(26.9%)	763	(73.1%)	1044	(100%)	0.148
	Yes	1	(8.3%)	11	(91.7%)	12	(100%)	
Administration to NICU	No	258	(26.3%)	724	(73.7%)	982	(100%)	0.248
	Yes	24	(32.4%)	50	(67.6%)	74	(100%)	
Hospitalization (days)	≤4	201	(26.8%)	550	(73.2%)	751	(100%)	0.945
	>4	81	(26.6%)	224	(73.6%)	305	(100%)	

RDS, Respiratory Distress Syndrome; TTN, Transient Tachypnea of the Newborn; NICU, Neonatal Intensive Care Unit; * Significant differences between CS groups were analyzed by chi-square test ($p < 0.05$).

Table 5. The association between emergent CS and neonatal sepsis

	p* value	OR	95% CI
Emergent CS	0.03	2.070	1.072-3.997

CS, Cesarean Section; OR, Odds Ratio; CI, Confidence Interval; *significant at p<0.05

DISCUSSION

The results of our study showed the absence of significant differences in short-term maternal and neonatal complications between subjects who had undergone emergent and elective cesarean section, with suspected or proven neonatal sepsis being the only significantly frequent neonatal complication in neonates born with emergent cesarean section. Subjects who had had an emergent cesarean section were significantly younger and more frequently primiparas compared to subjects who had had elective cesarean section. Finally, cephalopelvic disproportion or fetal macrosomia, previous cesarean section, and non-life-threatening maternal conditions that did not pose an immediate danger to the mother or the fetus were significantly more frequent indications for emergent cesarean section.

Several studies investigated maternal outcomes between emergent and elective cesarean sections. A systematic review and meta-analysis by Yang et al. (9) showed that both rates of maternal complication and fetal complication were significantly higher in emergent cesarean deliveries. Moreover, the same meta-analysis also observed a significantly higher infant mortality rate in the emergent cesarean section group compared to elective cesarean deliveries (9). These findings may be attributed to the extended preparation duration, improved surgical preparation of obstetricians, and the enhanced health status of pregnant women (9). The authors also concluded that the emergent cesarean section indications were typically urgent and crucial, hence impacting the likelihood of complications (9). In 2020, Darnal and Dangal conducted a cross-sectional study in Nepal to investigate the maternal and fetal outcomes of 1324 emergent versus 456 elective cesarean sections (10). The participants in this study who had undergone emergent cesarean sections were significantly younger and more

frequently primiparas compared to women who delivered with elective cesarean section (10). Benzouina et al. presented similar results in their 2016 comparative cross-sectional study in Morocco (11). Our results are in accordance with these studies. The more common occurrence of emergent cesarean section among younger mothers may suggest that the obstetrician in charge is inclined to suggest vaginal deliveries in these cases, as long as it is possible, to preserve mothers' future reproductive performance, while cesarean delivery is only considered when there is a potential risk to either the mother or the fetus. Moreover, in cases of prolonged vaginal delivery, it is essential to prevent any complications that would affect younger mothers' ability to reproduce. The study by Darnal and Dangal also showed significantly higher complication rates in the emergent cesarean section group (10). The rates of postoperative wound infection, blood transfusion, fever, and intensive care unit admission were higher in patients who had emergent cesarean sections (10). A retrospective study from 2018 by Agrawal and Agrawal in India (12) presented an overall rate of intraoperative complications following cesarean deliveries of 11.08 %. The authors concluded that complications were mainly attributed to patients who delivered by emergent cesarean section (12). Similarly, Patel et al. conducted a retrospective observational study to compare maternal and neonatal outcomes between emergent and elective cesarean deliveries (13). The authors stated that the incidence of complications was substantially higher in the emergent group, affecting both the well-being of the mother and the fetus (13). In an institution-based cross-sectional study of 382 patients who had undergone cesarean section by Negese et al (14), the most common complications were surgical site infection, anemia, and intraoperative bleeding. The results of this study showed that emergent cesarean section was statistically associated with maternal complications (14). Conversely, Al Riyami et al. conducted a retrospective cohort study in Oman to compare the outcomes between emergent and elective cesarean sections (15). There were no notable disparities in maternal and neonatal complications between emergent and elective cesarean section besides temporary low blood pressure during surgery, maternal fever after the operation, and anemia (15). Similarly, in a prospective study of 300 women, Farag et al. found no statistically significant differences in maternal postoperative complications between the subjects who had had elective and emergent cesarean sections (16). These results are in accordance with our study. In our opinion, the main reason behind the absence of significant differences in short-term maternal outcomes between the two groups was the indication for emergent cesarean section in our study. Most of the published studies on this topic included patients with hypertensive disorder, preeclampsia, and placental abruption (9). Moreover, in a previously mentioned meta-analysis by Yang and Sun, most of the included studies were conducted in developing countries (9). Firstly, our study did not include diseases or life-threatening maternal conditions that would affect maternal outcomes after cesarean section. Furthermore, one of the explanations behind our results could be improved aseptic and antiseptic techniques and preoperative and intraoperative antibiotics usage in cases of emergent cesarean deliveries.

A study of 77.888 deliveries showed that, compared to vaginal, instrumental, and elective cesarean delivery, emergent cesarean section was associated with the highest probability of severe neonatal outcomes (17). Furthermore, the authors stated that cord prolapse, failed instrumental delivery, and small for gestational age (SGA) babies, were associated with the greatest odds of composite outcome (17). The study from 2006 by Elvedi-Gasparovic et al. showed significantly better Apgar scores in newborns delivered with elective cesarean section, while neonates delivered with emergent cesarean section had more frequent asphyxia and resuscitation (18). In a retrospective study of 6.854 deliveries, the incidence of low birth weight, stillbirths, and admission to the intensive care infant unit was higher among fetuses delivered via emergent cesarean section compared to newborns delivered with elective cesarean section (19). Benzouina et al. also found that the incidence of fetal complications was significantly elevated in the emergent cesarean group (11). Respiratory morbidity emerged as the primary contributor to fetal morbidity, with birth asphyxia being a subsequent concern, predominantly observed within the emergent group (11). The incidence of prematurity, birth asphyxia, respiratory morbidity, and admission to the NICU was found to be significantly higher in the emergent cesarean group compared to the elective cesarean group (11). De Luca et al. conducted a study that revealed that the incidence of fetal morbidity was lower in the elective cesarean group compared to the emergent cesarean group (20). However, the rates of perinatal mortality and respiratory morbidity were found to be similar in both groups (20). There is an ongoing debate about the association between cesarean section delivery and the development of respiratory morbidity in neonates. Many studies demonstrated that newborns delivered with cesarean section, either elective or emergent, were at a greater risk of developing respiratory morbidities compared to the ones born via vaginal delivery (21). A meta-analysis by Li et al. concluded that both elective and emergent cesarean sections were associated with an increased risk of neonatal respiratory distress syndrome (22). Furthermore, Indraccolo et al. found that cesarean delivery in the absence of labor presented a persistent risk of respiratory complications in newborns, regardless of their gestational age during the near-term and early-term periods (23). The authors further stated that delayed timing of planned cesarean section was associated with improved respiratory outcomes in newborns (23). Kleiner et al. conducted a study to investigate the impact of elective cesarean section on respiratory morbidity in newborns compared to emergent cesarean section (24). The researchers observed that the severity of respiratory morbidity was greater in newborns delivered

via elective cesarean section (24). The authors suggested that the physiological changes that occurred in fetal lungs during labor may play a role in this disparity (24). Evidence from a randomized controlled trial indicates that the administration of prophylactic corticosteroids before an elective cesarean section at term was likely to decrease the need for NICU admission due to respiratory morbidity (25). The efficacy of antenatal corticosteroid administration in reducing the incidence of respiratory distress syndrome or transient tachypnea of the neonate remains uncertain (26). However, it is important to note that the overall certainty of the evidence for these primary outcomes was determined to be low or very low (26). None of the indications for the emergent cesarean section in our study included diseases that were an immediate threat to the fetal well-being, nor the cases of fetal asphyxia or fetal distress. That could be the explanation for the absence of differences in neonatal complications between emergent and elective cesarean deliveries in our study.

Sepsis, a condition characterized by systemic infection, continues to be a significant contributor to both mortality and morbidity rates among neonates (27, 28). Among term infants, group B streptococcus (GBS) remains the predominant pathogen identified in cases of sepsis (27, 28). Center for Disease Control and Prevention issued a recommendation for universal antenatal screening during the period between 35 and 37 weeks of gestation, as well as intrapartum chemoprophylaxis, for all women colonized with GBS at the onset of labor or premature rupture of membranes, including those who have planned cesarean section deliveries (29). The optimal timing for the administration of intrapartum antibiotic prophylaxis is a crucial factor in ensuring its effectiveness (28). It has been observed that intrapartum prophylaxis is most efficacious when administered at least four hours before the onset of delivery (30). An international multisite prospective observational study from 2022 showed that birth and neonatal factors that corresponded with an increased likelihood of laboratory-confirmed sepsis encompassed preterm delivery, premature rupture of membranes, and cesarean section delivery when compared to spontaneous vaginal delivery (emergent cesarean delivery carrying a higher risk compared to the elective cesarean section) (31). The study also concluded that the acquisition of extended-spectrum β-lactamase-producing Enterobacterales, bacteria that are frequently associated with sepsis in healthcare environments, had been identified as a reported risk factor following the performance of cesarean sections (31). Cesarean sections are associated with extended hospitalization durations in comparison to spontaneous vaginal delivery, thereby potentially elevating the risk of neonatal sepsis (31). Contrastingly, in their systematic review and meta-analysis, Seyoum et al. revealed that cesarean delivery was not associated with neonatal sepsis (32). On the other hand, Adatara et al. stated that neonates delivered via elective cesarean section were 85% less likely to have neonatal sepsis compared to those delivered with emergent cesarean section (33). In our study, the newborns delivered with emergent cesarean section had two times higher risk for developing sepsis. One of the explanations for this result could be the unavailability of GBS status in patients who had undergone emergent cesarean section, but our study did not include the patients' GBS status due to insufficient medical records data regarding this determinant.

Our study has several limitations. The first is the small number of subjects due to the study type and the single-center nature of the study. Moreover, we did not include the exact gestational age of the newborns, but since several studies highlighted the influence of gestational age on the neonatal outcome, even for term deliveries, we think that the results solely reflect the type of cesarean section on the investigated study outcomes. Finally, there is a lack of data regarding the decision to deliver in cases of emergent cesarean section. Numerous studies highlighted the essential role of this interval since it significantly affects neonatal and maternal outcomes (34-36).

This study was conducted in a university hospital. Being one of the two obstetrical tertiary institutions in Serbia, our results could reflect the nationwide trends and outcomes after cesarean deliveries. Our results could also be valuable for the design and further implementation of cesarean delivery national protocols. Finally, due to specific inclusion and exclusion criteria, our results could highlight the importance of indications, rather than the type of cesarean delivery, on short-term maternal and fetal outcomes.

CONCLUSION

The findings of our study indicated that there were no notable disparities in the initial maternal and neonatal complications among individuals who had had emergent and elective cesarean sections. However, it is worth noting that neonates born by means of emergent cesarean section had a significantly higher incidence of suspected or confirmed neonatal sepsis compared to other complications. Our findings suggest that the indications for cesarean birth had a greater impact on short-term maternal and fetal outcomes than the type of cesarean delivery. Further studies are required to confirm these initial findings.

Conflict of interest

None to declare.

Ethical approval

Ethical Committee of the Clinic for Gynecology and Obstetrics "Narodni front" (Ethical Committee number 22008/2023/024435

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POREĐENJE KRATKOROČNIH MATERNALNIH I NEONATALNIH KOMPLIKACIJA KOD ELEKTIVNOG I HITNOG CARSKOG REZA – ISKUSTVO JEDNOG CENTRA

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Sažetak

Uvod/Cilj: Registruje se povećanje stope carskog reza (CR) i posledičnih pojava ranih i kasnih maternalnih komplikacija (MK) i neonatalnih komplikacija (NK). Cilj ove studije je da uporedi rane MK i NK nakon elektivnog i hitnog CR.

Metode: Retrospektivno su analizirani podaci pacijentkinja porođenih CR u ginekološko-akušerskoj klinici „Narodni front". Kriterijumi za uključivanje su bili sledeći: niskorizične, terminske, monofetalne trudnoće sa postojećim akušerskim indikacijama i neugrožavajućim stanjima trudnica. Rane MK bile su: hirurške komplikacije, inflamacija, potreba za transfuzijom, hospitalizacija ≥5 dana. Rane NK su bile sledeće: respiratorni morbiditet, asfiksija, sepsa, povrede/laceracije, boravak u jedinici intenzivnog lečenja, hospitalizacija >4 dana.

Rezultati: Analizirano je 1056 jednoplodnih trudnoća. Prosečna starost je bila 32.63±5.38 godina, većina primipara 566 (53.6%). Od ukupnog broja CR, 774 (73%) je bilo hitnih. Značajno veći rizik hitnog CR je zbog cefalopelvične disproporcije/makrozomije ploda i ostalih obstetričkih indikacija koje vitalno ne ugrožavaju majku (oftalmološke, ortopedske, psihijatrijske, infekcije donjeg genitalnog trakta) (OR=3,943, 95%CI 2,036-6,591; OR=7,560, 95%CI 3,994-8,327). Hitnost CR nije značajno uticala na pojavu ranih MK. Primećena je značajno veća učestalost neonatalne sepse nakon hitnog CR (p=0,027), koji je nosio dvostruko veći rizik za pojavu navedenog ishoda (OR=2,070, 95% CI 1,072-3,997). Nije bilo fatalnih meternalnih i neonatalnih ishoda, niti potrebe za daljim zbrinjavanjem.

Zaključak: Nije bilo statistički značajne razlike u pojavi MK i NK u odnosu na urgentnost CR. Novorođenčad rođena hitnim CR su imala veći rizik za pojavu neonatalne sepse. Same indikacije za CR su pokazale veći uticaj na pojavu ranih MK i NK u odnosu na tip CR.

Ključne reči: elektivni carski rez, hitan carski rez, rane maternalne komplikacije, rane neonatalne komplikacije

Primljen: 28.12.2023. I **Revizija:** 22.01.2024. I **Prihvaćen:** 22.01.2024.

Medicinska istaživanja 2024; 57(2):57-64

Medicinska istaživanja 2024; 57(2):65-73 | DOI 10.5937/medi57-48691

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

The impact of chronic viral hepatitis on COVID-19: clinical course and risk factors for poor outcome

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Recived: 28 December 2023

Revised: 22 January 2024

Accepted: 22 January 2024

Check for updates

Funding information:
The authors recived no specific grant from any funding agency in public, commercial, ot not-for-profit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction/Aim: The impact of chronic viral hepatitis on COVID-19 has not been fully clarified yet. The aim of this paper was to analyze the clinical features, course and outcome of COVID-19 in patients with chronic viral hepatitis and to determine the risk factors for unfavorable outcome.

Methods: A case-control study was conducted in which the case group included patients with chronic viral hepatitis suffering from COVID-19, while the control group included patients with chronic liver diseases of other etiologies; the patients were matched according to the stage of the liver disease. All subjects were treated at the Clinic for Infectious and Tropical Diseases in Belgrade from 1st March 2020 to 1st March 1 2022.

Results: Seventy-five patients with chronic liver diseases suffering from COVID-19 were analyzed – 25 with chronic viral hepatitis (13 HBV, 12 HCV) - case group, and 50 in the control group. In the case group, there were more males (76% vs. 72%) and younger patients (53.5±15.1 vs. 57.9±13.4 years), whereas in the control group there were more overweight patients (36% vs. 20%). In relation to comorbidities, there were more subjects with endocrinological diseases in the control group. The groups did not differ in terms of the severity of clinical features and the outcome of COVID-19. Risk factors for severe form of COVID-19 and lethal outcome were: cirrhosis, active liver disease, high fever, dyspnea, whereas vaccination was a protective factor against COVID-19.

Conclusion: The course and outcome of COVID-19 is similar in people with chronic liver diseases. Risk factors for poor outcome include advanced liver disease and dyspnea, while vaccination is a protective factor.

Keywords: chronic viral hepatitis, COVID-19, risk factors, outcome

Cite this article as: Mitrovic N, Nikolic N, Filipovic A, Vujovic A, Karic U, Sabanovic M, Milosevic I. The impact of chronic viral hepatitis on covid-19: clinical course and risk factors for poor outcome Medicinska istaživanja 2024; 57(2):65-73 DOI: 10.5937/medi57-48691

INTRODUCTION

Corona Virus Disease of 2019 (COVID-19) presents a global public health challenge. Since the year 2019, when the first cases emerged, the World Health Organization (WHO) has reported the number of nearly 773 million infected individuals around the globe, and the number of 7 million people who have died (1). The first cases in Serbia were detected in March 2020 and more than 2.6 million people have been infected so far, while 18.000 people have died (1,2). The clinical spectrum of COVID-19 ranges from asymptomatic form (some reports say up to 40% of the people infected) to critical and fatal illness. The percentage of severe disease cases is estimated to be in the range of 15 to 20% of symptomatic infections in unvaccinated individuals, and the overall case fatality rate is 2.3% (3). Many underlying medical conditions which increase the risk for the severe form of COVID-19 and lethal outcome have been identified so far. Experts from Centers for Disease Control and Prevention (CDC) have compiled a list of underlying medical conditions: various chronic lung diseases (asthma, bronchiectasis, interstitial lung disease, pulmonary embolism, etc.), hematologic malignancies, neuropsychiatric conditions (cerebrovascular disease, dementia, schizophrenia), people receiving dialysis, diabetes mellitus, heart conditions (such as heart failure, coronary artery disease, cardiomyopathies), obesity (body mass index - BMI >30 kg/m^2), primary immunodeficiencies and HIV, pregnancy, and recent pregnancy (4). When it comes to chronic liver diseases (CLDs), risk factors that could be important are cirrhosis, non-alcoholic fatty liver disease (NAFLD), alcoholic liver disease (ALD), and autoimmune hepatitis. Regarding chronic viral infections caused by hepatitis B virus (HBV) and hepatitis C virus (HCV), those conclusions are not quite clear. This is why in order to define chronic viral hepatitis as an underlying medical condition or a risk factor for severe form of COVID-19 there has to be more proof and more researches (5,6). Reasons for possible severe form of COVID-19 and poor outcome in patients with chronic viral hepatitis include: coexisting direct damage to the liver by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and viral hepatitis, disturbed gut microbiota in these patients, immune system disorders (especially regulatory T cells), the impact of prescribed medications, extrahepatic manifestations of chronic HBV and HCV infections, and their effects on other organ systems (7-9). The main problem of the impact of COVID-19 epidemic on chronic viral hepatitis is postponed diagnosis and initiation of the treatment, along with impossibility of regular check-ups due to the pandemic. As a consequence, individuals more frequently progress to terminal phases of liver disease, such as cirrhosis and hepatocellular carcinoma (10). In addition, there is global inability to achieve the WHO goal for controlling viral hepatitis by 2030 (11).

The aim of this paper is to analyze clinical presentation, course and outcome of COVID-19 in patients with chronic viral hepatitis in comparison with patients with liver disease of another etiology. It is also investigated which risk factors are responsible for severe form and poor outcome of COVID-19 in patients with chronic viral hepatitis.

MATERIAL AND METHODS

In order to find answers, a retrospective study was conducted including patients with confirmed COVID-19 who had been previously diagnosed with CLD and had been followed for at least six months. They were hospitalized due to COVID-19 at the Department of Hepatology of the Clinic for Infectious and Tropical Diseases, the University Clinical Center of Serbia between 1st March, 2020 and 1st March, 2022. The treatment of COVID-19 was conducted in accordance with the Serbian National Treatment Protocol for COVID-19 Patients (12) and the recommendations of the European Centre for Disease Prevention and Control (ECDC) (13).

In the first part of the study, where the influence of viral etiology of CLD on the clinical presentation, clinical course, and outcome of COVID-19 was examined, a matched case-control study was conducted. The case group was made out of patients with chronic viral hepatitis (chronic hepatitis B or C) infected with SARS-CoV-2. The inclusion criteria were defined as follows: at least a six-month follow-up of chronic hepatitis B or C infection before COVID-19; the stage (presence or absence of liver fibrosis and the degree of fibrosis) and the activity of liver disease were known; COVID-19 was confirmed by PCR test; patients gave a written informed consent for being included in the study. The exclusion criteria were defined as follows: diagnosed or previously treated hepatocellular carcinoma, liver transplantation, and HIV co-infection. The control group was made from patients with previously diagnosed CLD of another etiology infected with COVID-19. They were matched with patients from the case group according to the severity of CLD (presence or absence of cirrhosis), which had been clearly defined as a negative prognostic factor in previously conducted researches. The same inclusion and exclusion criteria were applied to the control group. For every patient in the case group two patients from the control group were selected (ratio 2:1) to ensure a higher power of the study. When it comes to non-infective liver disease, the following were included: autoimmune liver disease (autoimmune hepatitis, primary biliary cholangitis - PBC, primary sclerosing cholangitis - PSC), ALD, metabolic liver disease, hereditary liver disease (hemochromatosis, Wilson disease, deficit of alfa-1 trypsin) or CLD of unknown origin (individuals who had had elevated liver enzymes more than 2x UNL for more than 6 months but without confirmed diagnosis even after complete diagnostics had been performed).

In the second part of the study, risk factors for severe clinical course and poor outcome of COVID-19 were examined in patients with chronic viral hepatitis (the study group). The severity of COVID-19 was determined based on the severity of pneumonia and the need for oxygen support in the following way: absence of pneumonia, mild pneumonia with no need for oxygen support, moderate pneumonia (involving less than 50% of lung parenchyma) requiring oxygen support, severe pneumonia (involving more than 50% lung parenchyma) requiring oxygen support, and critically ill patients treated in the intensive care unit (ICU) requiring high-flow oxygen support, non-invasive, or invasive mechanical ventilation. Risk factors that were analyzed were as follows: age and gender, constitution, severity of chronic hepatitis (assessed through the stage of liver fibrosis and the activity of the disease), comorbidity diseases previously defined as risk factors (heart diseases - cardiomyopathies, heart arrhythmias; endocrine diseases - thyroid disease, diabetes mellitus, chronic lung disease - asthma, chronic bronchitis, emphysem, chronic kidney disease, malignancy - carcinomas, hematologic malignancies), applied therapy for COVID-19 (antivirals, corticosteroids, immunomodulators), previous vaccination against SARS-CoV-2, symptoms and signs of COVID-19 (high fever, weakness and malaise, cough, dyspnea, diarrhea, vomiting). Neurological diseases as risk factors were not included, because none of the patients had any. The use of antibiotics, anticoagulants, or vitamins was not analyzed, as all patients received these medications. The study was approved by the Ethical Board of the University Clinical Centre of Serbia, under the reference number 87/3.

Statistical analysis

Methods of descriptive and analytic statistics were used. When it comes to statistical tests for comparing two groups of patients the following tests were used: Student's t-test, the Mann–Whitney U test, chi square test. The value $p<0.05$ was considered to indicate statistical significance. The statistical analyses were performed using the IBM SPSS software v21.

RESULTS

The study included 75 patients with CLD infected with COVID-19. The case group included 25 patients with chronic viral hepatitis: 13 (52%) with chronic HBV infection and 12 (48%) with chronic HCV infection. The other 50 patients had CLD of another etiology (the control group): 8 (16%) with autoimmune liver disease, 10 (20%) with ALD, 24 (48%) with NAFLD, and 8 (16%) with CLD of an unknown origine. The patients included in the study group were younger (53.5±15.1 years vs. 57.9±13.4 years), but a statistical difference was not reached ($p=0.754$). The male gender dominated in both groups, but there were still more individuals of male gender in the study group - ratio 3.2:1 vs. 2.8:1, respectively. In both groups, 64% of patients did not have cirrhosis, while 36% had diagnosed liver cirrhosis. Liver enzymes were found to be more elevated in the control group than in the study group (70% vs. 52%), but without significant difference ($p=0.315$). In terms of comorbidities that can impact the clinical course of COVID-19, the only notable difference was observed in endocrinological diseases, which had a higher prevalence in the group with nonviral liver diseases. There was no significant difference between the groups in terms of cardiovascular diseases, chronic lung diseases, chronic kidney diseases and malignancy. There was a higher percentage of vaccinated individuals in the group of patients with chronic viral hepatitis (40% vs. 22%), but without statistical significance ($p=0.102$). The main characteristics of patients are presented in **Table 1**.

COVID-19

Regarding symptoms and signs of COVID-19, there was a similar clinical course observed in both groups of patients with CLD. Therefore, there was no statistically significant difference ($p>0.05$) between the duration of the disease before hospital admission, the duration of hospital treatment, taste and smell loss, weakness and malaise, the presence of cough and a sense of dyspnea, diarrhoea and vomiting. On the other hand, a higher degree of body temperature was noted among patients with chronic viral hepatitis (38.7±0.9°C vs. 38.2±0.6°C) and it was a statistically significant difference ($p=0.036$). As for COVID-19 severity, the majority of patients in both groups had mild or moderate pneumonia (60% and 68%, respectively), and one-third of the patients had severe pneumonia or were critically ill, requiring treatment in the ICU. The difference in severity of COVID-19 in the case group and the control group was not statistically significant ($p=0.578$). When it comes to COVID-19 treatment, corticosteroids were used slightly more often in the control group (78% vs. 68%), such as immunomodulatory therapy - tocilizumab (55% vs. 48%). This difference was not statistically significant ($p=0.348$ and $p=0.126$, respectively). Antibiotics were used in all patients with pneumonia in both groups. The treatment with antibiotics, antivirals, vitamins C and D and anticoagulants was almost identical in both groups, without significant statistical difference ($p>0.05$). In the study group, there were more vaccinated patients (40%) compared to the control group (22%), although the difference did not reach statistical significance ($p=0.102$). Among vaccinated patients, the majority (61.9%) got an inactivated vaccine, 28.5% got an mRNA vaccine, and the remaining 9.6% got an adenovirus viral vector vaccine. Clinical characteristics and outcome of COVID-19 are presented in **Table 2**.

Table 1. The main characteristics of the subjects included in the study (n=75)

Variables	Patients with chronic viral hepatitis (case group); n=25	Patients with chronic nonviral liver diseases (control group); n=50	P value
Gender			
Male	19 (76%)	36 (72%)	0.712
Female	6 (24%)	14 (28%)	
Average age, y	53.5±15.1	57.9±13.4	0.754
Constitution			
Average constitution (BMI 18.5-24.9 kg/m^2)	17 (68%)	30 (60%)	0.212
Underweight (BMI <18.5 kg/m^2)	3 (12%)	2 (4%)	
Overweight (BMI>25 kg/m^2)	5 (20%)	18 (36%)	
Stage of liver disease			
No cirrhosis	16 (64%)	32 (64%)	1.000
Liver cirrhosis	9 (36%)	18 (36%)	
[a]Presence of active liver disease*			
Normal values of transaminases	12 (48%)	15 (30%)	0.315
Values of transaminases increased two- to three-times UNL	6 (24%)	16 (32%)	
Values of transaminases increased more than three-times UNL	7 (28%)	19 (38%)	
[b]Comorbidites			
Heart diseases (cardiomyopathy, arrhythmia)	10 (40%)	25 (50%)	0.413
Chronic lung disease (asthma, chronic bronchitis, emphysema)	2 (8%)	2 (4%)	0.665
Endocrine diseases (diabetes mellitus, thyroid disease)	3 (12%)	21 (42%)	**0.009**
Chronic kidney disease	2 (8%)	4 (8%)	1.000
Malignancy (carcinomas, hematologic malignancies)	5 (20%)	3 (6%)	0.108
Vaccinated against COVID-19	10 (40%)	11 (22%)	0.102

UNL - upper normal limit; [a]values of liver enzymes during follow up period before COVID-19; [b]presence of listed diseases

Table 2. COVID-19 in patients with chronic viral hepatitis (the case group) and with nonviral chronic liver disease (the control group)

Variables	Patients with chronic viral hepatitis (case group); n=25	Patients with nonviral chronic liver disease (control group); n=50	P value
[a]Clinical characteristics			
Fever	38.7±0.9°C	38.2±0.6°C	**0.036**
The loss of smell and taste	1 (4%)	3 (6%)	0.716
Weakness and malaise	15 (60%)	37 (74%)	0.215
Cough	14 (56%)	36 (72%)	0.166
Dyspnea	9 (36%)	18 (36%)	1.00
Vomitting	5 (20%)	5 (10%)	0.230
Diarrhea	4 (16%)	6 (12%)	0.631
Severity of disease			
No pneumonia	2 (8%)	3 (6%)	0.578
Mild pneumonia	3 (12%)	13 (26%)	
Moderate to severe pneumonia	12 (48%)	20 (40%)	
Severe pneumonia	3 (12%)	2 (4%)	
Critically ill	5 (20%)	12 (24%)	
[a]Treatment model			
Antiviral therapy applied	6 (24%)	15 (30%)	0.585
Corticosteroids applied	17 (68%)	39 (78%)	0.348
Tocilizumab applied	12 (48%)	15 (55.6%)	0.126
Antibiotics applied	23 (92%)	47 (94%)	0.743
Vitamin C or D applied	22 (88%)	43 (86%)	0.810
Anticoagulants applied	20 (80%)	45 (90%)	0.230
[a]Vaccination	10 (40%)	11 (22%)	0.102
Outcome			
Recovered	19 (76%)	37 (74%)	0.851
Died	6 (24%)	13 (26%)	

[a]the number of patients with listed characteristics

In terms of the outcome in the study group, the lethal outcome occurred in 6 patients (24%), while 19 patients completely recovered (76%). When it comes to patients with nonviral CLDs, 13 (26%) succumbed, while 37 (74%) recovered. The difference in the lethality rate was not significant (p=0.851). Further analysis showed that the majority of patients who died in both groups had liver cirrhosis and that difference was statistically proven. Thus, 5 (55.6%) out of 9 patients with viral cirrhosis died, while in those without cirrhosis only 1 (6.3%) died; p=0.006. In the control group, 12 (66.7%) out of 18 patients with cirrhosis died, and only 1 (3.1%) without cirrhosis died (p<0.001).

Risk factors for severe clinical course of COVID-19 and lethal outcome in patients with chronic viral hepatitis

Out of 25 patients with chronic viral hepatitis (designated as the study group), 16 individuals (64%) experienced a mild or moderately severe clinical form of COVID-19 and successfully recovered, while the remaining 9 patients (36%) faced a severe clinical presentation of COVID-19, resulting in either a critical condition or fatality. The presence of liver cirrhosis (66.7% vs. 18.8%, p=0.025) and active CLD (66.7% vs. 25%, p=0.041) were identified as statistically significant risk factors for the severe form of COVID-19 and fatal outcome. When it comes to clinical signs and symptoms of COVID-19, patients with dyspnea had significantly more severe clinical course and lethal outcome, in comparision with those without dyspnea (66.7% vs. 18.8%, p=0.025), while in respect to the presence of cough this difference was not noted (56.3% vs. 36.6%, p=0.648). Differences in other signs and symptoms between the groups were not registered. Severe clinical presentation and poor outcomes were more frequently observed in patients with comorbidities, and if there was no application of antivirals or immunomodulatory therapy, but without statistical significance (p>0.05). On the other hand, COVID-19 vaccination was shown to be a significant protective factor. Vaccinated patients with chronic viral hepatitis that had mild or moderate clinical courses of COVID-19 were registered in 56.3%, while there were 10% of vaccinated patients who had severe clinical presentation and lethal outcome (p=0.027). The analyzed risk factors are shown in **Table 3**.

DISCUSSION

CLDs generally present a risk factor for a severe form of COVID-19. Previous studies analyzing the etiology of CLD in relation to the course and outcome of COVID-19 have shown an increased risk in ALD, NAFLD and autoimmune hepatitis, while this relation is not so clear for chronic viral hepatitis (7,14,15). It was clearly defined that the severity of CLD had a direct influence on severity of COVID-19 and lethal outcome. It was demonstrated that liver cirrhosis carried a 3,31-4,90 times higher 30-day mortality rate. In addition, the lethality rate for those with decompensated liver cirrhosis was 43-63% (6,14,16). People with advanced liver diseases have a higher expression of the ACE2 receptor in the respiratory tract because the ACE2 gene is interferon-inducible. This could lead to a more severe form of COVID-19 in these individuals. Additionally, people with cirrhosis of the liver experience immune dysregulation known as cirrhosis-associated immune dysfunction (CAID), which affects both innate and acquired immune responses. This dysregulation causes damage to the synthesis and

Tabela 3. Risk factors for severe form of COVID-19 and lethal outcome

Risk factors	Patients with mild or moderate clinical course of COVID-19; n=16	Patients with severe clinical course or lethal outcome: n=9	P value
Gender - male	13 (81.3%)	6 (66.7%)	0.363
Constitution - overweight (BMI>25 kg/m^2)	3 (18.8%)	2 (22.2%)	0.835
Age >65 years	5 (31.3%)	2 (22.2%)	0.501
Severity of chronic liver disease - cirrhosis	3 (18.8%)	6 (66.7%)	**0.025**
[a]Active liver disease	4 (25%)	6 (66.7%)	**0.041**
[b]Comorbidities – one or more	9 (56.3%)	6 (66.7%)	0.470
Vaccination – not carried out	7 (43.8%)	8 (88.9%)	**0.027**
Fever >38°C	14 (87.5%)	7 (77.8%)	0.458
Weakness and malaise	10 (62.5%)	5 (55.6%)	0.530
Cough	9 (56.3%)	5 (55.6%)	0.648
Dyspnea	3 (33.3%)	6 (66.7%)	**0.025**
Dyarrhea and vomitting	3 (18.8%)	4 (44.4%)	0.181
Antiviral therapy – not applied	11 (68.8%)	8 (88.9%)	0.267
Corticosteroids – not applied	6 (37.5%)	2 (22.2%)	0.374
Tocilizumab – not applied	8 (50%)	5 (55.6%)	0.560

[a]prior to the occurence of COVID-19; [b]Heart diseases (hypertension, cardiomyopathies, heart arrhythmias), endocrine diseases (thyroid disease, diabetes mellitus), chronic lung disease (asthma, chronic bronchitis, emphysema), chronic kidney disease, malignancy (carcinomas, hematologic malignancies)

function of pattern recognition receptors (PRRs) and toll-like receptors (TLRs), disrupts the function of neutrophils and macrophages, leads to various T lymphocyte defects (such as CD4 and CD8 relationship disorder), and results in B lymphocyte hyperactivity. The function of the complement system (C3, C4, C5a) is also disturbed. Such immune disorders manifest, on one hand, as immunodeficiency (characterized by reduced bacterial opsonization, phagocytosis, and T lymphocyte-dependent antigen responses) and, on the other hand, as increased systemic inflammation (indicated by elevated serum levels of TNF-α, IL-1β, IL-6, IL-17, IL-18, and IFN-γ), resulting in a 2.6 times higher risk of sepsis. Consequently, the risk of a cytokine storm during COVID-19 is higher in people with advanced liver diseases, as well as the risk of the most severe form of respiratory complication, acute respiratory distress syndrome (ARDS) (17). Unrelated to COVID-19, advanced liver disease and cirrhosis were previously recognized as significant predisposing factors for ARDS because liver damage activates and enhances inflammation in the pulmonary intravascular compartment and lower respiratory tract, leading to important changes in the structure and/or functions of the lung (18).

In this study, COVID-19 patients with chronic viral hepatitis were mostly male and slightly younger than those with a nonviral cause of CLD. This finding was expected since the transmission of chronic viral hepatitis nowadays is mostly by means of intravenous drug use (HCV infection) or through sexual transmission (HBV infection) (19). The patients with nonviral cause of CLD were more often overweight (36% *vs*. 20%), and had more common endocrinological diseases (42% *vs*. 12%). The explanation lies in the fact that almost half of the patients in this group had NAFLD, and obesity and diabetes mellitus are primary risk factors for NAFLD. Although the sample of patients is too small to evaluate the prevalence of NAFLD in Serbia, it is important to note that the representation of these exceeds the prevalence in Europe which is 25% (20). No significant differences were registered between the examined groups regarding other comorbidities (cardiac, pulmonary, malignancies, chronic kidney diseases) that could have an impact on the course and outcome of COVID-19. There was no statistical difference in vaccination rate either. However, there is a notable difference in the vaccination rate, favoring the group with chronic viral hepatitis (40% *vs*. 22%). This can be explained by the fact that these patients were treated by infectiologists, who are well-known for advocating vaccination. Unfortunately, the total percentage of those vaccinated against COVID-19 in this study is low (only 28%). This low rate of vaccination is in collision with recommendations of the European Association for the Study of the Liver (EASL) as the most important organisation in Europe when it comes to liver diseases (21). The low number of vaccinated patients can be explained by the fact that the majority of included patients were treated at the beginning of the COVID-19 pandemic, before the introduction of vaccines. In the period when the vaccine became available, patients hesitated to get vaccinated for various reasons. A Chinese study from 2022 demonstrated that more than two-thirds of patients with advanced liver disease were unvaccinated. Besides fear and lack of support, a significant number of them did not want to disclose the reason for such a decision (22).

In addition to fever, which was a symptom in almost all patients, the dominant complaints were the feeling of weakness and malaise, as well as cough, which were reported by more than half of the respondents. The loss of smell and taste, gastrointestinal problems, dyspnea and skin rashes were rare in included patients. This distribution of COVID-19 symptoms is similar in patients without chronic liver disease (15,23,24). Almost a third of the patients (32%) with chronic viral hepatitis had severe pneumonia or were critically ill, but there was no statistically significant difference when compared to the control group. During SARS-CoV-2 infection, the largest number of patients had viral pneumonia, while the prevalence of bacterial superinfection was around 10% and was related to the severity of COVID-19. The greatest risk was found in patients who required treatment in the intensive care unit (25). However, given that people with advanced liver diseases are prone to bacterial infections, a higher percentage of superinfections can be expected in them during COVID-19, which is the main reason why the majority of patients in our study received antibiotic therapy.

When comparing the frequency of patients with severe COVID-19 in terms of the general distribution of disease severity, as expected, there was a higher percentage of severe cases in both groups compared to the general population. Thus, in the study published by Chinese Center for Disease Control and Prevention which included 44,500 patients, the percentage of people with severe pneumonia or critically ill was 19% (26). The reason for this might be the fact that the Chinese study included persons with confirmed COVID-19, disregarding the fact whether they were in need of hospitalization or not. In a study conducted in the UK analysing hospitalized patients with COVID-19, the majority had comorbidities and the rate of severe disease (26%) was similar to the one in our study. The main comorbidities in the UK study were chronic cardiac disease (30.9%), diabetes mellitus (20.7%), chronic pulmonary disease including asthma (32.2%) and chronic kidney disease (16.2%) (27).

The application of antiviral drugs in the study and control groups was 25% and 40%, respectively. This low percentage of antiviral therapy could be explained by the fact that antivirals became available in Serbia almost a year after the beginning of the study. In addition, the average duration of the illness before hospitalization was 6.5 days and the introduction of antivirals was advised until the fifth day of the illness. The other limitation for the application of antivirals was a warning that special caution was

needed in advanced liver diseases (12). Medications that decrease the possibility of the development of the cytokine storm (corticosteroids and tocilizumab) were used a bit more often in the control group of patients with nonviral CLD. We believe that the reason could be fear of worsening of other viral infections, such as chronic HBV and HCV infections. Almost the same lethality rate was registered in both the study and the control groups (24% and 26%, respectively). Similar lethality rate (20%) was noted analyzing 745 persons with CLD from the International registry of Great Britain in a study conducted by Major et al. In this study, it was also pointed out that there was a significantly higher percentage of deaths among people with liver cirrhosis (with Child-Pugh class C as much as 51% *vs.* 8% among those without cirrhosis), and the same conclusion was noted with our patients as well (14).

Risk factors for the development of severe COVID-19 and lethal outcome in patients with chronic viral hepatitis in our study were as follows: diagnosis of liver cirrhosis, active CLD, and dyspnea. These findings are consistent with previously published studies of other authors. Liver cirrhosis causes higher mortality rate probably due to CAID that leads to higher sensitivity to infection and aberrant inflammatory response during infection. All of this results in increased sensitivity, not only to bacterial and fungal infections, but also to viral infections such as SARS-CoV-2 infection (28,29). Active CLD with increased liver enzymes during continued follow up of our patients prior to COVID-19, was also a significant risk factor for adverse outcome. Elevated transaminases in chronic viral hepatitis are related to inflammatory process in the liver and necrosis of hepatocytes. During inflammatory process proinflammatory cytokines are released, also known as hepatokines (TNFα, IL-6, IL-1A, IL-33, pro-IL-18) leading to the development of cytokine storm in COVID-19 (30). Hepatocyte necrosis culminates in decreasing hepatic reserve making the liver more susceptible to injury in COVID-19. Liver injury itself influences the choice and dosage of drugs used in COVID-19 treatment (antivirals, antibiotics, anticoagulant therapy, analgopyretics, etc.) and also affects metabolism of the applied drugs (31).

In our study, vaccination against SARS-CoV-2 was noted as a protective factor for course and clinical outcome of COVID-19 in patients with chronic viral hepatitis. It was clearly demonstrated that aforementioned vaccines (inactivated, mRNA, vector with adenovirus as a carrier) had protective effect on all aspects of COVID-19, such as the infection itself, the need for hospitalization and oxygen support, the admission to ICU and overall mortality (32). Surprisingly, the administration of antivirals for COVID-19 in our study did not have a positive effect on the course and outcome of COVID-19. This might be accounted for by the fact that most of our patients (83.3%) were treated with favipiravir. Recently published meta-analysis conducted by Batool et al. showed that the administration of favipiravir was safe, but not efficient enough in COVID-19 treatment. This meta-analysis included 1,448 patients from eight randomized control trials (RCTs) and it showed that favipiravir did not exert any beneficial impact on reducing ICU admission, the need for oxygen therapy, and time to viral clearance (33). At the same time, antiviral therapy was mainly applied in our patients with liver cirrhosis which in itself was a negative prognostic factor for poor outcome of COVID-19 (6,14,34).

To our knowledge, this is the first study conducted in Serbia that analyzed the impact of chronic viral hepatitis on COVID-19. The characteristics of COVID-19 in these patients were compared with matched controls who had CLD of some other, non-infectious etiology, and the risk factors for a severe clinical course and poor outcome were analyzed, which gives special significance to the results obtained. The authors are fully aware of the limitations of the study. It included only hospitalized patients, and not those treated in outpatient settings, leading to a selection bias. This also leads to reporting bias, with over-representation of the cases with more progressive liver disease, or more severe COVID-19. All in all, a higher number of participants would certainly have increased the strength of the study and potentially discovered some other risk factors. Regardless of these imperfections, the authors believe that none of these facts have brought the study's validity into question.

CONCLUSIONS

COVID-19 in patients with chronic viral hepatitis has similar clinical course, severity and possibility of lethal outcome in comparison with patients suffering from some other nonviral CLDs. Administration of antiviral therapy and vaccination against COVID-19 was noted in low rates. Risk factors for severe clinical forms and lethal outcome in patients with chronic viral hepatitis are progressive liver disease (stage of cirrhosis), active necroinflammatory liver disease prior to COVID-19 and the presence of dyspnea. When it comes to protective factors, prior vaccination against SARS-CoV-2 was noted. The obtained results are significant for the prediction of course and outcome of COVID-19 in persons with chronic viral hepatitis, and the identified risk factors will help in their stratification and assessment of the need of hospital treatment. Protective effect of vaccination is also an important finding and should encourage patients with chronic viral hepatitis, and health professionals as well, to promote vaccination. This is in accordance with the recommendations of the most important world associations for liver diseases.

Author Contributions

Ivana Milosevic and Nikola Mitrovic conceived and designed the study. Nikola Mitrovic, Natasa Nikolic, Ana

Filipovic, Ankica Vujovic, Uros Karic and Milos Sabanovic collected the data. Ivana Milosevic, Nikola Mitrovic, Natasa Nikolic, Ana Filipovic, Ankica Vujovic, Uros Karic and Milos Sabanovic analysed and interpreted the data. Nikola Mitrovic, Natasa Nikolic, Ana Filipovic, Ankica Vujovic, Uros Karic and Milos Sabanovic drafted the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

Conflict of interest

None to declare

Ethical approval

The study was approved by the Ethical Board of the University Clinical Centre of Serbia, under the reference number 87/3.

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UTICAJ HRONIČNOG VIRUSNOG HEPATITISA NA KOVID-19: KLINIČKI TOK BOLESTI I FAKTORI RIZIKA KOJI UTIČU NA NEPOVOLJAN ISHOD

Nikola Mitrovic[1,2], Natasa Nikolic[1,2], Ana Filipovic[2], Ankica Vujovic[1,2], Uros Karic[1,2], Milos Sabanovic[2], Ivana Milosevic[1,2]

Sažetak

Uvod/Cilj rada: Uticaj hroničnih virusnih bolesti jetre na Kovid-19 do sada nije u potpnosti razjašnjen. Cilj rada je da se analizira klinička slika, tok i ishod Kovid-19 kod osoba sa hroničnim virusnim hepatitisima i da se utvrde faktori rizika za nepovoljan ishod.

Metode: Sprovedena je studija slučajeva i kontrola u kojoj su u grupu slučajeva uključene osobe sa hroničnim virusnim hepatitisom obolele od Kovid-19, a u kontrolnu grupu osobe sa hroničnim bolestima jetre neke druge etiologije, pri čemu su mečovani u odnosu na stadijum bolesti jetre. Svi ispitanici su lečeni u odeljenju hepatolgije Infektivne klinike u Beogradu od 1.3.2020. do 1.3.2022. godine.

Rezultati: Analizirano je 75 pacijenta sa hroničnim bolestima jetre obolelih od Kovid-19, i to 25 pacijenata virusne etiologije (13 HBV, 12 HCV) – grupa slučajeva i 50 ispitanika u kontrolnoj grupi. U grupi slučajeva bilo je više osoba muškog pola (76% nasuprot 72%) i bili su mlađeg uzrasta (53.5±15.1 nasuprot 57.9±13.4 godine), dok je u kontrolnoj grupi bilo više gojaznih (36% nasuprot 20%). Kada je reč o komorbiditetima, u kontrolnoj grupi je bilo više ispitanika sa endokrinološkim bolestima. Grupe se nisu razlikovale u pogledu težine kliničke slike i ishoda Kovid-19. Faktori rizika za težak oblik Kovid-19 i smrtni ishod kod ispitanika sa hroničnim virusnim hepatitisima su: ciroza jetre, aktivna bolest jetre, visoka febrilnost, prisustvo dispneje, a protektivan faktor vakcinacija protiv Kovid-19.

Zaključak: Tok i ishod Kovid-19 je sličan kod osoba sa hroničnim bolestima jetre, bilo da je u pitanju virusna etiologija ili neka druga. Najvažniji faktori rizika za nepovoljan ishod su uznaprdovala bolest jetre i dispneja, dok je zaštitni faktor vakcinacija.

Ključne reči: hronični virusni hepatitis, Kovid-19, faktori rizika, ishod

Primljen: 28.12.2023. I **Revizija:** 22.01.2024. I **Prihvaćen:** 22.01.2024.

Medicinska istaživanja 2024; 57(2):65-73

Medicinska istaživanja 2024; 57(2):75-83 | DOI 10.5937/medi57-48847

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

ORIGINAL ARTICLE

Multifractal characterization of grayscale histopathological images: unveiling patterns linked to metastases in breast cancer

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Recived: 22 January 2024

Revised: 26 March 2024

Accepted: 10 April 2024

Check for updates

Funding information:
This work was supported by the Serbian Ministry of Education, Science and Technological Development, grant number 451-03-66/2024-03/200110

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Breast cancer, a pervasive global malignancy, demands precise prognostication of the risk of metastases for personalized therapeutic strategies and enhanced survival rates. In pursuit of refined diagnostic methodologies, this study employs multifractal analysis on grayscale histopathological images, revealing distinctive patterns associated with the occurrence of metastases.

Aim: Analyzing the multifractal spectra of grayscale images for groups with and without metastases to assess the utility of this analytical approach in enhancing the diagnostic process.

Materials and methods: The study included 102 female patients treated at the Institute for Oncology and Radiology of Serbia in the same year (1993). Histopathological samples were immunostained with a pan-cytokeratin antibody and digitized with a high-resolution scanner, from which a specialist chose representative parts, thus leading to a total number of 519 images (418 in no-metastases group and 101 in metastases group). Images were subjected to multifractal analysis, assessing the generalized dimension, Hölder exponent, and singularity spectra.

Results: Statistical comparisons between groups with and without metastases unveil significant differences in the negative domains of both generalized dimension and Hölder exponent spectra, highlighting the influence of fine structures in tissue morphology that are linked to metastatic risk.

Conclusion: Multifractal analysis applied to images of histopathological samples from breast tumors demonstrates the ability to differentiate between groups of patients with and without metastases. While caution is warranted regarding image resolution limitations and immunostaining sensitivity, this method is a non-training-dependent approach with potential diagnostic significance and possible synergies with advanced neural network approaches.

Key words: multifractal, histopathology, breast cancer, metastasis

Cite this article as: Nedeljković Z, Milošević D, Radulović M, Milošević N, Rajković N. Multifractal characterization of grayscale histopathological images: unveiling patterns linked to metastases in breast cancer Medicinska istaživanja 2024; 57(2):75-83 DOI: 10.5937/medi57-48847

INTRODUCTION

Breast cancer is the most commonly diagnosed form of malignancy in the world and is the primary cause of cancer-related death in women (1,2). Metastases pose a significant challenge in breast cancer treatment, with the occurrence of distant metastases displaying exceptional variability. Accurately prognosticating the risk of metastases becomes pivotal for tailoring individual therapeutic regimens and improving survival rates. Precision medicine holds the potential to optimize treatment strategies, with less intense interventions for low-risk individuals and intensified therapies for those reliably identified as high-risk for metastases. Currently, the reliance on cytotoxic therapy to eliminate distant micro metastases, while effective for some, subjects many breast cancer patients to unnecessary toxic side effects (3,4). By refining prognostication, the field of oncology can move towards individualized treatment plans that enhance patient survival rates while mitigating the adverse effects of chemotherapy.

Non-invasive techniques, such as mammography and breast ultrasound, serve as valuable tools for tumor detection; however, histopathological images remain the gold standard in breast cancer diagnosis (5,6). The morphological information conveyed by histopathological images offers essential prognostic insights into the molecular biology of breast cancer (7). Nevertheless, visual assessment of tumor morphology by specialists presents challenges manifested in frequent non-reproducibility of results, thereby compromising the reliability of prognostic information (8). To address this, computer image analysis emerges as a potential solution, potentially offering enhanced reliability and reduced susceptibility to error in the diagnostic process. In recent years, various approaches within the realm of computer image analysis have been proposed to address these challenges in breast cancer diagnosis (9–12), among them multifractal analysis, which exhibits promise in distinction between abnormal and healthy tissue (13,14).

Multifractal analysis sprung from fractal geometry formulated by Benoit Mandelbrot with the aim of describing the complexity of living forms in nature (15). While histopathological images qualify as natural forms, they diverge from mathematically abstract fractal patterns by manifesting fractal properties solely at limited scales (16,17). Nonetheless, the morphology of natural patterns can be effectively quantified through the application of fractal and multifractal formalism. In contrast to monofractal analysis, multifractal approaches prove more adept at describing the irregularities inherent in natural objects. Given that natural entities typically lack universal or statistical self-similarity and exhibit an uneven distribution of complexity, multifractal analysis accommodates variations in fractal dimensions across different points within the object (18,19). The aim of this study is to analyze the multifractal spectra of grayscale images for groups with and without metastases to assess the utility of this analytical approach in enhancing the diagnostic process.

MATERIALS AND METHODS

In this study, we used the same sample from two previous studies (3,20), where a different approach to image analysis was applied. Patient group and image acquisition information were presented there in more detail.

The study was approved by the Ethics Committee of the Institute for Oncology and Radiology (#2794-01; 14. July 2016) and conformed with The Code of Ethics of the World Medical Association (Declaration of Helsinki) printed in the British Medical Journal (July 18, 1964) and its 7th revision in 2013.

Patient group

The patient group consisted of 102 female patients treated at the Institute for Oncology and Radiology of Serbia in the same year (1993). The data were obtained without identifiers that would allow the identification of an individual patient. The median age at diagnosis was 57 years (with a range of 37–80 years). The follow-up time of patients without metastases ranged from 77 to 165 months with a median of 147 months. Time to metastases ranged between 16 and 155 months with a median of 61 months. Out of 102 patients, metastases were observed in 20 cases.

Image acquisition

The tissue was obtained during surgical removal of the tumor. From the histopathological samples of 102 patients, the pathologist selected one sample per patient that best represented the tumor. The samples were immunostained with a pan-cytokeratin antibody for the purpose of labeling groups of epithelial cells, and subsequently were digitized with a *Hamamatsu NanoZoomer-XRC12000 high-resolution digital slide scanner*. The procedure was described in more detail in previous studies (3,20). In this way, high-resolution color images were obtained from which the pathologist selected representative parts of the same size (about five per patient) to represent the original images used in the analysis. **Figure 1a** shows an example of such an image. In this way, a total of 519 digital images were extracted, 418 in no-metastases group (group 0) and 101 in metastases group (group 1). All the images had the same resolution of 1278 x 753 pixels, to avoid the systematic error caused by the fractal calculations' dependence on image resolution (21).

Color images were then converted to 8-bit grayscale images using the image processing and analysis software *ImageJ* version 1.48v, using the command *"Image - Type - 8bit"* (22) **(Figure 1b)**. Multifractal analysis described in the following text was then applied to the images to obtain the multifractal spectra of each individual image.

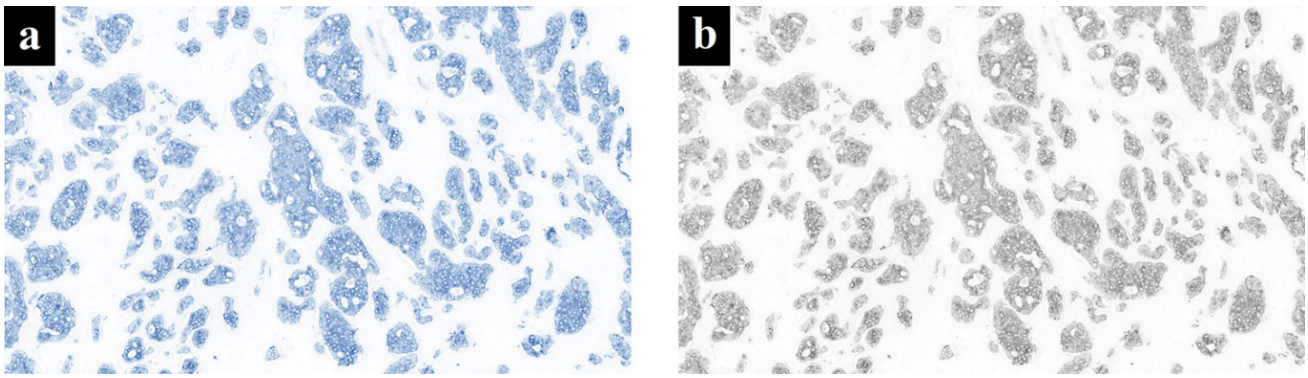

Figure 1. Representative image used in the study: a) Original color image, b) Grayscale image

Multifractal analysis

Multifractal analysis aims to quantify the morphological characteristics of an object that exhibits multiple scaling rules (23). A multifractal structure can be seen as a superposition of several homogeneous monofractal structures in a single object (24). Given the morphological nature of histopathological images, multifractal analysis can describe the statistical properties of such images that possess irregular spatial arrangements. In order to describe this "multifractality" of the objects, as well as to check whether the object is actually multifractal, we resort to the formation of a multifractal spectrum. For this purpose, an *ImageJ* plug-in called *FracLac* was used (25) whose calculations relied on the previous work of Chhabra and Jensen (26,27). We used two most commonly used spectrums – the spectrum of generalized dimensions D_Q vs Q, and singularity spectrum $f(\alpha)$ vs α, described in the following text. In addition, α vs Q and $f(\alpha)$ vs Q spectra are also represented in the study.

Analysis was implemented using the non-overlapping box count method. Spectrum of generalized dimensions D_Q vs Q is formed by using the so-called moments of order Q, which serve as a kind of distortion that mathematically emphasize different aspects of the morphology of the object (25,28). These moments are calculated for the measure $P_{(i,\varepsilon)}$ as

$$I_{[Q,\varepsilon]} = \sum_{i=1}^{N} \left[P_{(i,\varepsilon)}\right]^{Q} \quad (1)$$

where N is the total number of filled squares used in the box counting method, ε is the size of the box at the given scale, while $P_{(i,\varepsilon)}$ is the probability of a certain mass of pixels occurring in i-th box compared to the total mass at that box size ε. Hence, the generalized dimension of $D_Q(Q)$ is equal to

$$D_Q = -\lim_{\varepsilon \to 0} \frac{1}{1-Q} \frac{\ln I_{[Q,\varepsilon]}}{\ln \varepsilon} \quad (2)$$

This study is conducted on grayscale images, so the calculation of the mentioned moments is reduced to differences in pixel intensities described by the differential box counting method (25). This method calculates the difference in pixel intensities $\delta_{Ii,j,\varepsilon}$ in each square of a certain size, so it is

$$\delta_{Ii,j,\varepsilon} = max\ pixel\ intensity(i,j,\varepsilon) - min\ pixel\ intensity(i,j,\varepsilon) \quad (3)$$

This actual intensity range is increased by 1, to avoid a zero value in later calculations of the logarithm from which we have

$$I_\varepsilon = \sum \left[1 + \delta_{Ii,j,\varepsilon}\right] \quad (4)$$

Singularity spectrum $f(\alpha)$ vs α is another common type of multifractal spectrum. Parameter α is called Hölder exponent, also known as *singularity strength* (29), and it represents the degree of concentration of mass measure probability (30). Greater values of α indicate a smaller degree of concentration, and vice versa (29,30). In practice, following the calculations given by Chhabra and Jensen, we found the measure of mass probability for each box of the size ε (26,27,30)

$$\mu_{i(Q,\varepsilon)} = \frac{{P_{i(Q,\varepsilon)}}^{Q}}{\sum_{i=1}^{N} {P_{i(Q,\varepsilon)}}^{Q}} \quad (5)$$

which enabled us to calculate the coarse Hölder exponent (26,27,29,30)

$$\alpha(Q) = \frac{\sum_{i=1}^{N(\varepsilon)} \mu_{i(Q,\varepsilon)} \cdot \ln P_{i(Q,\varepsilon)}}{\ln \varepsilon} \quad (6)$$

and finally

$$f(\alpha(Q)) = \frac{\sum_{i=1}^{N(\varepsilon)} \mu_{i(Q,\varepsilon)} \cdot \ln \mu_{i(Q,\varepsilon)}}{\ln \varepsilon} \quad (7)$$

Parameter $f(\alpha)$ can be interpreted as a fractal dimension of a set of points with a singularity strength of α (30).

Values for parameter Q ranged from -10 to 10, with a step of 0.25, resulting in 81 points in the spectra of each image. Each of the 81 points in 3 different spectra (D_Q vs Q, α vs Q and $f(\alpha)$ vs Q) was treated as a separate variable for differentiation between the groups. The additional $f(\alpha)$ vs α spectrum was also presented for easier visual multifractal data comprehension, as it is the most commonly used multifractal spectrum.

Box counting algorithm was implemented with 12 different grid positions for each box size. Among the 12 positions, the one with the minimal number of boxes was chosen, reducing the possibility of error in calculations of the parameters (25). Theoretically, to avoid errors of this type altogether, the number of grid positions should be the maximum possible, depending on the image size. The number of positions in this study was chosen as a compromise between the computation time and further reducing the possibility of error, which is already quite low at 12 positions, rendering the increase unnecessary.

From the multifractal spectra $f(\alpha)$ vs α, additional parameters were extracted in order to further characterize the spectrum. Parameters included α_{min}, α_{max}, $\Delta\alpha$, $f(\alpha)_{min}$, $f(\alpha)_{max}$, $\Delta f(\alpha)$, where $\Delta\alpha$ and $\Delta f(\alpha)$ were calculated as $\alpha_{max} - \alpha_{min}$ and $f(\alpha)_{max} - f(\alpha)_{min}$, respectively.

Statistical analysis

Data analysis was performed using *IBM SPSS Statistics v25* software. The quantitative measure of the difference between the prognostic groups was evaluated by the non-parametric Mann-Whitney U test since normal distribution could not be guaranteed for all used variables. Results with p values ≤ 0.05 were considered statistically significant.

RESULTS

Considering the large number of variables in multifractal spectra, the data in this paper will be mainly presented graphically.

Spectrum of generalized dimensions $D_Q(Q)$

Figure 2 shows the median values of generalized dimension D_Q depending on the parameter Q, for both groups of patients. It is observed that with an increase in parameter Q, the values of the generalized dimension decrease, which is expected due to the sigmoid shape of this type of multifractal spectrum (25,30). The greatest drop in D_Q values is between Q = -1.5 and Q = -1.25 for group 0, and between Q = -0.75 and Q = -0.5 for group 1. Differences in median values between groups tend to decrease with increasing Q values and are greatest in the Q value range from -4.75 to -3. Statistically significant differences between groups were observed on practically entire spectrum with negative values of Q, (except for point Q = 0.25), with higher median D_Q values for group 1. The median values of the D_Q parameter are higher for group 1 on most of the spectrum except for the Q values in the range from 0.25 to 6.75, but it is worth noting that differences on the Q-positive part of the spectrum were negligible (differences were observed only in the third decimal place).

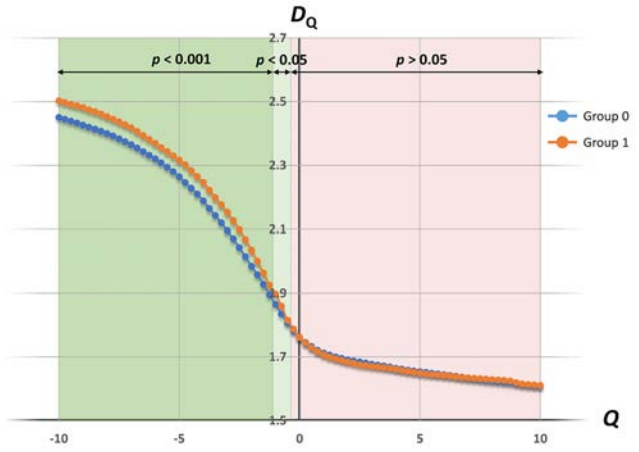

Figure 2. Spectra of median values of generalized dimensions $D_Q(Q)$. Significant differences were observed for Q values in the range of -10 to -1.25 ($p < 0.001$) and -1 to -0.5 ($p < 0.05$), indicated with green shades.

Spectrum of Hölder exponents α(*Q*)

The median value Hölder exponent spectrum $a(Q)$ is presented in **Figure 3** for both groups. This graph also exhibits a sigmoidal shape, where the median values of the Hölder exponent a decrease with increasing Q value. Statistically significant differences between the groups are found on the whole Q-negative part of the spectrum, including the Q = 0 point. On this part of the spectrum the median a values were higher for group 1, with the maximum difference in the Q range of -4 to -1. On the Q-positive part of the spectrum, similarly to $D_Q(Q)$ spectrum, the differences between the median values of a were negligible.

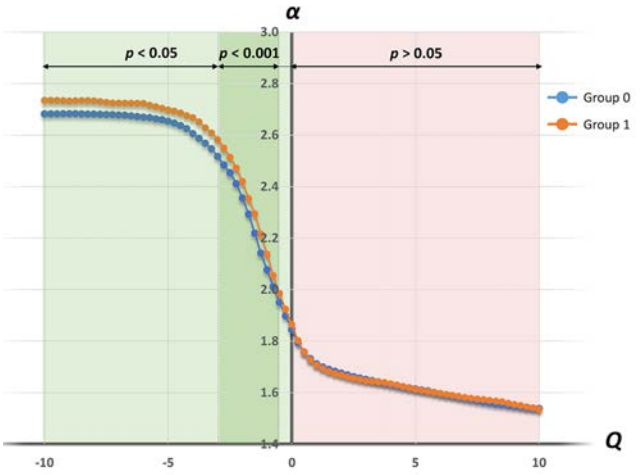

Figure 3. Spectra of median values of Hölder exponents $a(Q)$. Significant differences ($p < 0.05$) were observed for Q values in the range of -10 to 0, including the segment with $p < 0.001$ in the range of -2.75 to -0.5, indicated with green shades.

Singularity spectrum $f(\alpha)$

The graph $f(\alpha)$ vs Q is shown in **Figure 4** for both groups. Median values of $f(\alpha)$ were similar for both groups, except for three narrow parts of the spectrum: the extreme negative part ($Q < 9.5$), the middle part ($-2.25 < Q < -1.25$) and the extreme positive part ($Q = 10$), where statistically significant differences were observed. At the extreme negative part, the values of the parameter $f(\alpha)$ were higher for group 1, while on the other two segments the values were lower for the same group. This shows that both groups exhibited similar probability distribution of singularity strengths on most Q values.

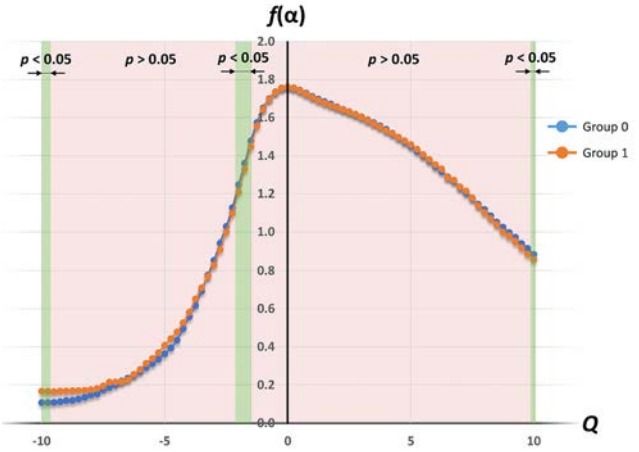

Figure 4. Spectra of median values $f(\alpha)$ vs Q. Significant differences ($p < 0.05$) were observed in narrow segments of Q values in the ranges of -10 to -9.75, -2 to -1.5 and on a single point $Q = 10$, indicated with green color.

Singularity spectra $f(\alpha)$ vs α, for both groups, are presented in **Figure 5**. Differences between the groups are observed in the right part of the spectrum, for median α values in the range of 1.844 (0.316) to 2.724 (0.792) where group 1 exhibited higher values of parameter $f(\alpha)$. This difference is mostly due to the differences in the singularity strengths α (as shown in **Figure 3**) as opposed to the differences in $f(\alpha)$ which were shown to be small **(Figure 4)**.

Table 1 contains median values and range for extreme value parameters in the $f(\alpha)$ vs α spectrum of each patient, for both groups. Parameters α_{max} and $\Delta\alpha$ showed statistically significant differences between the groups. This is consistent with the data in **Figure 3**, where we observed significant differences in the entire Q-negative part of the spectrum where the parameter α showed higher median values, including its maximum. On the other hand, minimal values of α parameter showed no significant difference between the groups. This value is contained in the Q-positive part of the spectrum where differences between the medians were very slight. None of the $f(\alpha)$ extreme value parameters (min, max and Δ) showed statistically significant differences, which is consistent with the data in **Figure 4**, where we observed differences on very narrow segments of the spectra, none of which contained these extreme values.

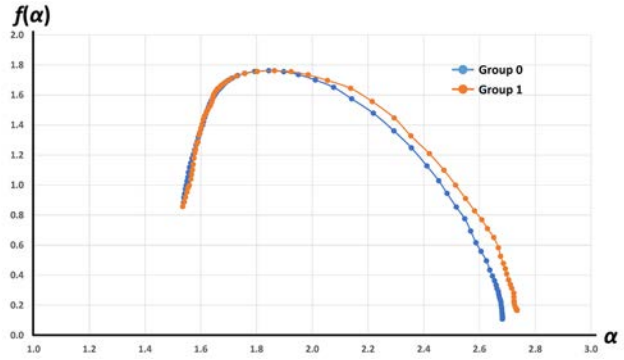

Figure 5. Singularity spectra of median values $f(\alpha)$ vs α for both groups.

Table 1. Extreme value parameters from the singularity spectra $f(\alpha)$ vs α of each patient

parameter	median value (range)		Mann-Whitney U	Z	P
	group 0	group 1			
α_{min}	1.538 (0.406)	1.535 (0.443)	20066.5	-0.771	0.441
α_{max}	2.687 (1.097)	2.736 (0.777)	25561.5	3.292	**0.001**
$\Delta\alpha$	1.153 (1.196)	1.213 (0.828)	25813.5	3.478	**0.001**
$f(\alpha)_{min}$	0.093 (1.403)	0.144 (1.296)	23553.5	1.807	0.071
$f(\alpha)_{max}$	1.762 (0.151)	1.762 (0.143)	22329.5	0.934	0.350
$\Delta f(\alpha)$	1.680 (1.489)	1.629 (1.253)	18839	-1.678	0.093

DISCUSSION

Multifractal analysis has proven to be a useful tool in quantifying the morphology of highly irregular two-dimensional objects. Images of histopathological tissue samples, including breast cancer samples, can also be included in such patterns, in a certain range of scales (13,23,28). By generating multifractal spectra, the properties of various aspects of the given object can be observed. The spectra are generated using moments of order Q, i.e., of numbers that serve as exponents that more or less emphasize the probability of the pixel distribution (Equations 1 and 5). In this way, a kind of distortion of the object is carried out, the aim of which is to accentuate different features of the object bringing them to the foreground. Thus, in the part of the spectrum with positive values of the exponent Q, the parts of the multifractal object that are more noticeable on the pattern and which contain a larger number of pixels, will prevail, while in the negative part of the spectrum, the "finer" features of the pattern that are not in the foreground will dominate (24,25). In case of the generalized dimension spectrum, the point in the central part of the spectrum (for the value $Q = 0$) is actually the Hausdorff-Besicovitch dimension, i.e., box count fractal dimension, and

describes the spatial complexity of the original object without the applied mathematical distortion (15,25). The multifractality of the object is reflected in the sigmoidal nature of its generalized dimension spectrum. The more pronounced the sigmoid shape the more heterogeneous the object in terms of the scaling rules. For example, the monofractal pattern would have a spectrum that is close to the straight line (25). We can also assess the multifractality of the object by observing the singularity spectrum ($f(\alpha)$ vs α), which exhibits a parabolic shape. The width of the spectrum (which is dictated by the range of values of the Hölder exponent α) is related to the scaling rules of heterogeneity, where wider spectra belong to the patterns with more scaling rules (i.e., multifractals) (25,30,31).

Two-dimensional histopathological images of breast tumors show multifractal characteristics (13,23,28). This multifractality can be considered a consequence of the fact that the given images contain sets of cells of various irregular shapes, so by observing an image as a whole, we include multiple scaling laws.

In the results presented in this paper, it can be noted that almost the entire part of the D_Q spectrum with negative Q moments showed statistically significant differences between the groups **(Figure 2)**. This indicates the possibility that parts of the image with a smaller number of pixels, and thus finer structures in the tissue, carry important prognostic information. The roughness of the pattern on the less pronounced parts of the image proved to be a separating factor for the two groups. This roughness is reflected in the values of the generalized fractal dimensions of D_Q, so it should be noted that the values of these parameters were slightly higher for the group with the appearance of metastases on the entire negative part of the spectrum. As all pixels of the pattern in the image represent immuno-staining with a pan-cytokeratin antibody, it can be assumed that precisely the fine morphological features of these structures in the epithelial cells contain essential information about the tumor itself. Our findings indicate that the rougher morphology of immunostained structures carries with it the higher prognostic risk.

Spectrum of Hölder exponents $\alpha(Q)$ **(Figure 3)** confirms the findings from the $D_Q(Q)$ spectrum, testifying to the dominance of fine details in the images. Regions with higher α value, i.e., the more homogenous regions in terms of local pixel intensity, showed significant differences between the groups on the entire Q-negative part of the spectrum. Observing the two spectra, $\alpha(Q)$ and $D_Q(Q)$ together, we conclude that small differences in the roughness of the finer regions of the patterns hold the key differences in terms of prognostic significance.

The biggest differences for the parameters D_Q and α were found on the parts of the spectra with Q values from around -4 to -1, which is located on the greatest slope of the sigmoidal spectra. Therefore, when trying to reduce the number of variables in this type of analysis, the mentioned region of the spectra could be of importance, as well as the variable α_{max}, which also showed statistically significant differences.

A multifractal object can be viewed as a superposition of several monofractal objects determined by a Hölder exponent α. Observed from this perspective, the function $f(\alpha)$ is actually the Hausdorff dimension of such a set of points with a monofractal structure and unique scaling law (24,30,32). The $f(\alpha)$ vs Q spectra were very similar for both groups, with only minor statistical differences **(Figure 4)**. From this we can conclude that groups had very similar probability distributions of Hölder's exponents α, i.e., they had similar distributions of scaling laws. On the other hand, the singularity spectra (presented in **Figure 5**) exhibit differences in width, with group 1 having a slightly wider spectrum. This difference $\Delta\alpha$ is statistically significant **(Table 1)**, and points to the slightly higher "multifractality" of the group 1 images. Thus, even though the overall distribution of complexity is rather similar between the groups, group 1 exhibited slightly more scaling rules. With all this taken into account, the group differences could be considered a consequence of the different degrees of complexity of individual regions, rather than fundamentally different distribution of the complexity of the overall images.

While the interpretation of our findings suggests that the fine structures within the pattern hold paramount morphological significance for diagnostic purposes, caution is warranted. The resolution of the images employed in this investigation, though relatively high (1278 x 753 pixels), introduces a potential limitation when confronted with larger distortions, as finer details are contingent upon the pixel density. Additionally, the preeminence of the immunostaining signal underscores its pivotal role in the analysis. Therefore, meticulous consideration must be given to the quality of staining, given its heightened sensitivity within the procedural framework. It is imperative to acknowledge that practical constraints limit our ability to regulate all contributing factors influencing the staining process.

It is noteworthy that, in terms of prognostic accuracy, the efficacy of the presented analysis method falls somewhat short when juxtaposed with convolutional neural networks (18,28,33–35). Conversely, neural networks exhibit drawbacks such as a requisite for substantial training datasets, high computational demands, and a narrow applicability confined to images closely resembling those encountered during training (36–38). In contrast, fractal and textural analysis methods possess the advantage of independence from training requirements, potentially offering a means to quantify investigated morphology and support specialists in decision-making (13,18,33,39). Subsequent investigations may explore synergies between these methodologies, integrating fractal and textural features as inputs into neural networks (40).

Examining the tissue holistically presents an additional advantage inherent to these analyses. This approach mitigates systematic errors that may arise from

further image segmentation or the isolation of individual elements. Furthermore, it affords a comprehensive overview of the entire information embedded in the image, avoiding oversight of critical details that could occur through the selective extraction of specific objects (28).

CONCLUSION

Multifractal analysis applied to gray-scale images of histopathological samples from breast tumors demonstrates the ability to differentiate between groups of patients with and without metastases. Statistically significant distinctions emerge in the negative domains of both the generalized dimension and Hölder exponent multifractal spectra between these patient groups. With further research, this type of analysis could potentially be a useful auxiliary tool in the diagnosis and the selection of treatment strategies for this disease.

Conflict of interest

The authors declare that they do not have any conflicts of interest.

Ethical approval

The study was approved by the Ethics Committee of the Institute for Oncology and Radiology and conforms with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

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MULTIFRAKTALNA ANALIZA NA HISTOPATOLOŠKIM SLIKAMA U SIVIM TONOVIMA: OTKRIVANJE OBRAZACA POVEZANIH SA POJAVOM METASTAZA KOD RAKA DOJKE

Zorana Nedeljković[1], Dejana Milošević[2], Marko Radulović[3], Nebojša Milošević[1], Nemanja Rajković[1]

Sažetak

Uvod: Rak dojke, sveprisutni globalni malignitet, zahteva preciznu prognozu rizika od metastaza za personalizovane terapijske strategije i povećane stope preživljavanja. U potrazi za unapređenim dijagnostičkim metodologijama, ova studija koristi multifraktalnu analizu na histopatološkim slikama u sivim tonovima, otkrivajući karakteristične obrasce povezane sa pojavom metastaza.

Cilj: Analizirati multifraktalne spektre slika histopatoloških uzoraka za grupe sa i bez metastaza kako bi se procenila korisnost ovog analitičkog pristupa u poboljšanju dijagnostičkog procesa.

Materijali i metode: Istraživanjem su obuhvaćene 102 pacijentkinje lečene iste godine (1993) na Institutu za onkologiju i radiologiju Srbije. Histopatološki uzorci su imunobojeni pancitokeratin antitelom i digitalizovani skenerom visoke rezolucije, od kojih je specijalista birao reprezentativne delove, što je dovelo do ukupnog broja od 519 slika (418 u grupi bez metastaza i 101 u grupi sa metastazama). Slike su podvrgnute multifraktalnoj analizi, procenjujući spektre generalizovanih dimenzija, Holderovih eksponenata i singulariteta.

Rezultati: Statistička poređenja između grupa sa i bez metastaza otkrivaju značajne razlike u negativnim domenima spektara generalizovanih dimenzija i Holderovih eksponenata, naglašavajući uticaj finih struktura u morfologiji tkiva koje su povezane sa rizikom od metastaza.

Zaključak: Multifraktalna analiza primenjena na slike histopatoloških uzoraka tumora dojke pokazuje sposobnost razlikovanja grupa pacijenata sa i bez metastaza. Iako je potreban oprez u pogledu ograničenja, poput uticaja rezolucije slike i osetljivosti na imunološko bojenje, ovaj metod ne zavisi od treninga na velikom uzorku i pokazuje potencijalni dijagnostički značaj kao i moguću sinergiju sa naprednim neuronskim mrežama.

Ključne reči: multifraktal, histopatologija, rak dojke, metastaze

Primljen: 22.01.2023. I **Revizija:** 26.03.2024. I **Prihvaćen:** 10.04.2024.

Medicinska istaživanja 2024; 57(2):75-83

Medicinska istaživanja 2024; 57(2):85-91 | DOI 10.5937/medi57-45708

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REWIEV ARTICLE

Beers criteria: an up-to-date tool for detecting inappropriate prescription in elderly

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Recived: 27 July 2023

Revised: 03 January 2024

Accepted: 10 April 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Potentially inappropriate medications (PIM) are drugs that can cause significant unnecessary harm to patients. Prescribing PIM can cause significant healthcare problems, especially if there is a safer, similar, or even more effective alternative for the treatment. They are the cause of significant health issues that lead to increased treatment costs and reduced life quality. The main problem when it comes to treating geriatric population is a lack of specific guidelines for the treatment. This is mainly because clinical trials which are the main pillars of clinical guidelines are usually aimed at people aged 18 to 65, leaving the geriatric population aside. For this reason, as well as to reduce the prescription of PIM in the geriatric population many guidelines have been created, among which the AGS Beers criteria were the first and have remained the leading and most comprehensive tool for this purpose. Since 1991, the Beers criteria have gone through several updates and changes in format, and in 2012 they came under control of the American Geriatric Society. The constant updates and work on these guidelines saved them the top position, compared to other guidelines in this field. Taking all this into consideration, it is imperative for every clinician who works with geriatric patients to be familiar with these guidelines and to utilize them properly as explained.

Keywords: AGS Beers criteria, potentially inappropriate medication, geriatric population

Cite this article as: Stojanović M, Banjanin N. Beers criteria: an up-to-date tool for detecting inappropriate prescription in elderly Medicinska istaživanja 2024; 57(2):85-91 DOI: 10.5937/medi57-45708

INTRODUCTION

Potentially inappropriate medication in the geriatric population

Potentially inappropriate medication (PIM) also known as potentially inappropriate prescribing (PIP) is defined as a medication prescribed to patients whose risk of harm outweighs its benefits (1). This is why PIMs are considered a significant healthcare problem, especially if there is a safer, similar, or even more effective alternative for the treatment (1-3). There is also a more basic definition of PIMs which defines PIMs as drugs that can cause significant unnecessary harm to patients. PIMs can endanger a patient's life, especially in elderly population where they significantly increase the risk of mortality (2-4). Several decades of intensive PIM research revealed the fact that in the geriatric population, PIMs can cause significant health issues that lead to increased treatment costs and impaired quality of life. In the next section of this paper, the focus will be on health and economic consequences of PIMs, as well as on the benefits of PIM reduction.

Health consequences of potentially inappropriate medication

Elderly patients, due to multiple simultaneously present conditions and diseases, are commonly prescribed more medications than the rest of the population. Polypharmacy, as this phenomenon is called, is one of the biggest health issues of the geriatric population (2,3). In this population, polypharmacy-related problems are more evident due to changes in physiological and cognitive function, as well as in pharmacokinetic and pharmacodynamic changes, that are consequences of aging. Studies in this field reported that in the elderly PIMs may increase the incidence of adverse drug reactions (ADR), hospitalization, poorer treatment outcomes, and death (5-8). A nationwide large-scale survey from Japan was conveyed to determine the prevalence of adverse drug reactions caused by PIMs; it showed that PIM was responsible for adverse drug reactions in at least 8% of patients (5). In another study that investigated the occurrence of ADR in elderly hospitalized patients, PIMs were detected in almost 24 % of patients whit ADR (6). The study managed to find a correlation between PIMs and the occurrence of ADR, marking PIMs a significant risk factor for ADR development (6). Varalo et al., (7) performed a study intending to determine the prevalence of PIMs in hospitalized elderly patients and to determine the connection between ADRs and PIMs. The study revealed that in hospitalized elderly patients 5% of ADRs were developed as a consequence of PIMs. All this literature evidence implies that prescribed PIMs mean worse health outcomes in elderly patients and drug-related problems occur significantly more often when compared to elderly patients without prescribed PIMs (8).

Economic consequence of potentially inappropriate medication

From an economic point of view, it is interesting to know that a single PIM prescribed to an elderly patient significantly increases healthcare expenditures (9). One of the estimates, which was done at the beginning of this century, revealed the severity of the economic burden that PIMs brought on the healthcare system (9). This study showed that in the United States of America (USA) around 7.2 billion US $ was spent on healthcare expenditures that had occurred as a consequence of prescribing PIMs to elderly patients (9). A study from Germany, which compared healthcare costs between the elderly with and without prescribed PIMs, showed that in the first quarter of the year since the beginning of the study, healthcare costs were significantly higher in PIM group. More precisely, in the first quarter health care costs were 1237 €/patient higher in the PIM group (10). Another finding in connection with PIM costs was obtained from the economic analysis performed in French nursing homes. This French study determined how much PIM cost per day, and how much would be saved if the number of prescribed PIMs would be reduced. The cost of a single PIM prescriber per resident per day was 2.8 €, which means that in France 25 million € could be saved per year, only by reducing the number of PIMs in nursing homes (11). A cost-utility analysis performed to determine the economic impact of three commonly prescribed PIMs in the elderly (i.e., non-steroid anti-inflammatory drugs, benzodiazepines, and proton-pump inhibitors) showed that in 2014, these three PIMs were associated with greater costs and reduced quality-adjusted life years (12). Out of these three drugs, benzodiazepines had the highest incremental costs, with 3470 € when compared to non-sedative medications (12). All these studies demonstrated that PIMs were not only associated with health problems but also with a significant economic burden for the healthcare system in every country and that significant effort should be invested in reducing PIMs for several reasons: prolonging life, increasing quality-adjusted life year (QALY), and reducing treatment costs.

GUIDELINES FOR THE REDUCTION OF PIMS IN THE ELDERLY

Clinical trials that include geriatric patients are rare. Mainly, the data that could be found in the literature is related to unwanted effects and therapeutic problems in this population. This is why the modest literature data related to the treatment of the geriatric population is used to create guidelines in a way in which we are focusing on what should be avoided when treating these patients instead of what should be a drug of choice for certain conditions or diseases.

Since the first guidelines created by Beers in 1990s, many new guidelines have been created with the same idea to reduce PIMs in the elderly population. The main reason for expansion and creation of different guidelines is the fact that each is regionally specific and focused on drugs sold in that specific market. Since its creation, the use of the Beers criteria have been evaluated in different countries, and apart from being created especially for the USA market it has been shown that this criteria is possible to use all over the world. Sometimes some minor changes were needed which led to the creation of new criteria like PIM-Taiwan which was based on the Beers criteria. This is why the Beers criteria are the most well-known and most widely used criteria for PIM reduction in older adults in the world (13). Nevertheless, after the Beers criteria there were a few specific criteria created with a different philosophy than the Beers criteria and for different geographic regions. Some of those criteria are STOPP/START criteria and EU (7)-pim criterion (2,3). All the above-mentioned criteria were created for the general geriatric population and were to be used mainly by medical doctors. By that time, there was a growing need for different guidelines, and this is why specific guidelines have been created for nursing home residences NORGEP-NH which are flaming in PIMs, and also there are now guidelines that could be used by pharmacists GheOP3S tool which facilitated pharmacists' inclusion in the struggle for PIMs reduction. All these guidelines enriched the field and helped medical professionals involved in the treatment of the elderly.

AMERICAN GERIATRIC SOCIETY BEERS CRITERIA

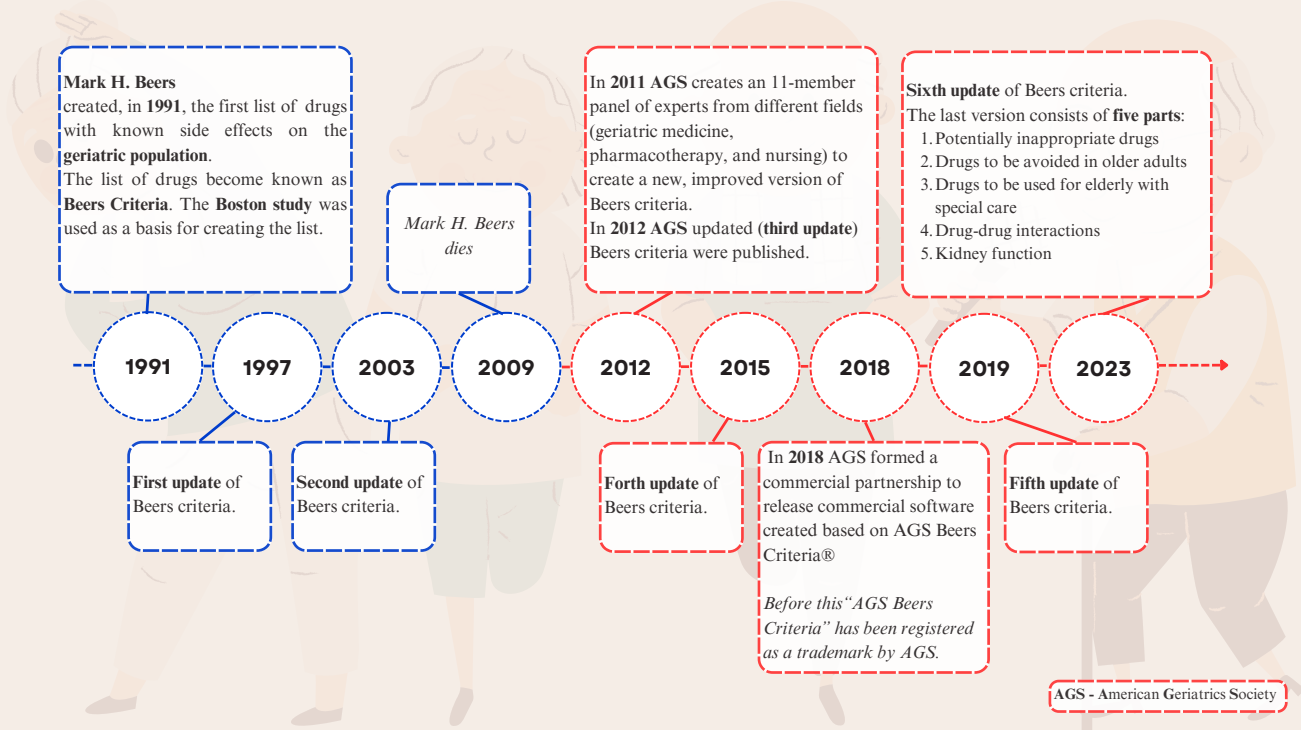
The American Geriatric Society (AGS) Beers criteria are widely used criteria by healthcare providers, researchers, educators, healthcare administrators, and regulators (14). These criteria are created as a list of medications that should be avoided by the elderly, always or in specific situations. The primary goal of these criteria is to manage and improve the pharmacotherapeutic part of healthcare for people older than 65 years (14). In clinical settings, the Beers criteria are used for all aspects of care, except for palliative and hospice care (1). The AGS Beers criteria are the most known and most widely used criteria for PIM reduction in older adults (> 65 years) (13). Apart from its purpose in reducing PIM in the elderly, AGS Beers criteria should be used for educating clinicians working in the field of geriatric medicine and for the evaluation of costs and quality of provided healthcare.

History of the AGS Beers criteria

The American geriatric society (AGS) Beers criterion was first created in 1991 by Mark H. Beers, a geriatrician whose research was focused on drug interaction in geriatric patients which led to the creation of the Beers guidelines. Beers deserves great credit for his criteria influenced a wave of much-needed innovation in geriatric pharmacology. The criteria were initially updated in 1997 and 2003. After Beer died in 2011, AGS became responsible for revisions and updates of the Beers criteria. The first criteria update under the supervision of AGS was published in 2012 (15). For this revision, AGS organized an eleven-member panel. The panel consisted of experts in different fields of geriatric medicine (medical doctors, nurses, pharmacists, and researchers). The panel created an update using the modified Delphi method. In the review process, the experts' panel decided whether new criteria, derived from literature data, should be added to the existing list of criteria, or if the existing criteria should be removed or revoked. The newly updated Beers criteria were based on experts' knowledge and literature review. Since 2012, the year when the first Beers criteria were updated under the supervision of AGS, there have been three more updates. Currently, AGS is trying to provide updates on the Beers criteria every 3 years. The last AGS Beers criteria update was published in May 2023. The detailed timeline of Beers criteria was presented in **Figure 1**.

Structure of AGS Beers Criteria

In this section, we will shortly present the most important parts of the AGS Beers criteria. The latest update is a product of a workgroup assembled of 12 experts with background in medicine, nursing, and pharmacotherapy. Ten members of this panel also worked on the 2019 update of AGS Beers criteria (16). The AGS Beers criteria 2023 is basically a list of medications that, if possible, should be always avoided, or should be avoided in certain situations (i.e. specific diseases or conditions) (14). The core of AGS Beers criteria are five parts that consist of several criteria. These parts are: (1) medications that are potentially inappropriate in most older adults; (2) medications that should typically be avoided in older adults with certain conditions (possible drug-disease or –syndrome interface); (3) medications that should be used by the elderly, but with special care; (4) drug-drug interactions that should be avoided in the elderly; (5) medication to avoid or adjust the dose based on kidney function. These five parts remained from the AGS Beers 2015 and AGS Beers 2019 versions of these criteria (14,15). In the last version, 38 drugs or medication classes were skipped when compared to the 2019 criteria (14,16). In total, 10 new criteria were added to AGS Beers 2023 criteria when compared to the last version (14,16). Also, 32 drugs/criteria from AGS Beers 2019 were modified in AGS Beers 2023 (i.e. switched from one part to another part of the guidelines based on new evidence, had clarified or modified language, were modified based on the newly discovered risks, etc.).



The first part of the criteria consists of the drugs which are potentially inappropriate in older adults, such as anticholinergic drugs (first-generation antihistamines, antiparkinsonian agents, and antispasmodics), antithrombotic drugs, anti-infective drugs, and drugs for pain; drugs affecting different organ systems (cardiovascular, endocrine, gastrointestinal, genito-urinary and central nervous system). The strength of recommendation for many elements of this part of criteria is marked as strong. The strength of the recommendation was weak only for androgens and estrogens (with or without progestins) for topical use. For this part of the guidelines, the quality of evidence was predominantly moderate to high.

The second part consists of the drugs that should be avoided in certain conditions or diseases since these drugs can aggravate the primary disease. For example, thiazolidinediones antidiabetic drugs with some pleiotropic effects (17) can promote fluid retention and thus are avoided in older adults with heart failure (16). The strength of recommendation for all criteria, except for alpha-1 blockers and antipsychotics in syncope (week), is marked as strong (16). The quality of evidence for this part of the criteria is predominantly moderate (16).

The third part of the AGS Beers 2023 criteria is dedicated to drugs that need to be used with caution in older adults. For example, prasugrel or ticagrelor should be used with caution due to a high risk of major bleeding in those 75 and older. Still, these drugs can have some benefits for selected patients. The strength of recommendation for all criteria of this part of the Beers criteria, except for SGLT2 inhibitors (week), is considered strong (16). The quality of evidence is moderate for all these criteria. The only exception is the trimethoprim-sulfamethoxazole combination for which the quality of evidence is low (16).

The fourth part of the criteria is dedicated to drug-drug interactions that are best to be avoided in the population older than 65 years (16). Most of the listed interactions are focused on the drugs that affect the central nervous system. For all the listed interactions, the strength of recommendation was marked as strong. The quality of evidence was moderate for all recommendations, while it was high only for criteria that considered a combination of CNS active drugs.

The fifth part considers drugs that should be avoided or drugs whose dosage should be adjusted regarding the kidney function. The kidney function is estimated based on creatinine clearance (mL/min). The strength of recommendation for this part of the criteria was strong for all, except for tramadol and duloxetine which should be avoided when creatinine clearance is under 30 mL/min. The quality of evidence for this part of the guidelines was moderate except for the mentioned tramadol criteria for which the quality of evidence was low.

EXAMPLES OF SUCCESSFUL UTILIZATION OF THE AGS BEERS CRITERIA AND THEIR LIMITATIONS IN PRACTICE

The AGS Beers criteria are created to be used in the USA. They may be used internationally, but still, a validation study needs to be performed. Previous versions were examined in different parts of the world and the results of these studies support the use of criteria outside of the USA. Also, these criteria are supposed to be used in any type of clinical care except hospice and end-of-life care such as nursing homes. Nevertheless, some data show that the AGS Beers criteria could be used in nursing homes as well

(3). The interpretation of the data from studies in nursing homes should be taken with special caution because in nursing homes there is a flaming issue of PIMs. This is why when using this guideline improperly it may seem that there are more PIMs than there really are. This is why it is always highlighted to use these guidelines properly, as explained in the AGS Beers criteria. Also, there are other guidelines specially created to address the most common problems in prescribing practice in nursing homes.

This and the previous version of the criteria were evaluated and validated in different clinical settings and in different populations. The AGS Beers criteria were created for primary health care, and they were evaluated for this purpose in a cross-sectional study conducted in two primary health care centers in Brazil (18). These criteria were also evaluated in a specific subgroup of the geriatric population, in so-called very old people (80 years and above) (19). The use of AGS Beers criteria was also validated in older (> 65 years) cancer outpatients with multimorbidity (20). There is evidence that the AGS Beers criteria can be used with great success for finding PIMs in psychiatry and internal medicine (21, 22). Also, the use of these guidelines has been proven to be successful in detecting PIMs in patients with chronic kidney disease (23). The use of these criteria was, among others, validated in Brazil, China, India, Jordan, Korea, Lithuania, Nigeria, Portugal, Serbia, South Africa, Spain, the USA (3, 18-30).

The main problem with the AGS Beers criteria is that they are constantly, in each new update, skipping the part related to supplement use. Supplement use is becoming more and more pronounced in different age groups. We are currently witnessing an increasing interest in research in the field of supplements. On a daily basis, we are getting new insights into where the use of different supplements could be useful (31 - 33). Still, their interaction with drugs and different diseases and conditions could be a serious health problem, especially in sensitive populations such as the geriatric population.

CONCLUSION

The AGS Beers criteria are certainly a powerful tool in reducing PIMs in the geriatric population, as well as in reducing the development of ADE and unintended consequences of inadequately prescribed drugs, while at the same time reducing the healthcare costs. Although initially created for the USA healthcare system, these criteria have been proven effective in various healthcare systems around the world. The most powerful feature of the AGS Beers criteria is their constant 3-year updates, based on the latest literature data. Considering all of these, it is imperative for every clinician who works with geriatric patients to know these criteria and to utilize them properly as explained in these criteria.

Acknowledgments

None

Conflict of interest

None to declare.

Author Contributions

Both authors contributed significantly to the conception and design of this review article and to preparing the draft of the manuscript.

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BIRSOVI KRITERIJUMI: MODERNA ALATKA ZA OTKRIVANJE NEADEKVATNO PROPISANIH LEKOVA U GERIJATRIJSKOJ POPULACIJI

Marko Stojanović[1], Nikolina Banjanin[2]

Sažetak

Potencijalno neodgovarajući lekovi su lekovi koji pacijentima mogu nepotrebno da naruše zdravlje tokom njihove redovne primene u terapiji. Propisivanje potencijalno neodgovarajućih lekova može izazvati značajne zdravstvene probleme, što treba izbeći, posebno ako postoji bezbednija, po efikasnosti slična, ili čak efikasnija alternativa za lečenje. Potencijalno neodgovarajući lekovi su uzrok zdravstvenih problema, dovode do povećanja troškova lečenja i smanjenja kvaliteta života. Glavni problem u lečenju gerijatrijske populacije je izostanak specifičnih smernica za lečenje. Kako klinička ispitivanja, koja su glavni izvor informacija za kliničke smernice, obično obuhvataju ljude uzrasta od 18 do 65 godina, nedostaje dovoljno relevantnih informacija na osnovu kojih bi se kreirale terapijske smernice za gerijatrijsku populaciju. Kako nema adekvatnih smernica za terapiju pacijenata u gerijatrijskoj populaciji, i kako bi se smanjio broj neadekvatno propisanih lekova, osmišljeni su vodiči sa tim ciljem. Prvi je ovakve vodiče osmislio Mark Birs 1991. godine. Nakon ovih vodiča osmišljen je veliki broj vodiča sa sličnom namenom. Međutim, Birsovi kriterijumi ostaju i do danas najkorišćeniji i najpoznatiji kriterijumi u svetu. Ovo je pre svega rezultat stalne dopune i osavremenjivanja ovih vodiča. Sam Birsov vodič je prošao kroz nekoliko dopuna i promenu formata, a od 2012. godine ovaj vodič uređuje Američko gerijatrijsko udruženje. Sve ovo čini Birsove kriterijume odličnom alatkom za pronalaženje neadekvatno propisanih lekova u gerijatrijskoj populaciji, a samim tim je od važnosti za sve lekare koji rade sa gerijatrijskom populacijom.

Ključne reči: Birsovi kriterijumi, neadekvatno propisani lekovi, gerijatrijska populacija

Primljen: 27.07.2023. I **Revizija:** 03.01.2024. I **Prihvaćen:** 10.04.2024.

Medicinska istaživanja 2024; 57(2):85-91

Medicinska istaživanja 2024; 57(2):93-102 | DOI 10.5937/medi57-47926

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Medical Research | Published by Faculty of Medicine University of Belgrade

REWIEV ARTICLE

Specific aspects of prognosis and treatment of elderly patients with large B-cell lymphoma

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Recived: 27 November 2023
Revised: 03 January 2024
Accepted: 03 January 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Large B-cell lymphoma (LBCL) is the most common type of non-Hodgkin lymphoma in the general population, constituting 40-50% of all NHL cases, and over 60% of lymphoma cases in the population of patients over 65 years. Given their increasing life expectancy, the prevalence of this lymphoma type is expected to grow in the upcoming years. Treating these patients is a significant challenge due to numerous factors that complicate the treatment and worsen the outcome of the disease. Elderly patients often have comorbid conditions, weakened organ function, altered drug metabolism, and reduced hematopoietic capacity for bone marrow recovery, which makes them less tolerant to chemotherapy. A poorer prognosis is attributed to a higher frequency of the non-GCB subtype and histologically unfavorable types of LBCL, such as EBV-positive LBCL, High-grade B-cell lymphoma (HGBL), and plasmablastic lymphoma, as well as extranodal localizations associated with worse outcomes. Treating elderly patients is complex because they represent a highly heterogeneous population with significant variations in health status, comorbid conditions, and expected lifespans. Therefore, when it comes to elderly patients, a comprehensive geriatric assessment is necessary, including the determination of a comorbidity index to differentiate between those in good general condition (fit), those in poor general condition (frail), and those who are in between. The treatment can be aimed at recovery, life extension, or symptom control. The standard therapy for elderly patients with good general condition and advanced disease is R-CHOP, while for patients with comorbidities and poor general condition, reduced protocols with or without anthracyclines are considered. Previously, elderly patients with relapsed or refractory LBCL faced a very poor prognosis due to limited treatment options. However, the treatment of elderly patients with R/R LBCL has improved in recent years due to the introduction of new drugs (polatuzumab, tafasitamab, bispecific antibodies, and CAR-T cells) that can be used in older individuals.

Key words: large B cell lymphoma, elderly, prognosis, therapy

Cite this article as: Markovic O, Bukurecki I, Stanisavljević N, Divac A, Todorovic Z, Cvetković Z. Specific aspects of prognosis and treatment of elderly patients with large b-cell lymphoma Medicinska istaživanja 2024; 57(2):93-102 DOI: 10.5937/medi57-47926

INTRODUCTION

Non-Hodgkin lymphoma (NHL) is the eighth most common malignancy in both sexes (1). Large B-cell lymphoma (LBCL) is the most prevalent type of non-Hodgkin lymphoma (NHL), accounting for 40-50% of all NHL cases and over 60% of lymphoma cases in the population of patients over 65 years (1). Its incidence gradually increases with age, and one-third of patients are older than 75 years (2). The median age of patients at the time of diagnosis is 67 years (1). Considering the increasing life expectancy, it is expected that the number of elderly patients with NHL will continue to rise in the years to come. Although the population over 65 years constitutes 13% of the total population, this group sees 25% to 35% of new lymphoma cases (2). According to the data obtained from the Institute of Public Health of Serbia "Milan Jovanovic Batut", the incidence rate of LBCL in the Republic of Serbia in 2021 was 6.9 for males and 5.7 for females, showing an increasing trend among the elderly (3). In the population over 75 years, it was 20.1 for males and 13.4 for females (3). Lymphomas in the elderly deserve special attention, considering the numerous factors that complicate the treatment and affect the outcome of the disease.

SPECIFIC NATURE OF ELDERLY PATIENTS

Large B-cell lymphoma (LBCL) in elderly patients deserves special attention due to numerous factors that complicate the treatment and affect the outcome of the disease (Table 1). The outcome of LBCL in patients aged ≥65 years is significantly worse than in patients under the age of 55, with a 5-year relative survival rate of 55.1% and 79.7%, respectively (4). The aging process alters drug tolerance, absorption, diffusion, and metabolism, thus affecting the response to therapy (5). Most chemotherapy protocols lead to myelosuppression, and in elderly patients, the recovery of bone marrow function takes longer (2). Elderly patients often have heart conditions, necessitating an assessment of cardiac function, especially when anthracycline-based protocols are employed. Aging increases the likelihood of dysfunction in various organs, often contributed to by the frequent use of non-cytostatic medications for controlling chronic diseases.

Table 1. Factors influencing the treatment of elderly lymphoma patients

Characteristics of elderly patients
Biological age diversity
Age-related stereotypes
Misconceptions among patients and healthcare professionals about the causes of the disease, disease progression, and treatment
A limited number of studies designed exclusively for elderly patients
Comorbid conditions in the elderly population
Decline in organ function
Age-related immune system weakening
Changes in drug pharmacokinetics associated with aging

Dysfunction pertains to the kidneys, liver, heart, and bone marrow (5). Changes in organ function affect treatment tolerance and the ability to apply protocols designed for lymphoma eradication, thus impacting the aim of the treatment (5). For example, in patients with renal insufficiency, cisplatin cannot be administered, adequate doses of doxorubicin cannot be given to patients with heart failure, and the presence of peripheral neuropathy prevents the use of vincristine at full doses. Age-related reduction in bone marrow reserves leads to marked myelosuppression and a higher incidence of infectious complications. Therefore, it is not surprising that advanced age is an independent prognostic factor for predicting the frequency of hospitalizations and febrile neutropenia.

Elderly patients may have poor general health, associated illnesses, and often take a large number of medications. The prevalence of severe comorbid conditions occurs in patients aged 60 to 69 and those over 70 years is 43% and 61%, respectively, and even more than 85% of patients over 80 years. In younger patients, comorbidity is present in only 20% of those affected (6). The most common comorbidities are other malignancies, diabetes, osteoporosis, arthritis, cardiovascular or pulmonary diseases, renal dysfunction, depression, Alzheimer's disease, and others. Patients with comorbidities are at a high risk of toxic treatment effects and a higher risk of mortality (6). Therefore, a comprehensive geriatric assessment (The Comprehensive Geriatric Assessment - CGA) is necessary for all elderly patients with lymphoma, along with an assessment of the comorbidity index (Charlson Index and Cumulative Illness Rating Scale - CIRS) (7). Geriatric assessment is a multidimensional diagnostic tool that evaluates nutritional status, cognitive abilities, and comorbidities, and its results help in defining the optimal therapeutic approach for elderly lymphoma patients (8).

Elderly patients may have misconceptions about the cause, progression, and treatment of the disease (2). On the other hand, diagnosing lymphoma in older individuals is associated with numerous end-of-life questions and a discussion about the necessity of curative treatment due to their age.

CLASSIFICATION OF LBCL AND THE FREQUENCY OF SUBTYPES AND ENTITIES IN OLDER PATIENTS

Based on the analysis of gene expression profiling (GEP), three types of LBCL have been identified. These include the germinal center B-cell (GCB)-like subtype, resembling the GEP of normal GCBs, the activated B-cell (ABC)-like subtype, resembling normal ABCs, and unclassifiable disease in the remaining 10-15% of samples (9). The distribution of these subtypes changes with age, with the highest frequency of the non-GCB subtype which has significantly poorer outcomes compared to GCB disease in the elderly patients (9). Patients with the GCB subtype

are, on average, 8 years younger than those with the non-GCB subtype (10).

LBCL is characterized by significant histological and clinical heterogeneity. According to the latest WHO-HEM5 classification in 2022, LBCL includes 18 different subtypes and entities. Some of them were categorized for the first time as separate entities in this classification (11). All subtypes of LBCL occur in older patients, but there is a higher incidence of histologically unfavorable types of LBCL, such as EBV-positive LBCL, high grade B cell lymphoma (HGBL), plasmablastic lymphoma, and extranodal localizations associated with a poorer prognosis, such as lymphomas of immune-privileged sites (brain, testes, vitreoretinal lymphomas), primary effusion lymphoma without HIV infection or adrenal lymphomas (12,13). One of the new LBCL subtypes added to the WHO-HAEM5 classification in 2022 is the so-called Fluid overload-associated large B-cell lymphoma. It most commonly occurs in elderly individuals who are not immunocompromised. Although it comes with exclusive localization in body cavities, it should be distinguished from primary effusion lymphoma which has a completely different genetic profile (11). It occurs in individuals with conditions characterized by fluid overload, such as heart and kidney failure, liver cirrhosis or protein-losing enteropathy. However, unlike PEL and most other lymphomas in older people, the prognosis of these lymphomas is quite favorable (11). EBV-positive LBCL is an aggressive lymphoma which occurs more frequently in the elderly and lacks distinctive morphology or immunophenotype. Diagnosis necessitates the demonstration of EBV-encoded small RNA through in situ hybridization (14). The median age at diagnosis is 71, with 70% of patients having extranodal disease. EBV-positive LBCL shows inferior survival across all IPI categories, with a median overall survival (OS) of 24 months (15).

HGBL represents a newly defined category of aggressive lymphomas encompassing DHL, as well as various prior cases of Burkitt-like or aggressive immunoblastic variants. These lymphomas exhibit poorer outcomes with R-CHOP immunochemotherapy and an increased risk of central nervous system (CNS) invasion (16). Advanced age correlates with a higher prevalence of high-risk molecular LBCL subtypes and MYC rearrangement (17).

PROGNOSTIC PARAMETERS IN LBCL

The significance of age as a prognostic parameter was recognized very early, and it is not surprising that it is one of the parameters contained in all clinical scores currently used in clinical practice. The basic and oldest prognostic score for LBCL is the International Prognostic Index (IPI) (18). It served as the basis for defining aa-IPI, the revised International Prognostic Index (R-IPI), and the International Prognostic Index for the elderly (E-IPI). However, in recent years, the NCCN-IPI has been increasingly used, developed in the era of rituximab, which distinguishes four different prognostic groups with four-year survival ranging from 33% to 96%. (19). The prognostic power of NCCN-IPI increases when the CCI is added to the prognosis assessment, especially in the elderly population (20).

However, when it comes to age, it is essential to define the boundary for defining "old age." There are conflicting views on what age constitutes the threshold for defining "elderly" patients with DBKL. The International Prognostic Index (IPI) classifies patients above 60 years of age as elderly patients because it is based on the results of studies in which there were very few patients over 80 years of age (18). Given that the median age of newly diagnosed DBKL patients exceeds 65 years, the age limit of 60 years is certainly not adequate for defining elderly patients. The age limit of 75 years should be the threshold included in the definition of "elderly patients" because the outcome in the group over 75 years is significantly worse than in the group of patients under 75 years (21). The National Cancer Network (NCCN) classifies elderly patients into "young elderly" aged 65 to 75 years, "elderly" aged 76 to 85 years, and "very elderly" patients over 85 years (19).

In recent years, the prognostic significance of numerous clinical, laboratory, and histological parameters has been analyzed, and many novel markers with potential prognostic significance in the elderly patients with LBCL have been identified (22). Laboratory parameters, such as the ratio of absolute lymphocyte count (ALC), absolute monocyte count (AMC), and histopathological characteristics, i.e., BCL2, surviving, XIAP, MYC, and CD5 expression, showed a significant impact on clinical outcomes (23-25). They were used to develop new models with an improved ability to discriminate prognosis.

ASSESSMENT OF THE ELDERLY PATIENTS' SUITABILITY FOR THE TREATMENT

The population of elderly patients is a heterogeneous group, since the physiological age does not always correspond to the patient's condition. Traditional measures like the Karnofsky index or ECOG (Eastern Cooperative Oncology Group) performance status are not precise enough to determine the treatment goal according to them, i.e., to avoid the risk of under- or overtreatment. ESMO guidelines recommend the use of geriatric assessment in patients with lymphoma (26). Comprehensive geriatric assessment (CGA) in patients with DBCL enables the prediction of tolerance to chemotherapy and mortality, independent of the performance status (27). However, the implementation of CGA is sometimes hardly possible due to the time and resources required for its implementation. The Fondazione Italiana Lymphomi

(FIL) has validated the simplified geriatric assessment (sGA) for people over 64 years old, whose score includes age (≥80/<80 age), CIRS-G (Cumulative Illness Rating Scale for Geriatrics), ADL (activities of daily living) and IADL (instrumental activities of daily living) (28). Based on the geriatric assessment, patients can be divided into three groups: patients in good general condition (fit), patients in poor general condition (frail) and those who are between these two groups (unfit) (28). One of the models for predicting the tolerability of therapy is a model developed by the Japanese group for the treatment of lymphoma that includes advanced age (>75 years), hypoalbuminemia (<3.7 g/dL), and a high Charlson Comorbidity Index score (≥3) (29). However, even simple gait speed tests can accurately identify frailty and predict the outcomes independent of the performance status as well as grip strength (every 5-kg decrease in grip strength was associated with worse survival) (30).

FIRST LINE THERAPY

Fit patients

The treatment goal for fit elderly patients <80 years is to cure them, using standard protocols, which has been the R-CHOP regimen for the last 20 years (31). In the population of elderly patients, complete remission in the era of rituximab is achieved in 60 to 80% of patients (31). However, the use of full doses of drugs within this protocol requires careful monitoring of the patient due to the possibility of side effects, primarily a regular check of cardiac function to minimize cardiotoxicity. A routine evaluation of the ejection fraction by echocardiography or by MUGA scan is suggested before therapy as well as after 4 cycles of anthracyclines with possibly more frequent monitoring if necessary (32). The International Society for Geriatric Oncology (SIOG) has given recommendations for the reduction of cardiotoxicity and the use of anthracyclines in the elderly in routine clinical practice (32).

The gold standard in the first line of treatment for fit elderly patients is 6 cycles of the R-CHOP protocol (33). Adding two more cycles or intense dose administration on 14 days did not improve treatment results but it increased toxicity (21.34). Shortening the treatment to 3 cycles of the R-CHOP protocol with consolidative radiation therapy (RT) in early stage DBCL showed identical survival outcome as full-course R-CHOP (≥6 cycles) and RT recipients experienced less acute toxicity (35.36). Also, a study of 592 patients showed the non-inferiority of the four-cycle regimen in comparison of six cycles of R-CHOP, but with relevant reduction of toxic effects (37).

The dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (DA-EPOCH-R) combination has been employed in patients with high grade LBCL, even those over the age of 80. However, no advantage of using DA-EPOCH-R in >65 years has been shown (38) and the treatment in this group of patients remains a significant clinical challenge.

Attempts to improve the treatment outcome compared to the application of the R-CHOP protocol by using new biological agents, especially in non-GCB, did not show an advantage. Polatuzumab is the first drug that improved the results of the treatment of patients with DBKL in the first line of treatment compared to R-CHOP. In the POLARIX study, it was shown that patients treated with the pola-R-CHP protocol had a longer two-year PFS compared to the group of patients treated with the R-CHOP protocol (with equal OS), while patients over 60 years of age, those with non-GCB type, "double" expressors, and patients with high IPI had the greatest benefit (39). In a phase II study, it was shown that chemotherapy-free IR2 (ibrutinib+rituksimab+lenalidomid) regimen in patients 75 years or older with de novo diffuse large B-cell was clinically effective and safe (40). A phase 2 trial, known as REAL07, which evaluated the safety and effectiveness of lenalidomide in combination with standard doses of R-CHOP21 for elderly fit patients with untreated LBCL has been shown to be both effective and safe, with a high rate of overall response and manageable side effects (41). The ongoing front-MIND phase III trial aims to compare the efficacy and safety of tafasitamab-lenalidomide plus R-CHOP versus R-CHOP alone in newly diagnosed LBCL patients aged 18-80 years, with high-intermediate or high-risk disease (42).

A new direction in treatment is being introduced by a group from MD Anderson under the so-called smart start study principle, which is based on the initial application of 2 cycles of a biological agent (RLI: rituximab, lenalidomide, ibrutinib), followed by the application of standard chemotherapy (R-CHOP or REPOCH) in non-GCB DBCL aged 29-83 year (43). The results are promising with 100% ORR and a 2-year PFS of 91 % (43).

Frail and unfit patients

Frail/unfit patients show a very poor tolerance to therapy and therefore require protocols with reduced doses **(Table 2)** or the application of palliative non-anthracycline protocols which significantly affects the outcome of the treatment. However, it should be acknowledged that some elderly patients show a poor performance status due to lymphoma itself. In such situations, introductory therapy can be started to improve the patient's functional condition. The German lymphoma study group suggests that in patients with an ECOG score of 2 or more, prednisone should be administered for 7 days or 1 mg of vincristine before the first cycle of therapy (44). If the patient's condition improves, the goal of the therapy can be modified. According to the ASCO guidelines, prophylactic white blood cell growth factors can reduce the risk of potentially life-threatening neutropenic infections,

and should be considered for patients aged 65 and over who receive immunochemotherapy for LBCL, as the risk of febrile neutropenia in this group is up to 3% (45). In the prospectic B-R-ENDA clinical study it was shown that the results of treatment with BR in elderly or frail aggressive B-cell lymphoma patients are similar with results received after the treatment with R-CHOP. In this study progression-free survival (PFS) and overall survival (OS) at 2 years were 45% and 46% for the patients age >80, as well 32% (13%-51%) and 37% (17%-57%) for frail patients aged 64–80, respectively (46).

In the treatment of this group of patients, a "chemo-free" approach, based on new antibodies and small molecules has been analyzed. The combination Rituximab - Lenalidomide was tested in phase II of the ReRi study with promising results: the ORR is 41%, while the 11-year OS is 69% (47). In phase I/II, as the first line of therapy in those >80 years or >60 years of age with comorbidities, it is being tested bispecific antibody mosunetuzumab with an ORR of 56% (48). The phase II study with epcoritamab alone or in combination with lenalidomide as the first-line treatment in elderly LBCL patients who are considered anthracycline ineligible (ClinicalTrials.gov Identifier: NCT05660967) is announced.

Treatment of very elderly patients (over 80 years old)

The main cause of death in patients over 80 years of age was lymphoma, which shows that the main goal for them should also be reaching a cure (33). Considering results of clinical studies R-mini-CHOP represents a good balance between efficiency and safety in very elderly patients with DBKL (33, 49-50). In a study by the French lymphoma

Table 2. Studies with reduced chemotherapy protocols or protocols adapted for elderly patients with DBCL

Study	No of patients	Age (years)	Regimen	Relative dose intensity	CR(%)	OS(%)	EFS/PFS
Zinzani **(56)** (prospective)	350	69(60-87)	VNCOP-B	NA	60-69: 61 70-79: 59	5-god. 53% 51%	
Hainsworth 2010 (retrospective) **(57)**	51	78	R-CNOP/R-CVP	NA	NA	**5-year** 72%	**2-year PFS 71%**
Peyrade **(58)** (prospective)	149	83(80-95)	R-miniCHOP	Doxorubicine 50%, cycklophosphamide 53%	63%	2-god:59%	2-god:47%
Corazzelli, 2011 **(59)** (prospective)	41	73 (62-78)	R COMP/14 days	88,6%	68%	**67%**	4 year DFS 72%
Hasselblom (retrospective) **(49)**	70	>80	Pre-R: 40 Posle-R:30	86%		3-god:17% 3-god:41%	3-god:17% 3-god:41%
Spina,2012 **(60)** (prospective)	100	75 (70-89)	R-CHOP/ CHOP	Fit: 100% Frail: 75% Unfit: 50%	70-80: 83% >80:80%	5- year (70-80):54%	5- year (70-80):67%
Olivieri,2012 **(61)** (prospective)	91	74(65-92)	R-CHOP ili R-CDOP ili miniCHOP	Fit: R-CHOP 100% Frail: R-CDOP: NPLD 50%, doxorubicine 50%	81% 64% 50%	5 year:46%	5- year:31%
Gimeno, 2011 **(62)** (prospective)	35	76(61-88)	RCMyOP	NPLD: 50% Vincristine: 24% Th delay: 8%	69%	2- year:70%	2- year:58% PFS
Fields, 2014 (63) (prospective)	62	77 (52-90)	R GCVP	Gemcitabine/Cyclophosphamide/ vincristine (75% dose)	39%	2 years OS 55,8%	2 year PFS 49,8%
Peyrade, 2017 (64)	120	>80	O+miniCHOP	1000 mg ofatumumab, 25 mg/m2 s doxorubicin, 400 mg/m2 cyclophosphamide, and 1 mg of intravenous vincristine, on day 1 of each cycle; and 40 mg/m2 of prednisone on days 1–5.		2-year 64.7%	
Park et al 2019 Multicentre, single arm **(65)**	53	(≥75y: 21) Med age 73	Dose-reduced R-CHOP 21	cyclophosphamide 600 mg/m2, doxorubicin 30 mg/m2, vincristine 1 mg, prednisone 40 mg/day) 6–8 cycles	64.1 %	3y: 62.7 %	3y EFS: 45.7 % 7.5 %
Merli et al 2020 (multicentric single arm) **(51)**	33	82 (62-89)	O+ miniCHOP	IPI≥3: 64% Cardiac comorbidity grade 1-2:54%	CRR 42%	2-years 68%	2-years 49%

group, which included 150 patients with DBKL over 80 years of age, it was shown that the reduced R-CHOP (R-miniCHOP) protocol has a 2-year PFS of 47% and 2-year OS of 59% (50). The only parameter that had a significant impact on survival in the multivariate analysis was the albumin level (≤35g/L), which indicates the great importance of nutritional status in these patients. In this study, lymphoma was the main cause of death, which shows that recovery should be the main goal. The results of the treatment of elderly patients using obinutozumab with mini CHOP in the LYSA study showed slightly better results than with R-miniCHOP as the 2-year OS is 64.7% (51). Considering the findings derived from the POLARIX research (39), a current study (ClinicalTrials.gov Identifier: NCT04332822.) conducted by the Nordic Group is in progress, wherein the integration of polatuzumab into the RminiCHOP protocol is being explored for patients aged 80 years and above or those who are above 75 and frail.

Treatment of patients when anthracyclines are contraindicated

According to ESMO recommendations, when the use of anthracyclines is contraindicated, it is suggested to replace doxorubicin with gemcitabine or etoposide (32). In a study by the British Columbia group, doxorubicin was replaced by etoposide, but the 5-year survival was 49%, which is shorter than the survival achieved with the R-CHOP protocol (52). The number of CRs was lower and involved less cardiotoxicity. An Italian phase II study evaluated the activity and safety of non-pegylated liposomal doxorubicin administered instead of doxorubicin within the R-CHOP protocol (R-COMP) (53). In this study, 3-year OS and PFS were 72% and 69%, respectively, with cardiotoxicity in 21% of patients. The results of a similar large and recently published Italian study showed that R-COMP had curative potential in elderly patients as three-year progression-free survival (PFS) was similar between R-CHOP and R-COMP (70% and 64%) and 3-year overall survival was 77%, and 71 (54). The risk of congestive heart failure associated with anthracyclines may also be reduced with the use of dexrazoxane, an iron chelator which is currently approved only for breast cancer (55).

TREATMENT OF ELDERLY PATIENTS WITH RELAPSED/REFRACTORY DIFFUSE LARGE B CELL LYMPHOMA

Despite new treatment strategies, a significant percentage of LBCL patients, around 30-40%, relapse or are refractory, i.e., they cannot achieve remission with the first-line treatment. The prognosis is particularly poor for primary and secondary refractory LBCL with an estimated median survival of only 5-7 months (66).

Intensive salvage chemotherapy followed by autologous stem cell transplantation (ASCT) is the standard second-line approach with curative potential and durable response with a 3-year progression free survival (PFS) achieved in 30% to 40% of patients after transplantation (4). Although almost half of relapsed/refractory (R/R) LBCL patients respond to platinum-based regimens, only 13% of patients who are scheduled for and receive salvage treatment eventually undergo ASCT (67). The choice of optimal treatment in elderly patients with R/R LBCL is particularly challenging and delicate, as only a minority are suitable for this traditional approach. There is paucity of data on the efficacy and safety of ASCT in the elderly as there are no clinical trials in this setting. Retrospective analysis of 484 patients with R/R LBCL, aged 60 years or over, who received ASCT identified in the Japan Society for Hematopoietic Cell Transplantation database, found that overall non-relapse mortality did not significantly differ among the three age groups: ages 60 to 64, 65 to 69, and 70 years or over. They concluded that older age alone should not be a contraindication for ASCT (68). A careful assessment of frailty, functional status, and comorbidities using CGA may help in therapy choices (69, 70, 71).

With recent new drug approvals, treatment options for patients with R/R LBCL with potential to cure have expanded. Anti-CD19 chimeric antigen receptor (CAR) T-cells therapy emerged as the standard of care in primary refractory LBCL and its early relapse (<12 months) and is the treatment of choice in third line and subsequent therapy if not previously given (4). Axicabtagene clioleucel (axi-cel), tisagenlecleucel (tisa-cel) and lisocaptagene miraleucel (liso-cel) are currently approved with reported high rates of initial response (CRR 40-54% and ORR 52-82%) as well as durable (>18 months) complete remissions in about a third of patients enrolled in ZUMA-1, JULIET and TRANSCEND NHL 001 registration trials (72, 73, 74). Although there are no prospective studies that have directly compared different CAR-T cell products, reported outcomes as well as associated substantial toxicity including cytokine release syndrome (CRS) and immune effectors cell-associated neurotoxicity syndrome (ICANS) are similar. In a matched control multicenter cohort study comparing 41 elderly (≥70 years) R/R LBCL patients and 41 younger patients who underwent CAT-T cell therapy with similar ECOG performance status and serum lactate dehydrogenase level, no differences in the incidence of grade ≥3 CRS (P=0.29), grade ≥3 ICANS (P=0.54), and duration of hospitalization (P=0.55) were found. Furthermore, there was no difference in response rates (CRR 46% and PRR 17% in the elderly group, non-relapse mortality at 1 and 3 months (0 in both groups), and 6- and 12-months PFS and OS survival in elderly patients compared to younger patients were 39% and 32%, and 74% and 69%, respectively with a median follow-up of 7 months (75). Furthermore, real-world experience indicates that, in specialized centers

with certificated medical stuff trained and equipped to timely recognize and treat CRS (with corticosteroids and tocilizumab - a humanized monoclonal Ab against interleukin 6 receptor - IL-6R), ICANS and other adverse events, and with longer rehabilitation therapy aimed at improving disabilities and long-term symptoms, CAR-T cell therapy can be safely used even on elderly patients with comorbidities (75).

Bispecific T cell engager (BiTE) antibodies bind a CD3 molecule on a T cell and target a B cell molecule (CD20) thus redirecting patients' T cells to eliminate malignant B cells. Glofitamab and epcoritamab are recently approved CD20xCD3 BiTEs and are promising new agents for the treatment of R/R LBCL patients. They can be used as a bridging option until CAR-T cell product is available, in case of relapse or disease progression after ASCT or CAR-T cell therapy, and for the treatment of elderly and frail patients non eligible for ASCT or CAR-T cell therapy (4, 71). The advantages of BiTEs are rapid availability and modest toxicity (most of documented CRS are grades 1-2), but with reported high response rates in registration studies (Glofitamab: CRR 39% and ORR 52%: Epcoritamab: CRR 39%, ORR 63%) (76, 77).

Cell-directed therapy is highly expensive and is still not widely available. Therapeutic options effective for R/R LBCL patients ineligible for ASCT include moAbs directed against surface receptors expressed by LBCL cells (CD20, CD19, CD79b) which are applied as monotherapy or in combination with chemotherapy and immunomodulatory drugs **(Table 3)**.

Table 3. Monoclonal antibodies in the treatment of R/R LBCL

R-GemOx (rituximab/ gemcitabine/oxaliplatin) (78)	CRR 44% Median OS of 10 months
Tafasitamab + lenalidomide (79)	CRR 43%, ORR 57.5% 22-month duration of response
Polatuzumab vedotine + bendamustine + rituximab (80)	CRR 38.7%, ORR 41.5%
Loncastuximab tesiren (71)	CRR 24% and ORR 48.3%

Responses to monotherapy with the immunomodulatory drug lenalidomide, Bruton-tyrosin kinase inhibitor Ibrutinib, and Selinexor, an oral selective inhibitor of nuclear export that functionally inactivates p53 and other tumor suppressor proteins, are modest. For very frail R/R LBCL patients, supportive and palliative end-of-life care is the only option.

CONCLUSION

Elderly patients with LBCL have a worse prognosis than patients who are 65 years old and younger. The elderly have a higher incidence of histologically unfavorable types of LBCL, comorbidities, they are generally in a worse condition and have poor tolerance to therapy. Due to these factors treating these patients represents a significant challenge. Treatment should be individualized according to clinical condition and present comorbidities. In the past few years, new therapeutic option cells have emerged and improved the course of disease and prognosis of LBCL in the elderly.

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POSEBNI ASPEKTI PROGNOZE I LEČENJA STARIJIH PACIJENATA SA KRUPNOĆELIJSKIM B-LIMFOMOM

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Sažetak

Krupnoćelijski B-limfom (KBL) najčešći je tip nehočkinskog limfoma (NHL) u opštoj populaciji, čineći 40-50% svih slučajeva NHL i preko 60% NHL u populaciji starijih od 65 godina. S obzirom na sve dužu očekivanu dužinu života, očekuje se da će prevalencija ovog tipa limfoma biti u porastu u narednim godinama. Lečenje ovih pacijenata predstavlja značajan izazov zbog brojnih faktora koji komplikuju lečenje i pogoršavaju ishod bolesti. Stariji pacijenti često imaju prateće bolesti, oslabljenu funkciju organa, izmenjen metabolizam lekova i smanjenu hematopoetsku sposobnost oporavka koštane srži, što ih čini manje tolerantnim na hemioterapiju. Lošija prognoza povezana je sa većom učestalošću non-GCB podtipa i histološki nepovoljnih tipova KBL, poput EBV-pozitivnog KBL, "high grade" B-ćelijskog limfoma (HGBL) i plazmablastnog limfoma, kao i ekstranodalnim lokalizacijama koje su povezane sa lošijim ishodom. Lečenje starijih pacijenata je kompleksno jer predstavljaju visoko heterogenu populaciju sa značajnim varijacijama u zdravstvenom statusu, pratećim bolestima i očekivanim životnim vekom. Stoga je potrebna sveobuhvatna gerijatrijska procena za starije pacijente, uključujući određivanje komorbiditetnog skora radi kategorizacije pacijenata na one dobrog opšteg stanja (fit), one lošeg opšteg stanja (fragilne) i one između. Zavisno od stanja bolesnika cilj lečenja može biti izlečenje, produženje života ili kontrola simptoma. Standardna terapija za starije pacijente dobrog opšteg stanja i uznapredovalu bolest je R-CHOP, dok se kod bolesnika sa pratećim bolestima i lošim opštim stanjem primenjuju redukovani protokoli sa ili bez antraciklina. U prethodnim godinama, stariji pacijenti sa recidivantnim ili KBL otpornim na lečenje imali su vrlo lošu prognozu zbog ograničenih mogućnosti lečenja. Međutim, uspeh lečenja starijih pacijenata sa R/R KBL značajno se poboljšao poslednjih godina zahvaljujući uvođenju novih lekova (polatuzumab, tafasitamab, bispecifična antitela i CAR-T ćelije) koje se mogu koristiti kod starijih osoba.

Ključne reči: krupnoćelijski B-limfom, starija populacija, prognoza, terapija

Primljen: 27.11.2023. I **Revizija:** 03.01.2024. I **Prihvaćen:** 03.01.2024.

Medicinska istaživanja 2024; 57(2):93-102

Medicinska istaživanja 2024; 57(2):103-111 | DOI 10.5937/medi57-45370

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REWIEV ARTICLE

History of the Institute of Pathology "Prof. dr Đorđe Joannović" – 100 years of experience and work

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Summary

One hundred years after its establishment, the Institute of Pathology "Prof. Dr. Đorđe Joannnović" remains the leading institution in Serbia and the region, where besides educating students and laboratory technicians, various state-of-the-art diagnostic procedures in the fields of histopathology, autopsy, and molecular pathology are conducted.

The Department of General Pathology and Pathological Anatomy was also founded in 1922. Dr Đorđe Joannnović was the first full professor and the head of the department. After World War II, a joint department of four subjects was formed. An independent Department of Pathological Anatomy was reestablished in 1978, with academician Prof. Vladimir Kanjuh as the first head of the newly formed department. Teaching at the Department of Pathology has been conducted according to a new program since the academic year 2006/07 academic year. Since its beginings, the Department of Pathology has supported students' scientific research work and has been involved in facilitating professional and scientific international student exchange. Teaching of basic academic medical studies in English at the Department of Pathology has been ongoing since 1997. Within the department, there are specializations in Clinical Pathology and Medical Cytology, and in the academic year 2022/23 academic, the first generation of students in the "Pathological Basis of Diseases" doctoral program was enrolled.

Today, the Institute of Pathology is listed among the reference institutions of the Republic of Serbia. It possesses a laboratory for routine histological processing of biopsy and autopsy material, an immunohistochemistry laboratory, and a laboratory for molecular pathology. Members of the Department and the Institute of Pathology are authors of numerous professional and scientific publications. They have been involved in founding and working within important professional, health, and scientific institutions in the country and the region. Owing to its exceptional contributions to the development of medical science and practice, the Institute of Pathology was awarded the Order of St. Sava, 3rd class, in 2017.

Key words: Institute of Pathology, Prof. Dr Đorđe Joannović, pathology, Faculty of Medicine University of Belgrade

Recived: 06 December 2023
Revised: 05 February 2024
Accepted: 05 February 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Cite this article as: Janković R, Emilija Manojlović Gačić E, Kanjuh V, Tatić S, Brašanac D, Tomanović N, Radojević Škodrić S, Glumac S. History of the Institute of Pathology "prof. Dr. Đorđe Joannović" – 100 years of experience and work; Medicinska istaživanja 2024; 57(2):103-111 DOI: 10.5937/medi57-45370

ESTABLISHMENT AND EARLY DAYS OF THE INSTITUTE OF GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY AND THE DEPARTMENT OF GENERAL PATHOLOGY AND PATHOLOGICAL ANATOMY

Currently, the Institute of Pathology at the Faculty of Medicine University of Belgrade is the largest and one of the oldest institutions in Serbia and former Yugoslavia. The history of the Institute of Pathology dates back to the year of 1920, with the comeback of Prof. Đorđe Joannnović **(Figure 1)** from the Faculty of Medicine in Vienna to Belgrade. His aim was participation in the establishment of the Faculty of Medicine in Belgrade. Two years later, in 1922, Prof. Joannnović founded the Institute of General Pathology and Pathological Anatomy (1,2,3). While some sources indicate that the Institute was founded in November 1921 (4), most sources cite the beginning of 1922 as the time of its establishment (1,2,3).

Figure 1. Prof. Đorđe Joannović

In its initial years, the Institute did not have its own building. Educational, scientific, and professional activities began very modestly, in a single room of the Main Military Hospital in Belgrade, and later continued at the premises of the Pathology Department of the General State Hospital in Belgrade (1,3). The most significant event in the development of the Institute was its relocation to a purpose-built new building on April 22, 1926 **(Figure 2)**. The construction of this building was started in 1923, according to the designs made by Prof. Đorđe Joannnović. The architects Petar Jovanović and Svetozar Jovanović, both professors at the Technical Faculty in Belgrade, designed the building. This facility met high standards of the time and was one of the best-equipped institutions of its kind in the world (3).

Figure 2. Newly built Institute of Pathology building in 1926.

In the newly constructed building, intensive scientific research work in the field of general and experimental pathology and pathological anatomy began. The Institute became the center of histopathological diagnostics. During this time, the Institute annually conducted thousands of clinical autopsies, referred from all clinics of the Faculty of Medicine, the departments of the General State Hospital, and the Hospital for Infectious Diseases (1).

Until the outbreak of the Second World War, the Institute of General Pathology and Pathological Anatomy experienced a rapid development, further expanding their activities. Prior to the Second World War, the Institute consisted of several departments: the autopsy department, pathological-histological department, experimental department, and biochemical department.

As the Institute was established as part of the Faculty of Medicine, all employed doctors were engaged in teaching from the moment of its inception. Before the Second World War, the Institute and the Department of General Pathology and Pathological Anatomy employed three professors, four associates, one laboratory technician, and one administrative officer (1,5). Prof. Joannnović simultaneously held the position of the Institute's director and the head of the Department of General Pathology and Pathological Anatomy. The department of experimental pathology was led by Prof. Ksenofon Šahović. Prof. Joannnović was also elected multiple times as the Dean of the Faculty of Medicine (1922/23, 1925/26, 1927/28, and 1928/29) (2,3). He was beloved and respected among students, went to excursions with them and served as the president of the Fund for Supporting Impoverished University Students (1,2). He even supported them during student demonstrations against government authorities in 1931, which aimed to preserve the autonomy of the University and improve students' material conditions. During the actions of the Progressive Student Movement in 1932, the Svetosavski Ball, traditionally organized on January 27th and planned to raise donations for the mentioned fund, was cancelled. There are different versions of what exactly happened that day, but it is certain that the events were linked to the tragic death of Prof. Joannnović, who

committed suicide in his office at the Institute building on January 28, 1932 (2,6,7,8).

After Prof. Joannnović's death, Professor Ksenofon Šahović assumed the leadership of the Institute and the Department. In collaboration with Associate Prof. Dimitrije Tihomirov, he published the "Atlas of Pathological Histology" in 1933, dedicating it to the late Professor Joannović (2). Several years before the start of the Second World War, Professor Šahović initiated the publication of two journals: "Acta Pathologica" and "Acta Cancerologica Yugoslavica". From 1937 until the war began, the journal "Acta Pathologica" was published. The authors of professional and scientific papers were mostly doctors from the Institute of Pathology and doctors from clinics they collaborated with. The papers were printed in German, French, or English and illustrated with high-quality photomicrographs of histopathological specimens. The journal "Acta Cancerologica Yugoslavica" was published from 1939 to 1941 (2). Before the outbreak of the Second World War, the Department of Pathology had three professors: Prof. Ksenofon Šahović, who was promoted to full professorship in 1940, Dr. Dimitrije Tihomirov, who held the position of an associate professor, and Dr. Živojin Ignačev, who held the position of an assistant professor (2,5).

TEACHING ACTIVITIES OF THE DEPARTMENT OF PATHOLOGY AFTER THE SECOND WORLD WAR

After World War II, the Department of Pathology continued its activities with new impulses and developments. It became a significant center for scientific research and education, both for medical students and for residents in pathology.

During this period, significant efforts were made to supply the Department with modern equipment and technology, which contributed to the improvement of histopathological diagnosis. The faculty members of the Department were engaged in scientific research in various areas of pathology, from histopathology to molecular pathology.

Educational programs at the Department of Pathology were refined and expanded in line with scientific advancements. In addition to educating students, the Department of Pathology provided training for doctors specializing in pathology.

With the advancement of technology, special emphasis was placed on molecular diagnostics and genetic research approaches in pathology. This field rapidly developed and became an essential part of modern medical pathology.

The Department of Pathology continued to actively participate in international collaborations, symposiums, and congresses. It further established itself as a hub for advancing knowledge in the field.

The Department's post-World War II activities were characterized by a commitment to excellence in both education and research, making significant contributions to the field of pathology and medical science as a whole.

During World War II, the building of the Department of Pathology suffered significant damage. Bombardments caused damage to the lecture hall, histology laboratories, and the museum with numerous exhibits. Some laboratories were completely destroyed. After the war, the building was reconstructed. A new lecture hall, the largest at the Faculty of Medicine, was built, and the 4th floor was added to the building. In 1945, the department changed its name to the Institute of Pathology and Pathological Anatomy (1). However, even in the renovated and expanded Institute building, the space for the work of pathologists remained limited and insufficient. This was due to the fact that the building also accommodated the Institute of Pathological Physiology, the Institute of Medical Research of the Serbian Academy of Sciences and Arts (Serbian – *Srpska akademija nauka I umentnosti - SANU*), and the Faculty of Pharmacy (2,5).

After World War II, the functioning of the Institute and the Department was challenged by significant financial problems and a shortage of skilled staff. Considering all these factors, in 1948, it was decided to establish a unified Department for four subjects: Pathological Anatomy, Pathological Physiology, Oncology, and Forensic Medicine. Professor Ksenofon Šahović was appointed as the first head of this unified Department and held this position until his death in 1956. Following his passing, the role was taken over by Professor Julijana Bogićević, a professor of forensic medicine (until the moment the Department for forensic medicine became independent in 1957). Later, Professor Marija Višnjić Frajnd, Professor Živojin Ignjačev, and Professor Branka Stojanović from the Department of Pathological Physiology were heads of the Department. During the period of the unified Department, teaching of pathological anatomy included three types of exercises alongside theoretical classes: macroscopic diagnostics, microscopic diagnostics, and histopathological exercises (2,5) **(Figure 3)**.

Figure 3. The team of the Institute of Pathology during the tenure of Prof. Živojin Ignjačev (head) and Prof. Marija Višnjić Frajnd.

The independent Department of Pathological Anatomy was reestablished in 1978, with Academician Profes-

sor Vladimir Kanjuh as its head. Changes were made to the teaching methods, including the introduction of macroscopic seminars in place of the previous macroscopic diagnostics exercises (5). Subsequent heads of the Department included: Professor Miomir Leposavić, Professor Milica Đorđević Tešanović, Professor Stevan Nešić, Professor Miomir Janjić, Professor Vesna Jovanović, Professor Mirjana Atanacković, Professor Ivan Boričić, Professor Milica Skender Gazibara, Professor Svetislav Tatić, Professor Dejan Oprić, Professor Jelena Sopta, and on the centenary, Associate Professor Radmila Janković)8,9] **(Figure 4)**.

Figure 4. Associate Prof. Radmila Janković, Head of the Department of Pathology on its centenary

Significant changes to the curriculum were introduced with the accreditation of the teaching program in the academic year 2005/6. Since then, students have had four types of mandatory exercises: autopsy exercises, macroscopic seminars, histopathological exercises, and exercises in the electronic classroom. In addition, a greater number of elective subjects from various areas of pathology were introduced for third-year students (9). Since its establishment Thet Department of Pathology has supported student scientific research, and it has also been involved in implementing professional and scientific international student exchange. In addition to the Department offering courses in the Serbian language, Studies in English were established in 1997, following the same curriculum as the Serbian-language studies. The first head of the Department of Pathology in English was Prof. Ana Laban, followed by Prof. Milica Skender Gazibara, Prof. Zorica Stojšić, and currently Prof. Nada Tomanović (8,9). Since 2013, the Department has been delivering teaching for Basic Academic Studies in Nursing, led by Assistant Prof. Dragan Mitrović and currently Associate Prof. Ljiljana Bogdanović (9).

Postgraduate education was officially introduced to the Faculty of Medicine in the academic yer 1947/48. Specialization in pathological anatomy was the first form of postgraduate education introduced in this period, and Dr. Milan Arambašić was the first physician at the Faculty of Medicine in Belgrade to officially pass a specialist exam (2). The two-semester theoretical teaching for specialist trainees in pathological anatomy was formally organized for the first time in 1979 (2,5). However, the Department for postgraduate education in pathological anatomy was officially established only in 2003 (5,8). Prof. Marija Havelka Đuković was appointed as the head of this Department. After her retirement, this role was taken over by Prof. Dejan Oprić, Prof. Tatjana Terzić, and currently Associate Prof. Emilija Manojlović Gačić. In 2010, the specialization name "Pathological Anatomy" was officially changed into "Pathology". Starting from the academic year 2008/9, two programs of sub-specialisation were introduced to the Department: Clinical Pathology and Medical Cytology. In the academic year 2022/23, the first generation of doctoral candidates was enrolled in the newly accredited module " Pathological Basis of Diseases" (9).

HEALTHCARE ACTIVITIES OF THE INSTITUTE OF PATHOLOGY AFTER WORLD WAR II

After World War II, the director of the Institute of Pathology was Prof. Ksenofon Šahović, and he remained in that position until his passing in 1956. Following him, the position of director was held by Prof. Marija Višnjić-Frajnd, and then by Prof. Živojin Ignjačev, who returned to Belgrade from Sarajevo (where he was the director of the Institute of Pathology). Subsequently, the directorship was held by Prof. Milan Arambašić, Prof. Miodrag Sinđić, Prof. Milorad Išvaneski, Prof. Miomir Janjić, Prof. Stevan Nešić, Prof. Milica Đorđević, Prof. Slobodan Dožić, Prof. Jovan Vasiljević, Prof. Dubravka Cvetković Dožić, Prof. Gordana Basta Jovanović, Prof. Sanja Radojević Škodrić, Prof. Nada Tomanović, Assoc. Prof. Duško Dunđerović, and Associate Prof. Sofija Glumac on the centenary of its founding **(Figure 5)**.

Healthcare activities of the Institute in the period after World War II followed an upward trajectory. The number of staff members was limited, which initially constrained the ability of pathologists to engage in highly specialized areas. However, the situation improved in 1960s with enhancements in human resources and material support, paving the way for focused endeavors within specific fields of pathology **(Figure 6)**.

Over time, specialized teams were formed to focus on pathology of various organ systems. Cardiovascular pathology was among the first fields in which individual pathologists at the Institute specialized. Academician Prof. Vladimir Kanjuh led this area, serving as the head of the cardiovascular pathology team (9).

Figure 5. Associate Prof. Sofija Glumac, Director of the Institute of Pathology on its centenary

Academician Kanjuh pursued training in cardiac pathology under the guidance of renowned figure Prof. J. E. Edwards in St. Paul/Minneapolis as a fellow of the American National Institutes of Health. He also pursued training in London, Paris, and Amsterdam. He discovered and described two new congenital heart defects, one of which was named after him - the Kanjuh anomaly. Numerous collaborators and educators passed through the "Vladimir Kanjuh School of Cardiac Pathology," which served as a Reference Center for cardiovascular pathology, and some of them continued successful careers abroad. Members of his research team included Prof. Gordana Tucaković, Prof. Sofija Lastić-Maletić, Prof. Dragan Velimirović, Prof. Jovan Vasiljević, and Assoc. Prof. Radmila Jovanović. Additionally, Academician Kanjuh founded and organized the Museum and Registry of Congenital Heart Defects, featuring over 1500 macroscopic specimens. He introduced histopathological analysis of lung biopsies in patients with congenital heart defects to determine operability. Notably, the discovery of "plexiform lesions" (by Heath-Edwards) became a contraindication for surgery in patients with congenital heart defects. Cases were received from all over former Yugoslavia.

Thanks to the "Vladimir Kanjuh School of Cardiac Pathology," the "Belgrade Cardiology School" gained significance and a higher level of integrated cardiology perspective. Numerous award-winning postgraduate textbooks were published (9,10,11). Also, owing to the efforts of Prof. Vasiljević, endomyocardial biopsies became part of the routine work of this team. This effort fostered intense collaboration with the Institute for Cardiovascular Diseases Dedinje and the Clinic for Cardiovascular Diseases at the University Clinical Center of Serbia (UCCS). Prof. Sofija Glumac and Assist. Prof. Milena Jovanović are continuing the tradition (9).

Many pathologists at the Institute were dedicated to the field of pulmonary pathology in its early decades. However, only Prof. Miomir Leposavić, Prof. Miloš Bašić, Prof. Stevan Nešić, and Prof. Mirjana Oklobdžija gained focused expertise in this area of pathology. After the untimely passing of Prof. Oklobdžija, Assistant Prof. Dragan Mitrović and his collaborator Assistant Prof. Ivana Savić continued to contribute to lung pathology. Today, Associate Prof. Sofija Glumac and clinical and research assistant Dr Milena Jovanović are subspecialized in pulmonary pathology, fostering close collaboration with the Clinic for Pulmonary Diseases and Tuberculosis

Figure 6. The stuff of the Institute of Pathology at the moment of the celebration of the centenary

at the UCCS, as well as Assistant. Prof. Ivana Savić (9).

Prof. Miodrag Sinđić was the founder of the Nephropathology and Uropathology Laboratory at the Institute of Pathology and he pursued advanced studies in Germany as a scholarship recipient of the Alexander von Humboldt Foundation. Prof. Sindjic was also a founding member of the Institute of Urology and Nephrology at the UCCS. Owing to Prof. Sinđić, the Institute of Pathology was the first to implement methods such as immunofluorescence, polarizing microscopy, and electron microscopy for the analysis of renal biopsies (10). Numerous collaborators passed through this laboratory, with Prof. Gordana Basta Jovanović and Prof. Jasmina Marković Lipkovski dedicating their entire careers to it, and leaving their personal imprint. Owing to Prof. Marković Lipkovski's dedication, various molecular methods were introduced at the Institute of Pathology. In 1990s, owing to the efforts of Prof. Dimitrije Brašanac and Prof. Marković Lipkovski, immunohistochemical staining was introduced into routine diagnostics. Today, the team for nephropathology and uropathology is led by Prof. Sanja Radojević Škodric, with other members including Associate Prof. Ljiljana Bogdanović, Assistant Prof. Jelena Filipović, Assistant. Prof. Maja Životić, and Teaching and research assistant. Dr Gorana Nikolić (9).

The field of bone and soft tissue pathology at the Institute of Pathology was established by Prof. Milorad Išvaneski, Prof. Mirjana Atanacković, and Prof. Dragoljub Bacetić. Currently, the members of this team are Prof. Jelena Sopta, with teaching and research assistants. Dr Ljubica Simić and Milena Mihajlović. The Institute of Pathology is the only specialized laboratory for bone and soft tissue pathology in the country and the region. Collaborations have been established with the Institute for Orthopedic Diseases Banjica (IODB), the Clinic for Traumatology and Orthopedics at the University Clinical Center, and the Department of Orthopedics at the University Children's Hospital Tiršova. Prof. Sopta has been a long-standing member of the sarcoma committee at the IODB (9,10).

The field of gastrointestinal pathology and pancreatic pathology developed as distinct areas owing to Prof. Ana Laban and Prof. Zorica Stojšić. Today, this team is composed of Associate Prof. Radmila Janković accompanied with clinical and research assistants Dr Jovan Jevtić and Miloš Đuknić. They collaborate with the University Children's Hospital Tirsova, the Infectious Clinic of the UCCS, the Clinical-Hospital Center "Dr. Dragiša Mišović," and the Clinical-Hospital Center Bežanijska kosa. Prof. Janković is a member of the Digestive Tumor Council at the Clinical-Hospital Center "Dr. Dragiša Mišović" (9).

Prof. Ana Begić Janeva is the founder of modern hepatopathology at the Institute of Pathology. She was a member of the first liver transplantation team in Serbia in 1990s, along with Prof. Ivan Boričić. The development of this area is continued by Prof. Ivan Boričić and Prof. Nada Tomanović, who collaborate with the Infectious Clinic of the UCCS and the Internal Medicine A Clinic of the UCCS (9,10).

Besides hepatopathology, the same team deals with head and neck pathology. Today, the team for hepatopathology and head and neck pathology consists of Prof. Nada Tomanović and her collaborator, teaching and research assistant Dr Ana Marija Tomić. Prof. Tomanović is a permanent member of the Tumor Council for Head and Neck Tumors at the Otorhinolaryngology Clinic of the UCCS (9).

Prof. Miomir Janjić was engaged in gynecological pathology at the Institute of Pathology. The current members of the team for gynecological pathology are Prof. Dejan Oprić and his collaborators, Assistant professor Danilo Obradović, who serve as consultants for the Gynecological-Obstetric Clinic of the UCCS. Recently teaching and research assistant dr Isidora Filipović jined the team. Dr Danilo Obradović is also specialized in placental pathology (9).

The pioneer of modern hematopathology at the Institute of Pathology is Prof. Vesna Jovanović, and her successors are Prof. Tatjana Terzić with her collaborator Assist. Prof. Novica Boričić. This team, due to the Institute's well-equipped facilities, can apply contemporary recommendations related to diagnosing hematologic neoplasms and detect specific markers of interest for targeted therapy (8,9).

The initiator of the team for endocrine pathology was Prof. Marija Jančić Zguricas. She was also one of the founders of the Department of Clinical Pathology at the Second Surgical Clinic of the UCCS (9,10). Prof. Marija Havelka Đuković and Prof. Svetislav Tatić were members of the team for endocrine pathology and pioneers of modern breast lesion diagnostics. They implemented immunohistochemical staining in the routine diagnostics of breast lesions. In this team today, alongside Prof. Tatić, work Associate Prof. Duško Dunđerović, Assoc. Prof. Maja Životić and Associate professor Mirjana Prvanović (9).

A large number of skin biopsies are diagnosed annually at the Institute of Pathology. This area was developed and improved by Prof. Dimitrije Brašanac. Prof. Brašanac predominantly focuses on tumor dermatopathology, especially melanocytic skin lesions. He collaborates daily with the Clinic for Burns, Plastic and Reconstructive Surgery of the UCCS. He is also a coordinator for histopathological diagnosis in the Intersectoral Board for Melanoma of the Serbian Medical Society. Assoc. Prof. Martina Bosić is also engaged in the dermatopathology team. Her specialization is inflammatory skin diseases, which leads to excellent collaboration with the Dermatovenereology Clinic of the UCCS (9).

There has been a long tradition of a neuropathology laboratory at the Institute of Pathology since 1970. Its founder was Prof. Slobodan Dožić. The Neuropathology

and Neuromuscular Diseases Laboratory was declared a reference laboratory in the territory of former Yugoslavia in 1986 (10,11). This laboratory applied all contemporary diagnostic methods, including enzyme histochemical staining and the use of an electron microscope that the Institute possessed in the 1980s and 1990s (11). Prof. Dubraka Cvetković Dožić and Prof. Milica Skender Gazibara were part of this unique team, and today this area is led by Associate Prof. Emilija Manojlović Gačić. The team members have a longstanding collaboration with the Neurosurgery Clinic of the UCCS. Associate Prof. Manojlović Gačić specializes in pituitary pathology and she introduced the latest diagnostic principles after training with eminent pathologists in this field, including Jacqueline Trouillas in Lyon, France, and Olivera Casar-Borota in Uppsala, Sweden, who authored the WHO classification. She actively participates in the International Pituitary Pathology Club and she contributed to changing the nomenclature of pituitary tumors, which was adopted in the latest WHO classification of endocrine tumors. Besides her collaboration with neurosurgery, Prof. Skender Gazibara established intensive cooperation with the University Children's Hospital Tirsova, particularly in diagnosing disorders of intestinal motility and Hirschsprung's disease. After her retirement, the diagnosis of intestinal motility disorders was taken over by Associate Prof. Radmila Janković (9).

Since 2006, the official name of the institute was the "Institute of Pathology". In 2017, the name of the institution was officially changed and extended to include the name of its founder, Prof. Dr. Đorđe Joanović (9). In the jubilee year of 2022, the Institute employed 24 doctors, 2 molecular biologists, 14 laboratory technicians, and 23 members of administrative and non-medical staff. The Institute currently has two autopsy rooms and a mortuary with cooling equipment for storing deceased individuals. The number of autopsies conducted in the first decades of the Institute's activity was quite high, amounting to several thousand annually. However, there was a significant decline in the number of autopsies during 1980s and 1990s, maintaining a lower count until the first decade of the 21st century. Around 2010, the number of autopsies started to rise again, positioning the Institute of Pathology as an institution that performed the highest number of clinical autopsies annually in the country and the region. The number of biopsy materials for histopathological analysis has consistently increased since the institute's founding, along with the complexity of diagnostic methods (5,6,12).

Presently, the Institute is equipped with three main laboratories: a laboratory for routine histological processing of biopsies and autopsy material, an immunohistochemistry laboratory, and a molecular pathology laboratory. Owing to these facilities, the Institute is capable of adhering to global diagnostic standards. The laboratory for routine histological processing of biopsies processes over 25.000 biopsies and cytological samples annually (including ex tempore biopsies). The immunohistochemistry laboratory stains preparations from the institute's biopsies and also provides staining services for numerous institutions in Belgrade and the region. On a daily basis, 500-600 slides are immunohistochemically stained. Most histopathological preparations are routinely stained with hematoxylin and eosin, and in addition, about 30 different special histochemical stains are used (9).

The Institute of Pathology employs licensed laboratory technicians. In addition to preparing specimens for diagnosis, they participate in preparing specimens used for educational purposes, as well as in the production of specimens that are part of scientific research and doctoral dissertations. Practical training for laboratory technicians from secondary medical schools takes place in the laboratories. Internships are offered to students who have completed secondary and higher medical schools. Supplementary training is also provided to laboratory technicians from other healthcare institutions in the country and the region (8,9).

Over the past decade, the Institute has experienced significant progress, particularly with the establishment of the Molecular Pathology Laboratory. The creation of this laboratory involved the participation of Associate Prof. Martina Bosić and a molecular biologist and physiologist Dr Sanja Ćirović, with the support of Prof. Jasminka Marković Lipkovski and the then director of the Institute, Prof. Sanja Radojević Škodrić. Since 2018, another molecular biologist and genetics specialist, Marija Denčić Fekete, has been employed in the laboratory. Finaly, molecular biologist Milica Radovanović Komnenić accompanied the team for molecular pathology in 2022. The Molecular Pathology Laboratory conducts a large number of analyses annually. In the year marking the centenary of the institute's founding, the Institute performed 2450 analyses of fluorescence in situ hybridization, 894 PCR analyses, and 88 cytogenetic analyses. The Institute of Pathology serves as a reference institution for molecular testing, which is significant not only for tumor diagnosis and prognosis (especially sarcomas, lymphomas, and central nervous system tumors) but also for personalized approaches and targeted therapy for various malignant tumors (9).

THE ROLE OF THE INSTITUTE OF PATHOLOGY IN THE DEVELOPMENT OF PATHOLOGY IN THE REGION

The Institute of Pathology has played a significant role in the support and the constitution of numerous educational and healthcare institutions throughout the region. The Faculty of Medicine and collaborators from the Department of Pathology were involved in establishing departments of pathology at medical faculties in Novi Sad, Kragujevac, and Pristina, as well as at the School of Dental Medicine in Belgrade. They also contributed to teaching

Figure 7. Order of St. Sava, Third Class, awarded to the Institute of Pathology in 2017.

at these faculties and at the Medical Faculty in Podgorica and the Dental Faculty in Novi Sad (2,5,10,12). Prof. Đorđe Joannović and Prof. Ksenofon Šahović were among the founders of the Yugoslav Society for the Study and Suppression of Cancer in 1927. Prof. Šahović was not only a founder of the Institute of Pathological Physiology but also its first director. He also founded the Institute of Oncology and Radiology of Serbia in 1948, establishing the foundations of modern oncology in the country (2,3). He remained at the helm of this institution until his death. Prof. Marija Višnjić Frajnd succeeded Prof. Šahović in this role (2,12). Members of the Department of Pathology were part of the founding committee of the Association of Yugoslav Pathologists. They were also involved in the establishment and work of the Pathologists' Section of the Serbian Medical Society (SMS), serving as presidents and secretaries of the section. Prof. Živojin Ignjacev served as the first president of the Pathologists Section of the SMS (12). Many faculties and collaborators from the Department of Pathology have received education and specialization in various areas of pathology both domestically and internationally. They have participated in numerous congresses, courses, schools, and symposia as both attendees and lecturers. Prof. Mirjana Atanacković and Prof. Jelena Sopta organized a course in bone pathology with international participation in 2005. European School of Pathology courses were held annually from 2009 to 2019 in Belgrade, under the organization of Prof. Jovan Vasiljević and certain faculty and collaborators from the Institute of Pathology. Owing to the efforts of Prof. Jovan Vasiljević, the European Congress of Pathology was held for the first and only time in Belgrade in 2015 (9).

Professor Joannovic was an associate member of the Serbian Royal Academy. Importantly, Professors Šahović and Kanjuh were elected as regular members of the Department of Medical Sciences of the *SANU* (2,3). Professors of the Department of Pathology have also been or are still members of various committees within SASA. For example, Professors Gordana Tucakovic and Sofija Lastic Maletic are members of the Committee for Cardiovascular Pathology at SASA, with Academician Vladimir Kanjuh serving as its president. Professor Miodrag Sinđic was a permanent member of the Committee for Endemic Nephropathy at SASA from 1971 until his passing (10,13). The Committee for Tumors of the Urinary System at SASA includes Academician Vladimir Kanjuh and Professor Jasmina Markovic Lipkovski (13).

Professors Marija Jancic Zguricas and Prof. Gordana Tucakovic were regular members of the Academy of Medical Sciences of the Serbian Medical Society (9,10). Professor Jelena Sopta is a member of the Scientific Society of Serbia, while Professor Jovan Vasiljevic has been not only a long-standing member but also the president of this society since 2016 (9).

Members of the Institute of Pathology have actively participated in expert committees at the level of the Republic of Serbia, such as the Republic Expert Committee for Pathology. The current president of this committee is Associate Prof. Dusko Dunđerovic, and several members of the Department of Pathology are part of it. Since December 2017, Prof. Sanja Radojevic Skodric has been the director of the Republic Health Insurance Fund (9).

It can be concluded that the Institute of Pathology "Prof. Dr. Đorđe Joannovic" has played a central role in the development of pathology in the country and the region since its establishment. Throughout its century-long history, the teachers and collaborators of the Institute of Pathology "Prof. Dr. Đorđe Joannovic" have successfully faced numerous challenges, preserving traditional values while simultaneously keeping up with modern trends in the field and science, continuously educating themselves and passing their knowledge to generations of doctors.

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ISTORIJAT INSTITUTA ZA PATOLOGIJU "PROF. DR ĐORĐE JOANNOVIĆ" - 100 GODINA ISKUSTVA I RADA

Radmila Janković[1], Emilija Manojlović Gačić[1], Vladimir Kanjuh[2], Svetislav Tatić[1], Dimitrije Brašanac[1], Nada Tomanović[1], Sanja Radojević Škodrić[1], Sofija Glumac[1]

Sažetak

Sto godina nakon svog osnivanja, Institut za patologiju „Prof. dr Đorđe Joannović" predstavlja vodeću ustanovu u Srbiji i regionu u kojoj se pored edukacije studentata i laboratorijskih tehničara vrše različite najsavremenije dijagnostičke procedure iz oblasti histopatološke, autopsijske i molekularne patologije.

Katedra za opštu patologiju i patološku anatomiju je takođe osnovana 1922 godine. Prvi redovni profesor i prvi šef Katedre bio je dr Đorđe Joannović. Nakon Drugog svetskog rata formirana je zajednička Katedra za četiri predmeta. Samostalna Katedra za patološku anatomiju ponovo je formirana 1978. godine, a prvi šef novoformirane Katedre bio je akademik prof. dr Vladimir Kanjuh. Na Katedri za patologiju nastava se po novom programu odvija od 2006/07. školske godine do danas. Katedra za patologiju od osnivanja podržava naučno-istraživački rad studenata, a uključena je i u realizaciju stručne i naučne međunarodne razmene studenta. Nastava osnovnih akademskih studija medicine na engleskom jeziku na Katedri za patologiju odvija se od 1997. godine. U okviru Katedre postoje uže specijalizacije Klinička patologija i Medicinska citologija, a školske 2022/23. upisana je prva generacija studenata doktorskih studija „Patološke osnove bolesti".

Institut za patologiju se danas nalazi na listi referentnih ustanova Republike Srbije. Raspolaže laboratorijom za rutinsku histološku obradu biopsijskog i autopsijskog materijala, imunohistohemijskom laboratorijom i laboratorijom za molekularanu patologiju.

Članovi Katedre i Instituta za patologiju su autori brojnih stručnih i naučnih publikacija. Učestvovali su u osnivanju i radu važnih stručnih, zdravstvenih i naučnih institucija u zemlji i regionu. Za naročite zasluge i doprinos u razvoju medicinske nauke i prakse Institut za patologiju je odlikovan Sretenjskim ordenom trećeg stepena 2017. godine.

Ključne reči: Institut za patologiju, prof. dr Đorđe Joannović, patologija, Medicinski fakultet

Primljen: 06.12.2023. I **Revizija:** 05.02.2024. I **Prihvaćen:** 05.02.2024.

Medicinska istaživanja 2024; 57(2):103-111

Medicinska istaživanja 2024; 57(2):113-119 | DOI 10.5937/medi57-46846

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REWIEV ARTICLE

Liquid biopsy as a source of potential biomarkers for checkpoint inhibitor treatment in non-small cell lung cancer

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Recived: 30 September 2023

Revised: 13 February 2024

Accepted: 01 April 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Lung cancer (LC) is the leading cause of cancer-related mortality around the world. Immune checkpoint inhibitors (ICIs) have revolutionized the treatment and improved clinical outcomes of non-small-cell lung cancer (NSCLC) patients. However, while some patients have good response to ICI others are refractory to therapy or have life threatening adverse reactions. There are still no good strategies to identify responders to ICIs. That is why personalization of ICI therapy based on a patient's unique genomic profile represents an attractive strategy to improve NSCLC treatment.

There are continuous efforts to find predictive biomarkers to identify patients who are likely to respond to ICIs. In turn, these strategies are required to spare patients the time, expenses, and toxicity while trying out therapies from which they will not derive any benefit. Based on this, non-invasive liquid biopsy has the potential to help identify the patients who may respond to ICI. Liquid biopsy derived circulatory tumor DNA, circulatory tumor cells, and immune cell-based biomarkers could be new biomarkers that will guide clinical decisions for checkpoint inhibitor treatment in NSCLC. Furthermore, these biomarkers can serve for monitoring the treatment response and unraveling the mechanisms of resistance.

Keywords: immunotherapy, immune checkpoint inhibitor, biomarkers, liquid biopsy, ctDNA, circulating tumor cells, tumor mutational burden

Cite this article as: Kontić M, Marković F. Liquid biopsy as a source of potential biomarkers for checkpoint inhibitor treatment in non-small cell lung cancer Medicinska istaživanja 2024; 57(2):113-119 DOI: 10.5937/medi57-46846

INTRODUCTION

Lung cancer is the leading cause of cancer-related mortality worldwide. Non–small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers. By targeting appropriate molecular targets in tumors, personalized medicine has helped improve survival in patients with NSCLC. With the advancement of technology, genetics and biomarker testing, specific biomarkers have been identified to better target treatment for individual patients who would benefit more from novel therapeutic approaches and thus have better survival (1).

Immune checkpoint inhibitors (ICI) targeting programmed cell death protein 1 (PD1) or programmed cell death ligand 1 (PD-L1) have made revolutionary changes in the clinical approach to managing NSCLC. However, only a minority of patients respond to ICI and biomarkers predicting response are still lacking (2).

Molecular, genetic and epigenetic information often stem from relatively small tissue sample which is obtained at the time of diagnosis only and which is mostly incomplete. Even at the time of diagnosis, up to 30% of NSCLC patients are unable to provide a tissue sample suitable for the foreseen molecular testing. (1). It is not always feasible for patients who are progressing on treatment rebiopsy, or it may not be suitable for molecular testing (2). Liquid biopsies provide an alternative or a complementary modality that can be utilized to better capture the molecular evolution of tumors and its spatial and temporal heterogeneity.

Various technologies and panel tests have emerged for analyzing molecular alterations for liquid biopsies. Among these, polymerase chain reaction (PCR)-based sequencing stands out, alongside the increasingly favored NGS-based sequencing methods due to their advanced capabilities.

PCR assays are a popular choice on a large scale due to their widespread use, high sensitivity, and cost-effectiveness. These technologies excel in identifying very low Mutant Allele Frequencies (MAF) of circulating tumor DNA (ctDNA). However, their limitation lies in their ability to detect only known point mutations, insertions, and deletions. That means the information about tumor DNA derived from this method is somewhat restricted. Despite this constraint, PCR-based assays are widely embraced in clinical practice for their simplicity, efficiency, and reliability.(3,4).

NGS assays have gained extensive adoption due to high sensitivity, the availability of commercial companion diagnostic and agnostic panels (capable of detecting low Mutant Allele Frequencies of circulating tumor DNA). Additionally, NGS is utilized in untargeted panels, eliminating the necessity for prior knowledge of molecular alterations and enabling the discovery of genome-wide DNA variations (5,6). NGS methods have reached a stage where both cost and performance align well with clinical diagnostic needs (7). Consequently, the increasing popularity of profiling circulating tumor DNA (ctDNA) using NGS technologies stems from their applicability throughout the entire cancer diagnosis and management process.(4).

cTDNA

Cell free DNA (cfDNA) represents extracellular strands of DNA that are present in body fluids. Specific type of cfDNA is circulating tumor DNA (ctDNA) which consists of DNA fragments that originate form tumor cells. The way in which they enter the bloodstream is not fully understood, but it has been suggested that they originate from apoptotic, necrotic tumor cells or are actively secreted via extracellular vesicles (1,8).

The major limitation for ctDNA use lies in its variable detectability (from 0.01% to more than 90%) of the total cfDNA (9). This variability depends on the type and microenvironment of the tumor, disease stage and anatomic location. However, multiple IO trials across the tumor types (including NSCLC) have validated the use of ctDNA for early diagnosis, identification of minimal residual disease, mutation detection and monitoring therapy response.

A decrease in ctDNA levels from baseline after initiation of IO therapy in NSCLC patients has been linked to immunotherapy benefit.(10). In a trial evaluating patients with advanced NSCLC undergoing pembrolizumab based therapies, a decrease in cfDNA levels at 9 weeks was associated with significantly better progression free survival (PFS) (median PFS 14.1 months v 4.4 months; hazard ratio [HR], 0.25; 95% CI, 0.13 to 0.50) and overall survival (OS) (median OS NR [95% CI lower bound 22.1 months] v 12.0 months; HR, 0.27; 95% CI, 0.12 to 0.64) (11).

Monitoring levels of ctDNA after first line of treatment is also helpful in guiding treatment decisions and monitoring disease activity as reflected in the results of IMpower010 trial that analyzed the effectiveness and safety of atezolizumab in the adjuvant setting compared to best supportive care after adjuvant platinum-based chemotherapy following resection of NSCLC (stage IB-IIIA). (12). More recently Assaf et al. reached a similar conclusion while analyzing the treatment outcomes of patients enrolled in IMpower 150 who received first-line IO-based combination therapy for advanced NSCLC. They found that patients with undetectable ctDNA levels at baseline and good ctDNA clearance derive most benefit from this treatment option in terms of median overall survival (13). Also, levels of ctDNA can help differentiate between pseudoprogression and progression as in the first case the radiographical increase in tumor size is not accompanied by the rise in ctDNA levels, while in case of progression it is (9).

An optimal treatment duration of IO therapy in advanced NSCLC patients has not been precisely established yet and is a matter of debate. Despite the fact that

most registrational studies limited the duration of IO therapy to two years, in the real-world clinical practice many patients' course of IO treatment exceeds this time frame. (14). Hellman MD et al. have found that among patients with durable response to IO therapy (>12 months) those with undetectable ctDNA at that time point have remained progression free as opposed to patients with detectable ctDNA levels whose disease ultimately progressed.(15). This concept may soon be adopted as a strategy to guide treatment de-escalation in this subset of patients.

On-treatment concentration of ctDNA could be a useful biomarker in assessing response to IO therapy. Its measurement may help differentiate those patients that are likely to benefit from IO treatment from those who are not likely to do so. It is beneficial to identify the latter in the early stages of treatment when the performance status allows for the use of other treatment regiments such as chemotherapy or perhaps targeted therapy. Identifying the former in the later stages of treatment may help reduce social, physical, and financial burden of unnecessary treatment extension.

Detection of somatic mutations in ctDNA may also serve to guide the IO treatment of NSCLC patients.

The presence of serine/threonine kinase 11 (*STK11*) mutation and a phosphatase and tensin homolog (PTEN) was associated with early progression in stage IIIb/IV NSCLC patients recieveing PD-1 inhibitors.(16) In the same study, Guibert N. et al. also found that the detection of transverse mutations in Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (*KRAS*) gene and tumor suppressor gene TP53 alone in these patients lead to better outcomes.(16). Similarly, Basher et al. found that stage VI NSCLC patients receiving IO therapy with ctDNA detectable co-mutations of KRAS and STK11 had longer OS compared to patients harboring only STK11 mutation.(17). In NSCLC patients treated with IO harboring mutations in kelch-like ECH-associated protein 1 (*KEAP1*) and nuclear factor erythroid-2-related factor-2 (*NFE2L2*) genes detected in ctDNA were associated with poorer OS and PFS (18). Also, a better response and prolonged PFS were observed in NSCLC patients with AT-rich interacting domain containing protein 1A gene (*ARID1A*) mutations or AT-rich interacting domain-containing protein 1B gene (*ARID1B*) mutations while undergoing IO based therapy regiments (19). Goldberg et al. found that NSCLC patients with more than a 50% decrease in variant allelic frequency (VAF) of the detectable somatic mutation at baseline had greater PFS and OS. Interestingly, the "ctDNA response" was registered 42.5 days in median before the radiological confirmation.(20).

SOLUBLE PD1 AND PD-L1

Programmed death-1 (PD-1) is a protein found on membranes of T Cells and functions as a checkpoint. It has a key role in downregulating the immune system, advancing self-tolerance and regulating T cell exhaustion.(21). Binding of this protein to its ligand, programmed death - ligand -1 (PD-L1) inhibits T cell activation. PD-L1 is found on the surface of tumor cells and its interaction with PD-1 facilitates tumor growth through immune evasion. Development of anti-PD-1/PD-L1 antibodies has been a major steppingstone in the field of cancer immunotherapy. Since then, surface PD-L1 has been perhaps the most studied biomarker in immunotherapy. In advanced NSCLC patients undergoing anti-PD-1 based ICI therapy, higher levels of PD-L1 expression led to better OS, as per Nikolic et al. (22).

Soluble forms of PD-1 and PD-L1 can also be easily detected in peripheral circulation. Soluble PD-L1 (sPD-L1) has been studied as a potential biomarker for patients undergoing ICI therapy across tumor types including NSCLC. Elevated levels of sPD-L1 have been associated with more advanced disease and worse outcomes.(9).

In a meta-analysis by Cheng et al. including 1188 advanced NSCLC patients, authors confirmed that high sPD-L1 post treatment was significantly associated with worse OS (HR = 2.20; 95%; $p < 0.001$) and PFS (HR = 2.42; 95% $p < 0.001$) in patients treated with ICIs.(23). More recently, Schirocchi et al. reached similar conclusions in their subgroup meta-analysis of NSCLC patients. Data for OS which were pooled from five studies and included 542 NSCLC patients suggested that a higher concentration of sPD-L1 was significantly associated with worse OS (HR = 1.81; (95%CI: 1.09–3.00, $p = 0.02$). The case was similar with PFS (HR = 2.18; (95%CI: 1.27–3.76, $p < 0.01$) when the data were pooled from seven studies that included 616 NSCLC patients.(24).

In a pan-cancer cohort that included 50 NSCLC patients, high pre-treatment sPD-L1 levels were associated with advanced stage disease. Surprisingly, the sPD-L1 levels did not correlate with the tumor PD-L1 levels (25). In advanced NSCLC patients this phenomenon has been observed in other studies and the relationship between tumor tissue PD-L1 and sPD-L1 remains to be defined (26,27). It has also been observed that any on-treatment increase in sPD-1 plasma level has been correlated with improved survival for various cancers including NSCLC.(28).

With all this in mind it seems as though monitoring sPD-L1 before and throughout the IO treatment may help in selecting patients that are likely to benefit from it. However, further effort is needed in the domain of standardization of sPD-L1 sampling and its quantifying before this potential biomarker can be further validated in large scale trials.

BLOOD CELL COUNT AND NEUTROPHIL TO LYMPHOCYTE RATIO

Peripheral blood cell counts and their ratios have also been evaluated as biomarkers for the response to immunotherapy in NSCLC patients.

Low concentration of circulating lymphocytes may correlate with lower levels of tumor-infiltrating lymphocytes (TILs) and a diminished anti-tumor T-cell response (29). Neutrophil to lymphocyte ratio (NLR) reflects systemic inflammation and could provide insight into balance of the immune system in a patient with a malignant neoplasm (30,31). The fact that these analyses are easily accessible (in a sense that they can be obtained via simple blood test that is available anywhere in the world), cost-effective and reliable make them an attractive biomarker candidate.

Ye Jin Lee et al. found that increased pre- and post-treatment peripheral lymphocyte count in NSCLC patients undergoing ICI therapy was associated with favorable PFS and OS (32).

A meta-analysis that included 1225 NSCLC patients on nivolumab from 14 retrospective studies concluded that elevated pretreatment NLR was associated with poor PFS (HR = 1.44; 95% confidence interval (CI):1.18–1.77; $p < 0.05$) and OS (HR = 1.75; 95% CI: 1.33–2.30; $p < 0.05$).(33).

More recently another meta-analysis evaluated NLR in 1719 advanced NSCLC patients undergoing IO therapy and a similar conclusion was made. Elevation of NLR at baseline as associated with worse outcomes, both in PFS and OS (HR PFS 2.21 [95% CI: 1.50–3.24; $p < 0.0001$] and HR OS 2.68 [95% CI: 2.24–3.6; $p < 0.0001$) (34).

NLR ratio as a prognostic indicator is not unique to ICI-treated patients as NLR may be a prognostic indicator for different cancer treatment modalities as well as other conditions.(9). Also, there are plethora of factors that may influence and distort NLR such as age, gender, ethnic, environmental factors and lifestyle (34). While cut-off value for NLR of 5 has been used in most of the studies in the aforementioned meta-analyses, it is yet to be standardized and thus find its way into the daily clinical practice.

TMB

The overall count of somatic mutations occurring within one million bases of DNA (1 megabase, Mb), referred to as tumor mutational burden (TMB), plays a significant role in predicting how well a patient responds to immunotherapy (IO) in various types of cancer. Elevated TMB can stem from various biological processes, including the exposure to environmental factors like cigarette smoke or ultraviolet radiation. It can also arise from harmful mutations in mismatch repair genes leading to microsatellite instability or in the DNA repair system. These factors collectively influence the TMB and consequently impact the effectiveness of immunotherapy treatment.

Although tissue biopsy remains a standard for TMB assessment, obtaining sufficient tissue from advanced cancer patients is challenging, and archived primary tumor samples might not fully represent the evolving tumor during advanced stages. In such cases, a minimally invasive approach using ctDNA-based TMB becomes crucial to identify patients who may benefit from ICI immunotherapies. Some studies show good agreement between ctDNA-based TMB and tissue TMB, suggesting that cTMB testing is feasible and predicts the outcomes of IO therapies.

To better determine which patients will respond positively to IO treatments, more research is required to establish specific cutoff values for cTMB and tTMB and fully evaluate the predictive value of cTMB.

To determine the tumor mutational burden (TMB), a considerable number of genes, usually more than 300, need to be sequenced. The purpose is to analyze these genes and calculate the number of non-synonymous mutations per mega base pair (Mbp). Researchers are currently investigating the potential association between TMB, specifically ctDNA-based TMB (cTMB), and clinical outcomes in various studies like BF1RST, MYSTIC, and OAK trials (30-32).

Overall, patients with detectable ctDNA and higher cTMB at the time of diagnosis (greater than 10–16 mutations per Mbp) tend to experience a longer median overall survival (OS) when treated with first-line immune checkpoint inhibitor (ICI) therapy. It was observed that patients with less than 10 mutations per Mbp detectable from ctDNA did not benefit significantly from immune checkpoint inhibitor treatment in this study.

High cTMB (ctDNA-based TMB) predicts better responses to immune checkpoint inhibitor (IO) treatments compared to chemotherapy. The greater the number of mutations per mega base pair (Mb) at a cutoff of ≥20, the more significant the benefit is when using IO therapies.

However, in some studies, the agreement between cTMB and tTMB (tissue-based TMB) for the patients involved was low. This difference could be due to varying amounts of ctDNA released by the tumor and normalizing for ctDNA versus cfDNA might enhance the reliability of cTMB. Additionally, technical variations arising from different methods of ctDNA isolation and sequencing could also lead to discrepancies in genomic coverage.

A high TMB is generally defined as having at least 10 mutations per Mb. However, the determination of TMB can vary significantly depending on whether panel sequencing (with more than 300 genes) or whole exome sequencing is used, necessitating adaptation of the TMB score based on the sequencing method. Through clinical validation efforts, researchers have determined specific TMB cutoff values that can predict the response to ICI treatment. This demonstrates that TMB is an independent predictive biomarker, complementing other markers like PD-L1, for assessing the effectiveness of ICI therapy (27).

For instance, in the NSCLC CheckMate-227 trial, it was evident that higher TMB levels predicted longer progression-free survival (PFS) in patients receiving a

combination of nivolumab and ipilimumab, but it did not show the same benefit in patients receiving chemotherapy alone (28).

Furthermore, elevated TMB was also found to be a predictor of improved survival in patients with various types of tumors receiving ICI treatment. However, the specific TMB cutoff values varied significantly depending on the type of cancer being treated.

TISSUE OR LIQUID? BOTH?

Acquiring a tissue sample is often imperative for a conclusive diagnosis and the identification of tumor histology. Additionally, as previously mentioned, tissue sample is the standard for TMB assessment as well as PD-L1 tumor proportion score. Tumor heterogeneity, both spatial and temporal, make accurate assessment of resistance and driver mutations based on biopsy of a single metastatic site challenging. Liquid biopsy with its capacity to address these challenges and provide a faster turnaround time, emerges as a potential complement and even an alternative in certain scenarios. Good concordance between the two methods and the high specificity and the moderate sensitivity of liquid biopsies has been established across cancer groups including NSCLC.(35–37)

In a prospective study of 323 advanced NSCLC patients, Aggarwal et al. found that in case of inadequate tissue DNA, liquid NGS biopsies are an adequate surrogate for molecular profiling. They found therapeutically targetable mutations were detected in 113 patients (35.0%), 66 (58.4%) had a mutation in plasma and there were only 8 patients that had negative concurrent tissue tests carried out. Furthermore, 101 patients in the mentioned study tissue testing was not possible highlighting the importance of liquid biopsy as an adequate alternative.(36).

CONCLUSION

Newly developing predictive biomarkers for immune checkpoint inhibitors (IO) encompass the evaluation of PD-L1 expression on circulating tumor cells (CTCs) and/or peripheral blood mononuclear cells (PBMCs), as well as the assessment of tumor mutational burden (TMB). However, the reliability of predicting patient responses using these biomarkers is still uncertain, similar to tissue-based markers, often due to technical limitations in terms of sensitivity and specificity. Overcoming these challenges is crucial in order to enhance and ensure the reproducibility of these biomarkers, ultimately improving their effectiveness in predicting treatment outcomes.

Using a comprehensive genomic profiling (CGP) approach offers the benefit of generating combined biomarkers. These composite biomarkers can help categorize patient groups more effectively, identifying those who are most likely to experience significant clinical benefits from immune checkpoint inhibitors and other targeted treatments that are matched to their specific genomic profiles.

Conflict of interest

None to declare.

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NEINVAZIVNA TEČNA BIOPSIJA KAO IZVOR POTENCIJALNIH BIOMARKERA ZA LEČENJE NESITNOĆELIJSKOG KARCINOMA PLUĆA INHIBITORIMA KONTROLNE TAČKE

Milica Kontić[1,2], Filip Marković [1]

Sažetak

Karcinom pluća (LC) je vodeći uzrok smrtnosti od malignih bolesti širom sveta. Iako je terapija inhibitorima imunoloških kontrolnih tačaka (ICI) dovela do revolucije u lečenju i poboljšanju kliničkih ishoda pacijenata obolelih od nesitnoćelijskog karcinoma pluća (NSCLC) i neki pacijenti imaju dobar odgovor na nju, drugi su rezistentni na ovu terapiju ili imaju neželjena dejstva opasna po život. Još uvek ne postoje dobri biomarkeri za predikciju odgovora na ICI. Zato personalizacija ICI terapije na osnovu jedinstvenog genomskog profila pacijenta predstavlja atraktivnu strategiju za poboljšanje ishoda lečenja bolesnika sa NSCLC.

Postoje kontinuirani napori da se pronađu prediktivni biomarkeri za selekciju pacijenata koji će reagovati na ICI, sa ciljem da se izbegne gubitak dragocenog vremena, troškova i toksičnosti pri isprobavanju terapija od kojih pacijent neće imati nikakve koristi.

Neinvazivna tečna biopsija ima potencijal da pomogne u otkrivanju pacijenata koji mogu da reaguju na ICI. Utvrdjivanje cirkulišuće DNK tumora, cirkulišućih tumorskih ćelija i drugih biomarkera iz tečnih biopsija mogli bi da budu novi biomarkeri koji će uticati na izbor ICI u lečenju NSCLC. Pored toga, ovi biomarkeri mogu da posluže za praćenje odgovora na tretman i otkrivanje mehanizama rezistencije.

Ključne reči: imunoterapija, inhibitori kontrolne tačke, biomarkeri, tečna biopsija, ctDNA, cirkulišće tumorske ćelije, TMB

Primljen: 30.09.2023. I **Revizija:** 13.02.2024. I **Prihvaćen:** 01.04.2024.

Medicinska istaživanja 2024; 57(2):113-119

Medicinska istaživanja 2024; 57(2):121-126 | DOI 10.5937/medi57-47676

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Complications of pneumococcal meningitis in a child with proteus syndrome: a case report and literature review

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Recived: 13 November 2023
Revised: 25 December 2023
Accepted: 10 April 2024

Check for updates

Funding information:
The authors did not receive specific grants from any funding agency in public, commercial, or nonprofit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Proteus syndrome (PS) is an exceptionally rare disorder characterized by asymmetrical overgrowth of the skin, bones, muscles, adipose and connective tissues as well as blood and lymphatic vessels.

Case presentation: We describe the clinical case of a 6.5-year-old girl with PS diagnosed and treated at the Institute for Mother and Child Health Care of Serbia "Dr Vukan Čupić". When she was 11 months old, she was treated for pneumococcal sepsis and meningitis. The disease was complicated by intracranial thromboses of venous sinuses, subdural empyema, brain infarction and a severe neurological deficit in the acute phase. Additionally, portal and mesenteric venous thromboses were identified. At 2.5 years of age, echocardiography and cardiac magnetic resonance revealed an ascending aortic aneurysm. The patient suddenly passed away when she was 6.5 years old and the cause of death has remained unknown. **Conclusion:** Despite aggressive antibiotic therapy, our patient with PS experienced multiple life-threatening complications associated with pneumococcal disease. Considering the previously documented immune disturbances in PS patients, it is plausible to speculate that our patient's immune system was compromised due to the primary diagnosis. However, as data on the immunological response in PS patients are scarce, conclusive evidence regarding the predisposition to serious infections necessitates further comprehensive studies.

Keywords: Proteus syndrome, pneumococcus, meningitis, pulmonary embolism, aortic aneurysm.

Cite this article as: Ostojić S, Kravljanac R, Kovačević G, Vučetić Tadić B, Kuzmanović M, Prijić S, Gazikalović S, Paripović A, Sarajlija A. Complications of pneumococcal meningitis in a child with proteus syndrome: a case report and literature review; Medicinska istaživanja 2024; 57(2):121-126 DOI: 10.5937/medi57-47676

INTRODUCTION

Proteus syndrome (PS) is an exceptionally rare and intricate disorder characterized by asymmetric overgrowth of various body parts (1-4). Clinical manifestations of this condition exhibit remarkable variability, with disproportional overgrowth in diverse organs and tissues, primarily the connective tissue, bone, skin, adipose tissue and central nervous system (CNS) (4). The following are also found in PS patients: hyperostosis, specific progressive cerebriform connective tissue nevi, epidermal nevi, scoliosis, other skeletal abnormalities, splenomegaly, vascular malformations, benign and malignant tumors (1-6). Notably, deep venous thrombosis with pulmonary embolism was also documented in multiple patients with PS (7,8). The mosaic expression of somatic mutation in the AKT1 gene results in random distribution of affected tissues, contributing to significant phenotypic variability among patients (9). The estimated global prevalence of PS is 1 per one million live births, with approximately 200 reported cases in the medical literature (10,11). In this report, we present the case of a Serbian child diagnosed with PS and experiencing complications related to bacterial meningitis. To the best of our knowledge, this constitutes the first genetically confirmed case of PS in Serbia.

CASE PRESENTATION

We present a case of a 6.5-year-old girl, who was initially admitted to our hospital at the age of 11 months in a comatose state. She is the third child of non-consanguineous parents who was born following an uncomplicated pregnancy, with uneventful perinatal period. Linear skin hyperpigmentation of extremities emerged when she was 6 months old. Psychomotor development remained normal in the first year of her life.

The onset of acute infection symptoms occurred the day before her admission to our hospital, marked by a fever up to 38°C, vomiting, dehydration, and somnolence. The progression of the illness included convulsions and a rapid decline in the level of consciousness from somnolence to coma, as reflected by a Glasgow Coma Scale (GCS) score of 6. Elective intubation was conducted before transferring the child to the intensive care unit, yet spontaneous respiration persisted, requiring synchronized intermittent mandatory ventilation. Throughout the initial days of treatment, the patient continued to exhibit a persistent fever, sinus tachycardia (170 bpm), hypertension (140/110 mmHg), and decreased breath sounds on the right side. There were some noteworthy phenotypic characteristics that included dolichocephaly, hyperostosis of the parietal bone, right hemihypertrophy, macrodactyly of the second finger on the left hand, and linear skin hyperpigmentation of the extremities. Upon admission, inflammation parameters were elevated, including C-reactive protein (CRP) level of 196.2 mg/L and fibrinogen concentration of 15.06 g/L. Additionally, the patient displayed anemia (hemoglobin: 80.7 g/L) and leucopenia (3.03 x 10^9/L). Findings in cerebrospinal fluid (CSF) were suggestive for bacterial meningitis: white blood cells (WBC) of 128/cubic millimeter, protein concentration of 4956 g/l, glucose concentration of 0.1 mmol/l, chloride concentration of 98 mmol/l. Streptococcus pneumoniae was isolated in blood and CSF cultures. Antibiotic therapy was immediately initiated (ceftriaxone and vancomycin). Two days later, laboratory assessments revealed signs of disseminated intravascular coagulation (DIC), including a prolonged prothrombin time (PT) of 32.1 seconds, a reduced antithrombin (AT) level of 43.1%, and a high D-dimer concentration of 8675 ng/mL. Chest radiography revealed consolidation of pulmonary parenchyma and pleural effusion. Computerized tomography (CT) of the brain on admission showed wide subarachnoid space, dilatation of the left ventricle frontal horn and thickening of the right parietal bone, while the brain parenchyma appeared normal. However, after six days, a contrast-enhanced brain CT was done due to worsening clinical condition. The scan revealed thrombosis of the sagittal sinus and cortical veins in the left parietal lobe, resulting in a hemorrhagic infarct and perifocal edema. On the eighth day of the treatment, the enlargement of the abdomen was notice, prompting suspicion of the ascites. Subsequently, a repeated ultrasound (US) examination of the abdomen revealed the presence of portal vein thrombosis and its branches, along with the observation of free abdominal fluid. The management of the thrombosis involved the administration of low molecular weight heparin, along with concurrent screening for thrombophilia. D-dimer concentration was found to be constantly elevated, while rotational thromboelastometry (ROTEM) showed extended time in INTEM with subsequent inadequate function of platelets and fibrinogen.

The patient's overall condition remained poor, marked by coma and dependence on mechanical ventilation, throughout the initial two weeks following admission. On the fifteenth day of her hospital stay it was possible to extubate the patient. A neurological assessment at this time revealed right-sided hemiparesis. The MRI examination of the endocranium on 20th day of the hospitalization indicated signs of the sagittal venous sinus recanalization, with the residual lumen narrowing, partial thrombosis of the superficial cortical sinuses in the frontal region on both sides and the parietal region on the left side. Additionally, evidence of massive cortical necrosis in the left frontal, parietal, and occipital gyri, representing sequelae of cortical infarct with hemosiderin deposits, was observed **(Figure 1B)**. Changes observed in relation to the CT scan included epidural and subdural effusions on the right (up to 3 mm) and left side (16 mm), exerting compressive effects on the left frontal lobe **(Figure 1A)**. Neurosurgical intervention was employed to drain the subdural and epidural collections. Partial

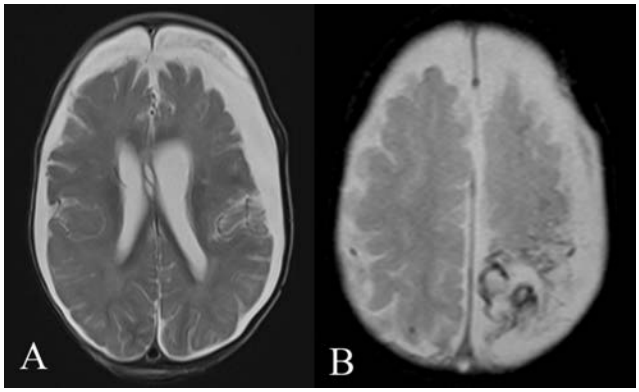

Figure 1. Axial Brain MRI T2W image shows (A) frontal epidural and subdural empyema. (B) Cortical infarction of the left frontal parietal gyrus with hemosiderin deposits.

convulsions stopped due to intravenous administration of midazolam and phenobarbital, and the treatment continued with levetiracetam, successfully achieving control of epileptic seizures. However, the resolution of meningitis was notably slow, resulting in neurological sequelae. The patient was discharged after a two-month treatment.

Regrettably, the child regressed in all acquired milestones of the early development, including sitting, standing, and walking, as well as speaking.

FOLLOW-UP AND OUTCOME

Asymmetric overgrowth was prominent in child throughout the-follow up period, with the following measurements: (1) at the age of one year, 95.2 cm (13.2 cm above +3SD); (2) at 2.5 years, 116 cm (15 cm above +3SD); (3) at 6 years, 148 cm (23 cm above +3SD), accompanied by reduced weight (20.8 kg) and BMI (9.49 kg/m2, below -3SD). The child exhibited atrophy of the adipose tissue and muscles, along with pale skin featuring linear hyperpigmentation along the neck, chest, and extremities, capillary malformation, and evident macrodactyly of the second finger on the left hand **(Figure 2)**. She had craniofacial dysmorphia characterized by an elongated face, dolichocephaly and prominent forehead. Visual impairment, severe myopia, and bilateral sensorineural hearing impairment were confirmed, necessitating the use of eyeglasses and hearing aids. Speech development remained incomplete. Neurologically, a flaccid quadriparesis, more pronounced on the right side of the body, dominated the presentation.

Full head control and the ability to sit unsupported or stand were not fully established. No epileptic seizures were recorded post-discharge, and there were no occur-

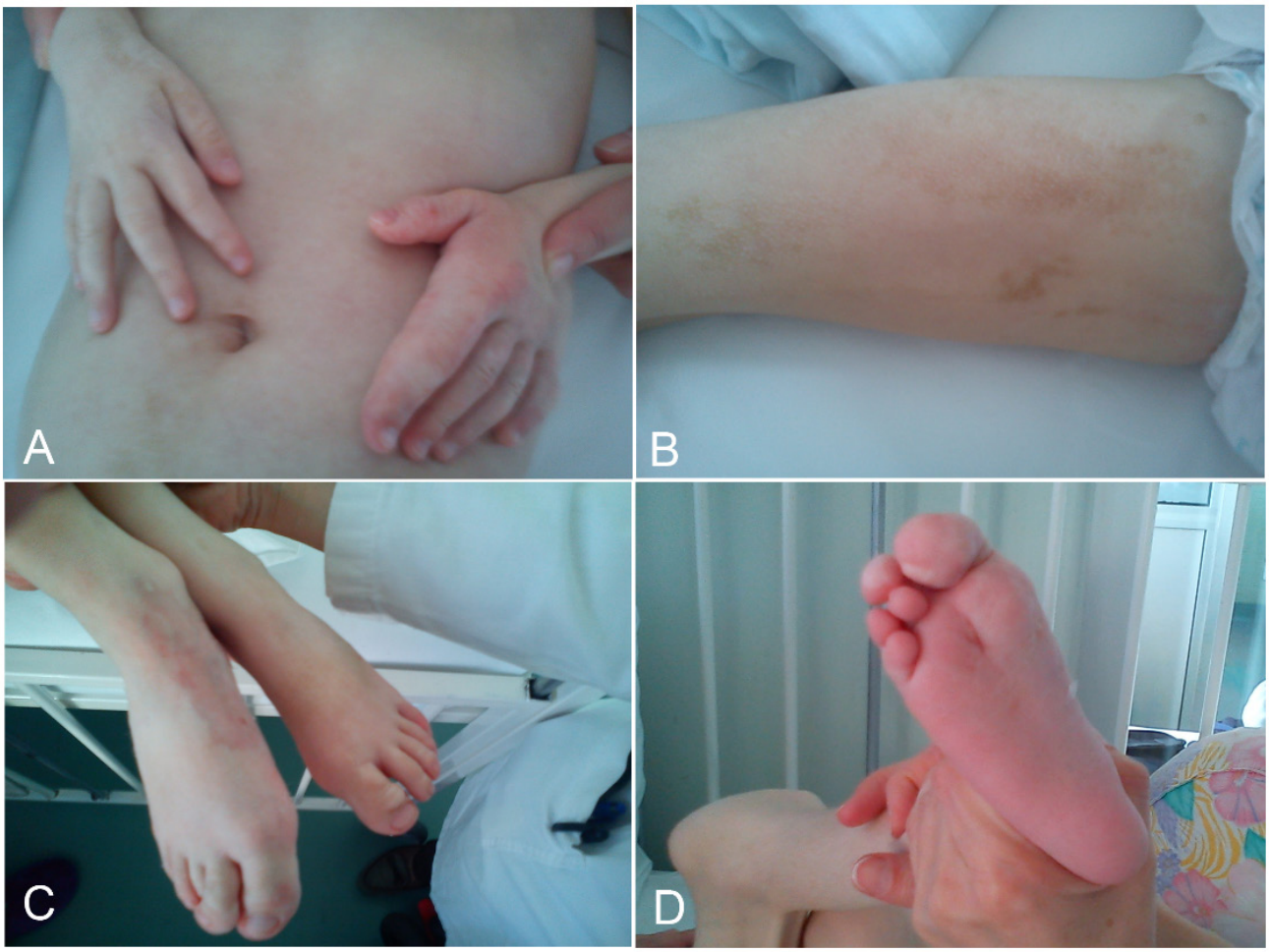

Figure 2. Phenotypic characteristics of the patient. (A) macrodactyly of the second finger on the left hand; (B) dark linear skin hyperpigmentation of the skin; (C) right hemihypertrophy and vascular skin nevus on the right leg; (D) limb hypotrophy and incipient cerebriform nevus on the plantar side of the right big toe.

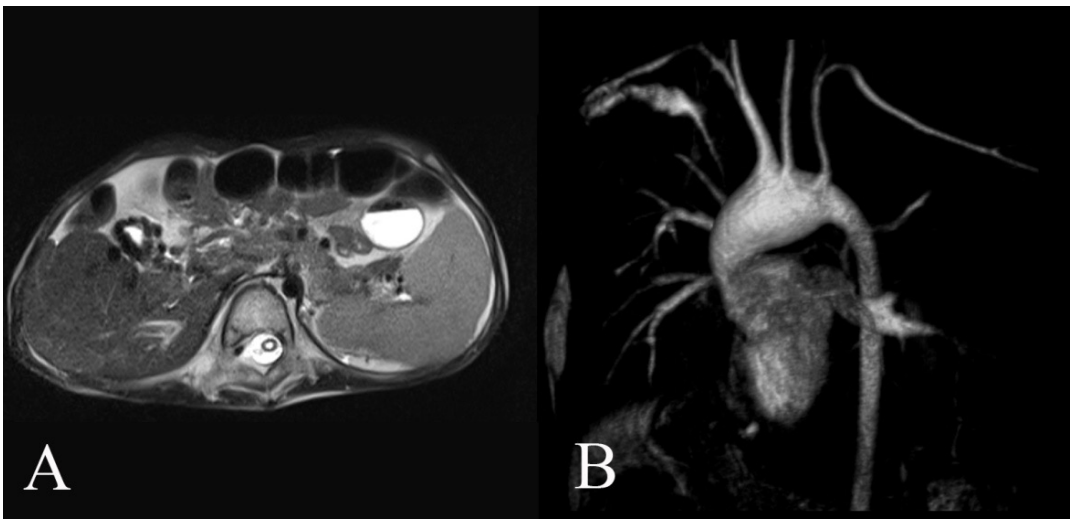

Figure 3. MRI of the abdomen and the heart. (A) Transverse T2W abdominal tomograms showing cavernous portal vein transformation and dilated portosystemic collateral blood vessels in the cholecystic lobe in the form of low IS ("flow void" structures in the blood vessels) and subsequent splenomegaly; (B) MR of the aortic arch (contrast aortography). Fusiform dilatation of the ascending aorta, aortic arch, and part of the isthmic region with a maximum diameter of 34 mm.

rences of severe infections during the follow-up period.

At the age of 2.5 years, an MR examination of the abdomen with angiography revealed splenomegaly, esophageal varicosities, and a portal vein aneurysm (Figure 3A). The examination also confirmed complete occlusion of the mesenteric artery, leading to mesenteric hypertension, and demonstrated cavernous portal vein transformation. Echocardiography and cardiac magnetic resonance disclosed an ascending aortic aneurysm (diameter up to 34 mm) and mild mitral regurgitation **(Figure 3B)**, in contrast to the normal aortic findings observed at 11 months of age. Propranolol and valsartan were prescribed to manage these cardiovascular abnormalities. A thoracic MR examination revealed fatty tissue expansion of the anterior thoracic wall, corresponding to a lipoma or a lipoblastoma. There was hyperplasia of other tissues as well. Fiber optic laryngotracheobronchoscopy indicated extreme hyperplasia of the adenoid and tonsil, with tumor tissues observed in the vocal cords and arytenoids, indicating mucosal metaplasia. Pelvic MRI confirmed the presence of large multilocular ovarian cysts measuring 49x71x41 mm and 93x63x60 mm, corresponding to multilocular cystadenomas. Elevated tumor marker levels (CA-125: 4694 U/ml) prompted gynecologists to perform bilateral adnexectomy, with histopathological findings confirming ovarian cystadenoma. In the sacral region, a surgically removed polyp was pathologically identified as an angiomatous hamartoma. The most appropriate management for suppressing growth was considered since overgrowth and the tumor masses in different tissues were impeding normal functioning of this child.

Clinical suspicion of Proteus syndrome (PS) arose when the child reached 2.5 years of age. A skin biopsy was performed, followed by the culture of fibroblasts. Samples of the DNA and the fibroblast culture from clinically affected areas were sent to the genetic laboratory at the National Institute of Health (Bethesda, USA). Here, the diagnosis of PS was confirmed at the genetic level by analyzing the samples isolated from three cell lines. A missense mutation c.49G>A (p.Glu17Lys) in the AKT1 gene, was identified as a pathologic one.

Despite regular health check-ups at our clinic, the child passed away suddenly at home at the age of 6.5. The immediate cause of death remained unknown.

DISCUSSION

Proteus syndrome (PS) is an exceptionally rare and intricate disorder characterized by malformations and abnormal overgrowth of various tissues (1-3). Affected individuals typically appear normal at birth, with abnormalities progressively manifesting during childhood. Due to the variable nature of clinical manifestations, diagnosing PS in infancy can be challenging, often resulting in initial misdiagnoses (1, 2).

In our patient with PS, meningitis caused by Streptococcus pneumoniae emerged as a severe and life-threatening complication. Initially, the child's neurological deficit was attributed solely to the severity of the infection. However, by the second year of her life, objective signs indicative of a genetically determined syndrome became apparent. Specifically, she exhibited all major and specific clinical criteria for PS, except for the presence of lung cysts (1-4). The diagnosis was genetically confirmed at the National Human Genome Research Institute in Bethesda during her third year of life.

According to the literature data, bacterial meningitis as a complication have not been reported in a patient with PS before. However, we did not prove immunodeficiency in a child with PS. There are limited publications on the immune profile of individuals with PS (12,13). The first reported case involved a 10-year-old boy with PS and mild hypogammaglobulinemia. Immunological in-

vestigations showed low serum IgG and IgA levels, along with reduced specific antibodies to pneumococcus and Haemophilus type B polysaccharides. Notably, post-immunization antibody concentrations after administering the pneumococcal vaccine in the same patient were within normal limits (12). Our patient was not immunized with the pneumococcal conjugate vaccine, as it was not mandatory in our country's immunization program at that time (2011). The patient received all other vaccines stipulated by the mandatory vaccination calendar in Serbia, including BCG, Hep B, DTaP, Hib, and IPV polio. Transitory leucopenia and lymphopenia were accounted for by sepsis. Hodge and Loungaris previously reported cases of individuals with PS exhibiting lymphopenia, which resulted in a reduction of total T and B cell counts (12,13). Notably, in contrast to our case, these patients did not experience severe infections. The role of AKT kinase appears to be significant in B cell biology, influencing apoptosis and survival, as suggested by previous research (14). Evidently, the most severe complications in our patient stemmed from a predisposition to develop deep venous thrombosis during serious bacterial infections, such as pneumococcal sepsis and meningitis. We lack evidence supporting the notion that the occurrence of thrombosis in our patient was linked to well-known prothrombotic conditions, such as factor V Leiden, prothrombin mutation in F2, deficiency of antithrombin III, protein C, or protein S. These conditions typically elevate the rate of thrombin production and fibrin clot formation. Additionally, our patient exhibited platelet dysfunction according to the ROTEM test. A similar conclusion was reached by Keppler-Noreuil *et al.*, who highlighted potential mechanisms for thromboembolism in PS, suggesting specific vascular and platelet dysfunction resulting from the AKT1 p.E17K mutation (7).

Our patient passed away at the age of 6.5 years, and the exact cause of death remains unknown as the death occurred at home, and no autopsy was performed. In addition to her predisposition to deep venous thrombosis and tumorous tissue affecting the vocal cords and thorax, compromising respiratory function, she also had an aortic aneurysm, representing a significant risk factor for fatality. This raised the question of whether an aortic aneurysm is a clinical feature of PS. In their investigation of cardiothoracic findings in PS, Mirmomen *et al.* demonstrated mild aneurysmal dilatation of the ascending aorta (with the diameter of 42–43 mm) in two out of 38 individuals (6%), with the mean age of 23 and a range of 9–61 years (15). More than half of the individuals who underwent cardiothoracic imaging of the chest exhibited pulmonary venous dilation (62%) (15). While Streptococcus pneumoniae is considered a rare cause of mycotic aneurysms (16), we can only speculate that our patient's lifespan was shortened due to, the sequelae of pneumococcal disease, to mention only one risk. Clinical studies indicate that the greatest risk of death in patients with PS occurs during childhood and adolescence (10). Early causes of death are diverse, including deep venous thromboembolism, pulmonary thromboembolism, pneumonia, respiratory failure, and others (10,17,18). Additionally, individuals with PS are at an increased risk of developing malignancies in various tissues and organs, most commonly meningiomas and gonadal tumors (15,17), as observed in our patient.

CONCLUSION

In conclusion, despite the administration of aggressive antibiotic therapy, our patient experienced multiple life-threatening complications due to pneumococcal disease. Considering the previously documented immune disturbances in patients with PS, it is plausible to speculate that the compromised immune system in our patient resulted from the primary diagnosis. However, the fact that there are scarce published data on the immunological response in PS patients, primarily due to the rarity of the disease, hinders reaching definitive conclusions about their propensity for serious infections. Further studies are essential to provide a comprehensive understanding of the immunological characteristics in PS patients.

Abbreviations:

PS - Proteus syndrome; GCS - Glasgow Coma Scale; CNS - Central Nervous System; CRP - C-reactive protein; DNA - Deoxyribonucleic Acid; ICU - Intensive Care Unit.

Acknowledgements: We express our gratitude to the Biesecker lab at the National Human Genome Research Institute at the National Institutes of Health for their molecular analysis of this patient's samples.

Conflict of interest: None to declare.

Authors' contributions: All authors have thoroughly reviewed and approved the final manuscript.

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KOMPLIKACIJE PNEUMOKOKNOG MENINGITISA KOD DETETA S PROTEUSOVIM SINDROMOM: PRIKAZ SLUČAJA I PREGLED LITERATURE

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Sažetak

Uvod: Proteusov sindrom (PS) je veoma retko oboljenje, za koje je karakterističan asimetrični prekomerni rast kože, kostiju, mišića, masnog i vezivnog tkiva, krvnih i limfnih sudova.

Prikaz slučaja: Prikazujemo kliničku sliku devojčice uzrasta 6,5 godina sa PS, koja je dijagnostikovana i lečena u Institutu za zdravstvenu zaštitu majke i deteta Srbije. U uzrastu od 11 meseci lečena je od pneumokokne sepse i meningitisa. Bolest se komplikovala intrakranijalnim trombozama venskih sinusa, subduralnim empijemom, infarktom mozga i teškim neurološkim deficitom u akutnoj fazi. Registrovane su tromboze mezenterijalnih krvnih sudova i vene porte. U uzrastu od 2,5 godine, ehokardiografijom i magnetnom rezonancom srca otkivena je aneurizma ascendentne aorte. Pacijentkinja je iznenada preminula u uzrastu od 6,5 godina kod kuće. Uzrok smrti je bio nepoznat.

Zaključak: Uprkos primeni agresivne antibiotske terapije, naša pacijentkinje sa PS je imala brojne, po život opasne, komplikacije pneumokokne bolesti. Imajući u vidu prethodno opisane imunološke poremećaje kod pacijenata sa PS, možemo da pretpostavimo da je imunski sistem našeg pacijenta bio kompromitovan zbog osnovne bolesti. Publikovani podaci o imunološkom aspektu bolesnika sa PS su malobrojni, zbog toga što je bolest veoma retka, te su neophodna dalja istraživanja sklonosti ovih bolesnika ka teškim infekcijama.

Ključne reči: Proteus sindrom, pneumokok, meningitis, plućna embolija, aneurizma aorte

Primljen: 13.11.2023. I **Revizija:** 25.12.2023. I **Prihvaćen:** 10.04.2024.

Medicinska istaživanja 2024; 57(2):121-126

Medicinska istaživanja 2024; 57(2):127-130 | DOI 10.5937/medi57-46608

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Erosive pustular dermatosis of the scalp – a case report

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Recived: 18 September 2023
Revised: 17 October 2023
Accepted: 03 November 2023

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Erosive pustular dermatosis of the scalp (EPDS) is rare inflammatory dermatosis of unknown etiology, which mainly affects the scalp. The condition is characterized by sterile pustules, often with secondary bacterial colonization, erosions and crusts which lead to scarring alopecia. EPDS occurs in the elderly, more frequently in women.

Patient report: A 55-year-old Caucasian female presented with a large atrophic erythematous plaque on the scalp, coated with pustules on the sides and covered with thick crusts and erosions. The patient reported a history of painless, moderately pruritic, enlarging lesions during three months prior to the admission to our department. She had no history of trauma, excessive sun exposure or some medical or cosmetic treatment (topical and systemic). Serological and immunologic tests (Hepatitis B and C, HIV; antinuclear antibodies, ANA) were negative. Fungal cultures were negative. Bacterial cultures grew *Staphylococcus aureus*. A scalp biopsy revealed polymorphous inflammatory infiltrate in the dermis of neutrophils, lymphocytes and plasma cells. Periodic acid Schiff (PAS) stain was negative. Direct immunofluorescence test was negative. Treatment with an oral antibiotic (rimfapicine) and potent topical corticosteroids led to marked and quick improvement but with remaining scarring alopecia.

Conclusion: EPDS is a diagnosis of exclusion, based on clinical presentation, disease course and histopathological findings**.** It is necessary to exclude other differential diagnoses - autoimmune bullous disorders, malignancies, neutrophilic dermatoses, bacterial and fungal infections**.** A prompt diagnosis and treatment will reduce scarring.

Key words: erosive pustular dermatosis, trauma, diagnosis, treatment

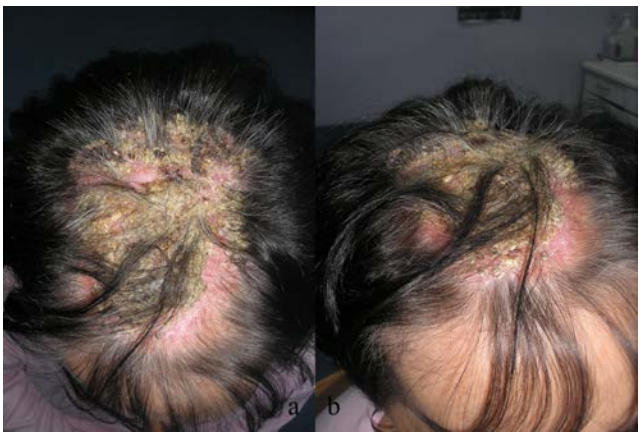
Cite this article as: Cakić J, Tanasilović S, Vuković J, Živanović D. Erosive pustular dermatosis of the scalp – a case report
Medicinska istaživanja 2024; 57(2):127-130 DOI: 10.5937/medi57-46608

INTRODUCTION

Erosive pustular dermatosis of the scalp (EPDS) is a rare inflammatory disease, with a slow onset (1). EPDS has been reported after local injuries as well as some medication treatments (1). The disease tends to develop on sun-damaged skin of the scalp, in the elderly, more frequently in women (1, 2). Pathogenesis is not well known, predisposing factors (skin atrophy, androgenetic alopecia) and triggering factors such as trauma or damage are possibly involved (3, 4). EPDS is a diagnosis of exclusion (2, 4). The condition is characterized by sterile pustules that often become secondarily colonized, erosions or superficial ulcerations and crusts (1, 4, 5). The choice of treatment depends on age, severity and the extent of the disease.

CASE REPORT

A 55-year-old healthy Caucasian female presented with a large atrophic erythematous plaque on the scalp, coated with pustules on the sides and covered with thick crusts and erosions **(Figures 1a and b)**.



Figures 1a and b. Clinical features of a patient showing erythematous plaque covered with hyperkeratotic crusts, erosions and pustules

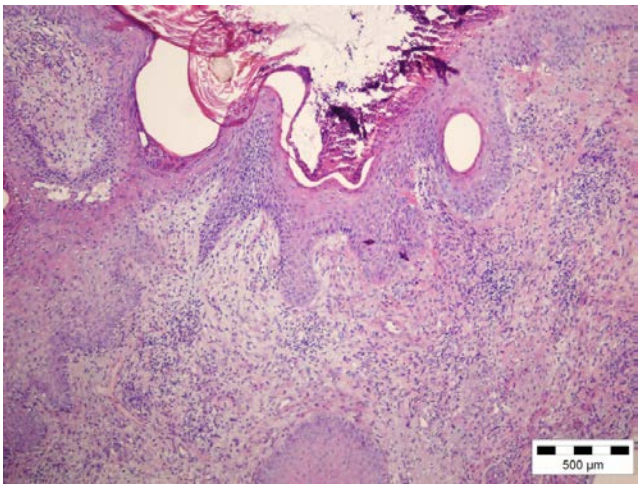


Figure 2. Histopathological finding (hematoxylin-eosin stain; original magnification x 200)

The patient reported a three-month history of painless, moderately itching, enlarging lesions. She had no history of trauma, sun exposure or a cosmetic treatment. She was initially treated with oral antihistamines and antibiotics with no or minimal improvement. Except for an elevated erythrocyte sedimentation rate (40 mm/h) and C- reactive protein (5.9 mg/L), all other tests were within the normal range. Serological and immunologic tests were negative. Fungal stains were sterile. Bacterial cultures grew *Staphylococcus aureus*. A scalp biopsy revealed polymorphous inflammatory infiltrate in the dermis consisting of neutrophils, lymphocytes and plasma cells **(Figure 2)**.

Direct immunofluorescence test was negative. Treatment with oral antibiotics (Rimfapicine, 600 mg daily, during 2 weeks) and potent topical corticosteroids led to a marked and quick improvement **(Figures 3a and b)**

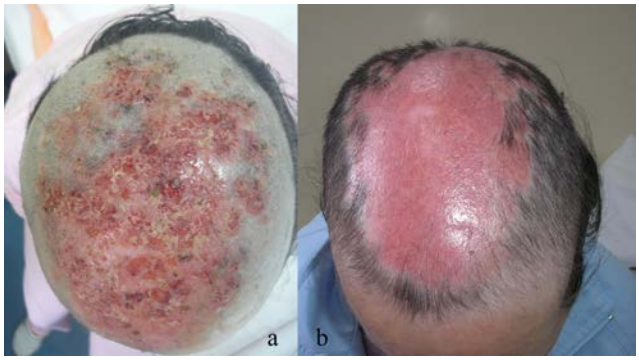


Figure 3a and b. Clinical presentation after 2 weeks of therapy with remaining scarring alopecia (Figures 4a and b).

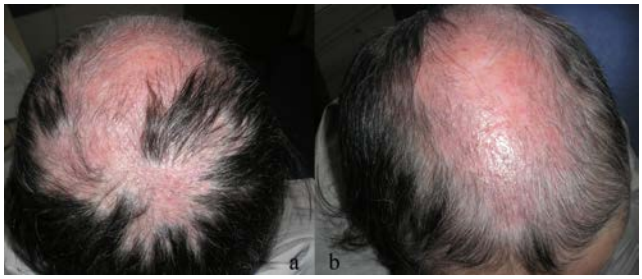


Figure 4a and b. Complete clinical regression of skin lesions with remaining scarring alopecia

In the follow-up period of 18 months she experienced only one mild recurrence and was treated shortly with topical steroids. The patient was later lost to follow-up.

DISCUSSION

EPDS predominantly occurs on the scalp, mostly on the vertex and the crown, but other sites may also be affected, including the extremities (5). EPDS is usually characterized by the association of pustules, erosions, and serous, yellow- brownish or hemorrhagic crusts on atrophic skin (6). The clinical course is protracted, with intermittent improvement and recurrences, resulting in scar formation.

The etiology and pathogenesis of EPDS is still not clear. Some factors are assumed to lead to the development of the disease. Predisposing factors include areas of actinic damage, skin atrophy and androgenetic alopecia (4). It is postulated that the immune mediated reaction after skin trauma resulting in secondary inflammation and impaired microcirculation may play a role in etiopathogenesis (6). EPDS is mostly a disease of the elderly, so a term called 'immunosenescence' (a decrease in the specificity and the efficacy of the immune response that develops with age) has been implicated (4). Aberrant immune response-loosing tolerance to self-antigens, leading to increased "self-reactivity", along with other predisposing factors may account for the development of EPDS (4, 7). Although similar to bacterial or fungal infection, infectious etiology is not thought to play a role in inflammation in EPDS, as cultures often demonstrate only occasional colonization (6, 8). However, *Herpes zoster* is reported as a possible trigger for EPDS (9). According to literature, local trauma, burns, contact dermatitis, laser treatment, cryotherapy, topical photodynamic therapy (PDT), radiotherapy, as well as some medications, cosmetic or surgical treatments are mentioned as provoking factors (2, 3, 10). The recurrence of EPDS following trauma such as skin graft, has been described in the literature indicating that removing affected skin did not control the disease (11). The presence of neutrophils in the inflammatory infiltrate is probably the consequence of skin trauma triggering autoimmune reaction resulting in a secondary inflammation (6). Recently, systemic medications such as epidermal growth factor receptor (EGFR) inhibitors have been reported as triggers (12). Most patients, however, present with a history of spontaneous onset of one or multiple confluent skin lesions (1) which was also the case in our patient.

Establishing an EPDS diagnosis can be challenging because numerous other diseases, such as primary scarring alopecia, skin malignancies, bacterial and fungal infections, inflammatory conditions (autoimmune blistering disorders in particular) may clinically mimic EPDS (13,14). Biopsy and clinicopathologic correlation are required to distinguish between EPDS and aforementioned entities. Although histopathology of EPDS is nonspecific, it is mandatory for the diagnosis to differentiate ESPD from other clinically similar diseases. The fact that chronic nonspecific inflammation is present in skin biopsies indicates that the inflammation plays a significant role in the pathophysiology of this disease (15). Considering that neutrophils are almost always present in the histopathological findings, it suggests that EPDS could or should be classified as part of neutrophilic dermatosis, but they are not predominant in the same way as they are in other conditions (5, 15).

Trichoscopy and dermoscopic examination can be useful in the diagnosis of EDSP and excluding other disorders, especially non-melanoma skin cancer (basocellular and squamocellular carcinoma) (3, 6, 13, 14). We did not use these diagnostic tools in our case.

The treatment depends on age, severity and the extent of the disease. Local treatment is given in early stages of the disease. According to literature, the best results have been obtained with high potent topical steroids, as the first line of treatment for EPDS (1, 3, 14). Besides topical steroids, topical tacrolimus and calcipotriol cream has been suggested as alternate treatments for EPDS (2, 7, 16). Although PDT has been reported as a successful treatment option it should be considered with particular attention as data also suggest PDT is a triggering factor for EPDS (2). Other treatment alternatives include oral retinoids, oral and topical dapsone and, in more severe cases, oral steroids (18, 19). Systemic antibiotics are also used to treat bacterial superinfection that occurs frequently in patients with EPDS, which was the case in our patient.

CONCLUSION

EPDS is a diagnosis of exclusion based on clinical presentation and course. The disease is likely underreported. The nonspecific histopathological pattern, the evolution leading to scarring alopecia, and the frequent response to topical steroids, favor the diagnosis. Once the other causes of the inflammatory process have been ruled out, the diagnosis of EPDS must be seriously considered. Given the lack of a large comprehensive case series and the unknown etiology, there is no general treatment recommendation for EPDS. Avoidance of precipitating factors and understanding the risk of development of actinic damage is very important.

Conflicts of interest: None to declare.

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

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EROZIVNA PUSTULARNA DERMATOZA SKALPA – PRIKAZ SLUČAJA

Cakić Jelena [1], Tanasilović Srđan[1, 2], Vuković Jelena[1], Živanović Dubravka [1,2]

Sažetak

Uvod: Erozivna pustularna dermatoza skalpa (EPDS) je retka, inflamatorna dermatoza, nepoznate etiologije, koja se uglavnom javlja u kapilicijumu. Karakteriše je pojava sterilnih pustula, često sa sekundarnom kolonizacijom, erozijama i krustama, što dovodi do ožiljne alopecije. EPDS se javlja kod starijih osoba, češće kod žena.

Prikaz slučaja: Predstavljamo slučaj pacijentkinje uzrasta 55 godina, sa velikim atrofičnim eritematoznim plakom na kapilicijumu, prekrivenim brojnim pustulama, erozijama i debelim krustama. Anamnestički, tokom tri meseca pre prijema dolazi do uvećanja promena, koje su bile bezbolne, praćene umerenim svrabom. Negirala je postojanje prethodne traume, intenzivnije izlaganje suncu, upotrebu medicinskih ili kozmetičkih sredstava (topikalnih i sistemskih). Serološki (Hepatitis B i C, HIV) i imunološki testovi (antinuklearna antitela, ANA) su bili negativni. Mikološke kulture su bile sterilne. U bakteriološkim kulturama izolovan je *Staphylococcus aureus*. U histopatološkom preparatu promene dobijene biopsijom, nađen je polimorfni zapaljenski infiltrat u dermu, sačinjen od neutrofila, limfocita i plazma ćelija. Bojenje po PAS-u (eng. *periodic acid Schiff,* PAS) je bilo negativno. Direktni imunofluorescentni test je bio negativan. Terapija oralnim antibiotikom (rimfapicin) i topikalnim potentnim kortikosteroidima dovela je do značajnog i brzog poboljšanja, ali sa posledičnom ožiljnom alopecijom.

Zaključak: EPDS je dijagnoza *per exclusionem*, na osnovu kliničke prezentacije, toka bolesti i histopatološkog nalaza. Potrebno je isključiti druge bolesti - autoimunske bulozne dermatoze, malignitete, neutrofilne dermatoze, bakterijske i gljivične infekcije. Brzo postavljanje dijagnoze i lečenje smanjuju stepen ožiljavanja.

Ključne reči: erozivna pustularna dermatoza, trauma, dijagnoza, lečenje

Primljen: 18.09.2023. I **Revizija:** 17.10.2023. I **Prihvaćen:** 03.11.2023.

Medicinska istaživanja 2024; 57(2):127-130

Medicinska istaživanja 2024; 57(2):131-135 | DOI 10.5937/medi57-47257

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Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Recurrent liver cyst: case report and literature review

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Recived: 21 October 2023

Revised: 05 December 2023

Accepted: 10 April 2024

Check for updates

Funding information:
Ministry of Science of the Republic of Serbia (grant no. 200110)

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Liver cysts are defined as cavities within the liver tissue, surrounded by a layer of epithelium and filled with liquid or semi-liquid contents. They are mostly asymptomatic and usually diagnosed by ultrasonography, CT or MR diagnostics. Symptoms occur as a result of complications such as bleeding, rupture, infection or compression of the biliary tract. All liver cysts can be divided into (1) infectious and (2) non-infectious liver cysts. The most common non-infectious cysts are simple congenital biliary cysts.

Case outline: We present a 74-year-old female patient. She had upper right abdominal pain and dyspepsia several weeks before the first examination. Abdominal ultrasound and CT verified a large simple cyst of the right lobe of the liver of about 14cm in size. She underwent minimally invasive surgery when a partial cyst resection was performed. Seven months later, abdominal ultrasound, MR and MRCP verified a simple recurrent cyst in the right lobe of about 13cm in size with no communication with the biliary system. She underwent open surgery when pericystectomia cum omentoplastica was performed. The pathohistological result showed a benign biliary cyst. There was no recurrence at regular follow ups 12 months upon the procedure.

Conclusion: There are many treatment modalities, and each one is accompanied by certain disadvantages. In recent years, conventional open surgical procedures have been replaced by minimally invasive surgical procedures. Laparoscopic surgery is the method of choice in carefully selected patients. Open conventional surgery is reserved for patients with giant cysts, recurrent cysts, deep intraparenchymal cysts, and right lobe subphrenic cysts.

Key words: recurrent cyst, cystis hepatis, liver cyst, biliary cyst, benign cyst, pericystectomy

Cite this article as: Mitrović N, Trifunović N, Stevanović D, Jašarović D, Aleksandrić G. Recurrent liver cyst: case report and literature review Medicinska istaživanja 2024; 57(2):131-135 DOI: 10.5937/medi57-47257

INTRODUCTION

Liver cysts are defined as cavities within the liver tissue, surrounded by a layer of epithelium and filled with liquid or semi-liquid contents. They are mostly asymptomatic and usually diagnosed by ultrasonography, CT or MR diagnostics. Symptoms occur as a result of complications such as bleeding, rupture, infection or compression of the biliary tree (1).

In Europe, the incidence is between 5 and 10%, and in the USA it is between 15 and 18%. In more than 85% of cases, patients are asymptomatic, and cysts are diagnosed unintentionally, with one of the radiological methods. About 15% of patients complain of upper right abdominal discomfort or pain, nausea, vomiting, and rarely icterus and acute abdomen (1, 2, 3).

According to etiology, all liver cysts can be divided into (1) infectious and (2) non-infectious liver cysts. Infectious cysts of the liver include echinococcosis of the liver (hydatid cyst) and pyogenic liver abscesses (4, 5, 6).

Non-infectious liver cysts can be further divided into a) benign, b) premalignant and c) malignant liver cysts. The most common benign lesions are simple liver cysts (biliary cysts), pseudocysts, biliary hamartoma and polycystic liver disease. Premalignant lesions include biliary cystadenomas, intrapapillary ductal biliary neoplasms and Caroli's disease. Malignant liver cysts can be in the form of biliary cystadenocarcinoma, undifferentiated embryonal sarcoma or mesenchymal hamartoma (7, 8, 9).

According to radiological diagnostics, there are simple cysts and complex liver cysts. Ultrasound is the method of choice. CT and MRI have better sensitivity and specificity and more precisely describe cystic lesions of the liver (10, 11).

Treatment decision depends on many factors such as the cyst type, clinical and radiological manifestations and the general condition of the patient.

CASE REPORT

We present a 74-year-old female patient. She had had upper right abdominal pain, nausea and vomiting several weeks before she was examined in a private health institution in June 2021. During diagnostic abdominal ultrasound a large cyst in the liver right lobe was verified. The cyst was 13 cm in diameter, and it was filled with echogenic content with floating particles.

An abdominal CT verified a large simple cyst, occupying most of the right lobe of the liver, measuring 137x98x137mm (APxLLxCC), with gracile internal septa. The right hepatic vein arcuately surrounded the described lession, with a slightly narrowed lumen, as a result of the compression effect. Intrahepatic bile ducts for both lobes were incipiently accentuated.

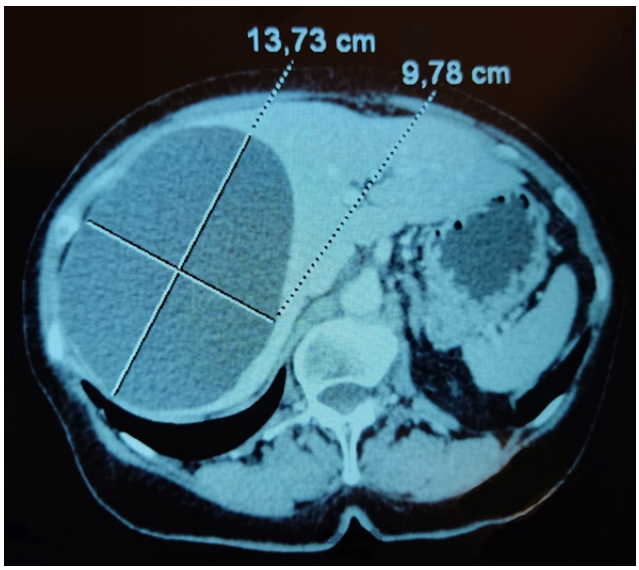

Figure 1. CT verified liver cyst

After complete preoperative preparation, the patient was operated on under general anesthesia in September 2021. The following operation was performed: resectio partialis cystae hepatis laparoscopica. Drainage. Pathohistology result: Solitary hepatic (Nonneoplastic) Cyst. Postoperatively without complications.

During the regular follow up in February 2022, abdominal ultrasound verified a cyst with a diameter of 12x10 cm in the right lobe of the liver, with intracystic hyperechoic fibrous band lessions and dense content. Intra and extrahepatic bile ducts were not dilated.

Abdominal MR was performed after i.v. applications of paramagnetic contrast. The liver was of the appropriate shape and position. The right lobe was largely altered by a well circumscribed, incompletely septated, cystic lession of diameter 125mm x 93mm x 110mm (APxLLxCC), with partial sparing of the V and VI segments. The cystic lession had a slightly lobulated contour, well limited by the capsule, filled with content of higher signal intensities corresponding to serohemorrhagic or dense protein content, with the presence of floating detritus. The described cystic lession only suppressed the intrahepatic bile ducts without signs of communication.

Magnetic resonance cholangiopancreatography was also done. MRCP verified a large cystic lesion of the right lobe of the liver, which initially only suppressed the intrahepatic bile ducts, without certain MR detectable signs of communication with the same.

The patient was then hospitalized at the Department of general surgery of the Clinical-Hospital Centre Zemun for surgical treatment of a recurrent liver cyst in March 2022. Having completed preoperative preparation, she was operated on under general anesthesia, when the following surgery was performed: J lap. Pericystectomy hepatis cum omentoplasty. Drainage spatii subhepatici.

A large cyst of the right lobe of the liver was verified intraoperatively. Pericystectomy was performed. The

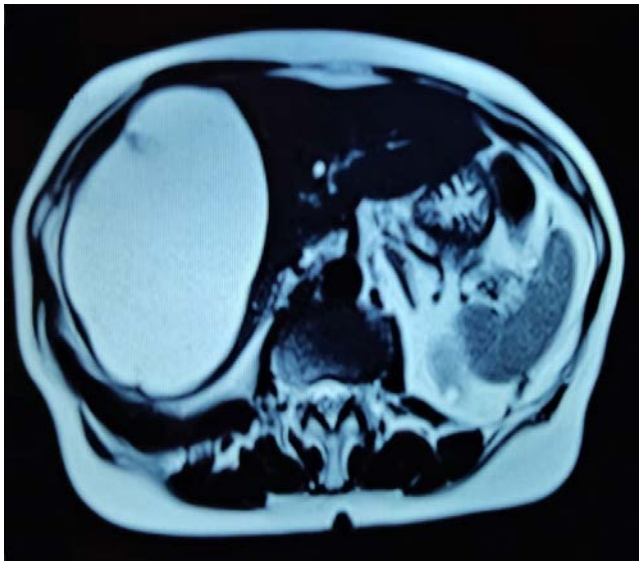

Figure 2. MR verified liver cyst

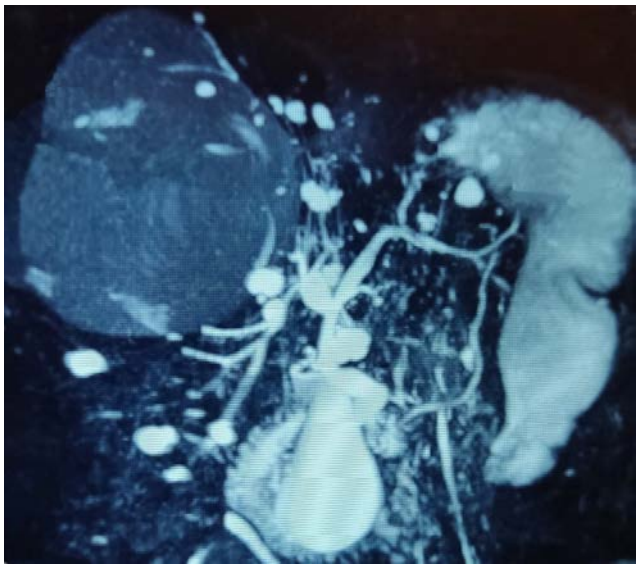

Figure 3. MRCP verified liver cyst

back wall of the cyst in contact with the liver parenchyma was not excised. Macroscopically, the existence of contact between the lumen of the cyst and the intrahepatic bile ducts was not verified. The wall of the cyst as well as the lymph gland from the hepatopancreatic angle were sent for ex tempore diagnostics - the results showed a benign cyst. In the next step, omentoplasty of the cyst was performed. One drain was placed in the right subphrenia. Samples were sent for definitive PH diagnosis.

The result of pathohistological diagnostics were as follows: the analyzed sample material constituteed the wall of a cystic lesion without an epithelial layer. The wall consisted of hyalinized, hypocellular connective tissue under which small biliary ducts and blood vessels could be observed in places in the stroma. This result may correspond to a solitary non-neoplastic liver cyst. Immunohistochemical analysis: CK PAN, CD 31, CD 68, S100.

Postoperatively without complications. At regular clinical and echosonographic check ups 12 months after surgery there was no recurrence.

DISCUSSION

The exact incidence of liver cysts is unknown due to the absence of symptoms in most patients. The development and greater use of more sophisticated diagnostic methods has increased the incidence of accidentally discovered liver cysts, and now it is slightly more than 5%. Simple liver cysts are significantly more common in women, depending on different authors, the ratio ranges from 1.5:1 to 9:1 (12, 13, 14). Large liver cysts are almost exclusively found in women over 50 years of age (15,16). Simple liver cysts are most often congenital and arise from various mutations on chromosome 16, which lead to aberrant development of the bile ducts. In some studies, there is evidence that the presence of an increased concentration of estrogen in women between 40 and 60 years of age can be the cause of the occurrence of simple non-parasitic liver cysts (17, 18). We presented a female patient aged 74 years, which is in accordance with other studies according to which she belongs to the group of patients with the highest incidence of large non-parasitic liver cysts. Our patient was asymptomatic for a long time, and only a few weeks before the diagnosis, she developed non-specific symptoms in the form of discomfort and occasional abdominal pain upper right, as well as dyspeptic complaints.

The diagnosis of a liver cyst is made by abdominal ultrasound, and a more precise description of the cyst, its characterization and communication with the bile ducts is obtained using CT or MR diagnostics.

There are many treatment modalities for simple liver cysts, and each one is accompanied by certain disadvantages. Non-surgical methods such as simple percutaneous aspiration are not adequate due to possible infection and early recurrences. The results are slightly better with the aspiration and the application of phenol alcohol, but relapses are also common, and there is a possibility of developing sclerosing cholangitis if there is unverified communication with the biliary tree (19, 20). In recent years, conventional open surgical procedures have been replaced by minimally invasive surgical procedures. Laparoscopic surgery is the method of choice for cysts up to 20 cm. Frequent postoperative recurrences of giant cysts represent a major obstacle in selecting patients for a laparoscopic or conventional open surgery (21). Factors that increase the incidence of recurrence are incomplete removal of the cyst wall, previous surgical treatment, deep intraparenchymal liver cysts, cysts located in segments VII and VIII, diffuse form of polycystic liver disease as well as biliary cystadenomas. Large cysts in the right posterior lobe have a high recurrence rate due to the intimate contact between the liver and the diaphragm blocking adequate drainage of the cyst into the peritoneal cavity and causing reaccumulation of its contents (22, 23, 24).

Open surgery is indicated in selected patients with giant cysts whose size increases intra-abdominal pressure and exerts compression on nearby organs. Open surgery

is the method of choice in patients with large recurrent cysts due to the more frequent recurrence. Nevertheless, the percentage of recurrence does not differ significantly between laparoscopic and open surgery and is about 10%. However, the postoperative morbidity associated with open surgery and the length of postoperative hospital stay are the main limitations of open surgery. (25)

CONCLUSION

Liver cysts are relatively common, mostly asymptomatic and mostly benign. The diagnosis is made using ultrasound and CT diagnostics. MR diagnostics allows a more detailed characterization of the cyst, usually preoperatively. The modality of treatment depends on many factors such as the characteristics of the cyst, the clinical manifestations, radiological manifestations and the general condition of the patient. The treatment is basically surgical due to frequent recurrences after non-surgical procedures. Laparoscopic surgery is the method of choice in carefully selected patients, because it is an effective method and causes minimal surgical trauma. Open conventional surgery is reserved for patients with giant cysts, recurrent cysts, deep intraparenchymal cysts, and right lobe subphrenic cysts. According to the current data, in carefully selected patients, the percentage of recurrence does not differ significantly between laparoscopic and open surgery.

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RECIDIVANTNE CISTE JETRE: PRIKAZ SLUČAJA I PREGLED LITERATURE

Nebojša Mitrović[1,2], Nemanja Trifunović[2], Dejan Stevanović[1,2], Damir Jašarović[1,2], Goran Aleksandrić[1,2]

Sažetak

Uvod: Ciste jetre definišu se kao šupljine unutar tkiva jetre, oivičene slojem epitela i ispunjene tečnim ili polutečnim sadržajem. Uglavnom su asimptomatske i dijagnostikuju se najčešće uzgredno ultrasonografskom, CT i MR dijagnostikom. Simptomi se javljaju kao posledica komplikacija poput krvarenja, rupture, infekcije ili kompresije bilijarnog stabla.

Prikaz slučaja: Prikazali smo pacijentkinju starosne dobi 74 godine sa recidivantnim cistama jetre. Imala je tegobe u vidu bola u gornjem desnom kvadrantu trbuha i dispeptične tegobe nekoliko nedelja pre prvog pregleda. Ultrazvuk i CT abdomena verifikovali su veliku prostu cistu desnog režnja jetre veličine oko 14cm. Podvrgnuta je laparoskopskoj operaciji kada je učinjena parcijalna resekcija ciste jetre. Na redovnoj kontroli 7 meseci nakon operacije ultrazvukom abdomena, a potom i MR abdomena i MRCP verifikovana je recidivantna prosta cista desnog režnja jetre veličine oko 13cm bez komunikacije sa bilijarnim stablom. Odlučuje se za konvencionalnu operaciju kada je učinjena pericistektomija sa omentoplastikom. Patohistološki verifikovana benigna bilijarna cista jetre. Urednog postoperativnog toka. Na redovnim kontrolama bez recidiva, 12 meseci nakon operacije.

Zaključak: Modaliteti lečenja prostih cisti jetre su brojni, a svaki je praćen određenim nedostacima. Poslednjih godina konvencionalne otvorene hirurške procedure zamenjene su minimalno invazivnim hirurškim procedurama. Laparoskopska operacija je metod izbora kod pažljivo odabranih pacijenata, jer je efikasna metoda, a prouzrokuje minimalnu hirušku traumu. Otvorena konvencionalna hirurgija rezervisana je za pacijenate sa džinovskim cistama, recidivantnim cistama, cistama lokalizovanim duboko intraparenhimski i cistama u desnom režnju subfrenično.

Ključne reči: recidivantna cista, cista jetre, bilijarna cista, benigna cista, pericistektomija

Primljen: 21.10.2023. I **Revizija:** 05.12.2023. I **Prihvaćen:** 10.04.2024.

Medicinska istaživanja 2024; 57(2):131-135

Medicinska istaživanja 2024; 57(2):137-141 | DOI 10.5937/medi57-48645

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CASE REPORT

Agenesis of dorsal pancreas as incidental finding in a COVID-19 85-year-old patient: a case report with a review of literature

Marija Branković[1,2], Tijana Gmizić[1], Marija Dukić[1], Jovana Lalatović[1], Davor Mrda[1]

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Recived: 11 January 2024
Revised: 27 March 2024
Accepted: 27 March 2024

Check for updates

Funding information:
The authors did not receive specific grants from any funding agency in public, commercial, or nonprofit sectors.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Introduction: Polysplenia syndrome is a very rare congenital condition with multifactorial inheritance. It is characterized by the malposition of the thoracic and abdominal organs with or without vascular abnormalities. It comprises multiple cardiac, gastrointestinal, hepatosplenic, pancreatic and renal disorders. It is usually an incidental finding on an abdominal ultrasound or computed tomography (CT) scan performed for other reasons.

Patient review: In this case, an 85-year-old male patient is presented, who tested positive for coronavirus disease 2019 (COVID-19) infection, and he was admitted to hospital for bilateral COVID-19 pneumonia. CT scan was performed and revealed polysplenia, various vascular abnormalities, gastrointestinal malformations and agenesis of the dorsal pancreas, so heterotaxy syndrome was diagnosed. Interestingly, this patient did not have diabetes mellitus in spite of agenesis of the dorsal pancreas, neither had he ever have pancreatitis.

Conclusion: This present case shows that the quality of life in patients with polysplenia syndrome does not have to be seriously impaired and that these patients can experience old age. Moreover, the awareness and prior knowledge of anomalies included in this syndrome are crucial in order to avoid complications during surgical procedures and/or interventions.

Keywords: pancreas, polysplenia, syndrome

Cite this article as: Branković M, Gmizić T, Dukić M, Lalatović J, Mrda D. Agenesis of dorsal pancreas as incidental finding in a COVID-19 85-year-old patient: a case report with a review of literature Medicinska istaživanja 2024; 57(2):137-141 DOI: 10.5937/medi57-48645

INTRODUCTION

The first case of polysplenia syndrome was described in 1781 (1). Polysplenia is a very rare heterotaxy disorder with a reported incidence of 1 per 250,000 live births (2). Additionally, it is encountered in elderly even more rarely (3). Asians have a higher prevalence of heterotaxy syndrome (4) and polysplenia syndrome is more common in females (5).

In complete heterotaxy there is an exact mirror-image of all organs, whereas in partial heterotaxy only some organs are displaced (6). The synonym of heterotaxy is situs ambiguous, and it is usually accompanied by left isomerism (7). Situs ambiguous is defined as the malposition of the thoracic and abdominal organs with or without vascular abnormalities (8). It is subclassified to polysplenia (left isomerism) and asplenia (right isomerism) syndromes (8).

Unfortunately, due to severe cardiovascular abnormalities, a reported mortality for polysplenia syndrome is 75% by the age of five (3) and only those with mild anatomical abnormalities survive into adulthood. It is usually an incidental finding on an abdominal ultrasound or a CT scan performed for other reasons.

CASE REPORT

An 85-year-old male, who tested positive for coronavirus disease 2019 (COVID-19) infection, presented to our hospital with fever and cough. The patient had a past medical history of asthma and arterial hypertension. Blood tests only revealed elevated inflammatory markers. Patient's admission chest radiography demonstrated bilateral opacities throughout the lung fields.

Further evaluation implied computed tomography (CT) scan of the thorax and it showed bilateral ground glass opacities consistent with bilateral COVID-19 pneumonia, but also as incidental finding situs ambiguous was described.

CT scan demonstrated inferior vena cava (IVC) interruption with azygous continuation and suprahepatic segment of IVC drains into the right atrium via azygos continuation **(Figures 1 and 2)**.

Other vascular anomalies include left retro-aortic renal vein, arising of hepatic artery from the aorta, above the diaphragm **(Figure 3)**, arising of lienal artery from the aorta, bellow the hepatic artery and diaphragm **(Figure 4)** and preduodenal portal vein **(Figure 5)**.

Also, levocardia was described and normal atrial situs. In addition, bronchial anatomy is normal in relation to the pulmonary arteries, with eparterial (superior to the main pulmonary arteries) right and hyparterial (below the pulmonary arteries) left bronchus. What is also important, there are four nodular peripherally calcified spleen structures in the left upper quadrant of different sizes **(Figures 6 and 7)**.

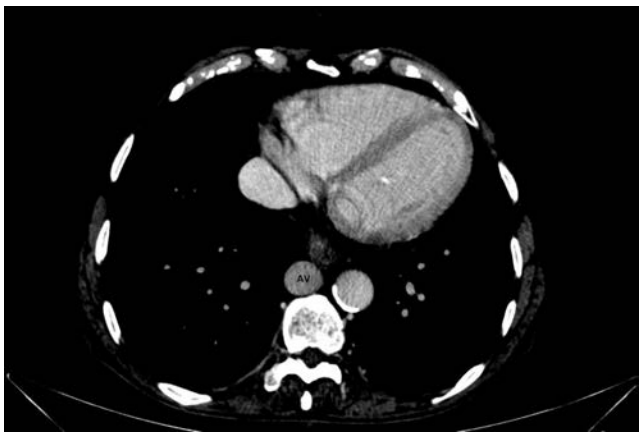

Figure 1. The abdominal computed tomography (CT) scan showing the azygos vein (AV).

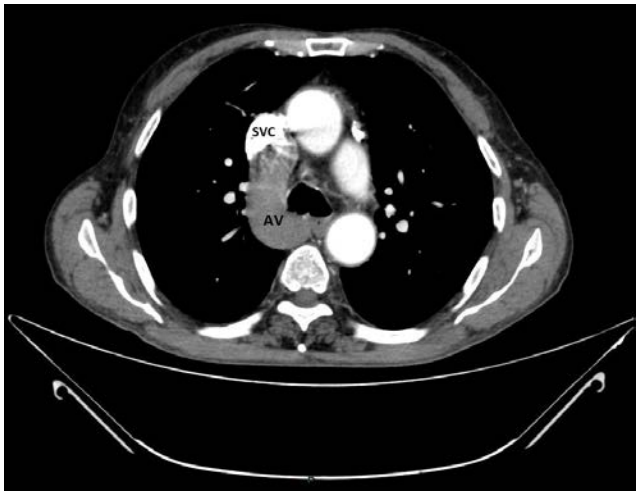

Figure 2. The abdominal computed tomography (CT) scan showing the superior vena cava (SVC) and the azygos vein (AV).

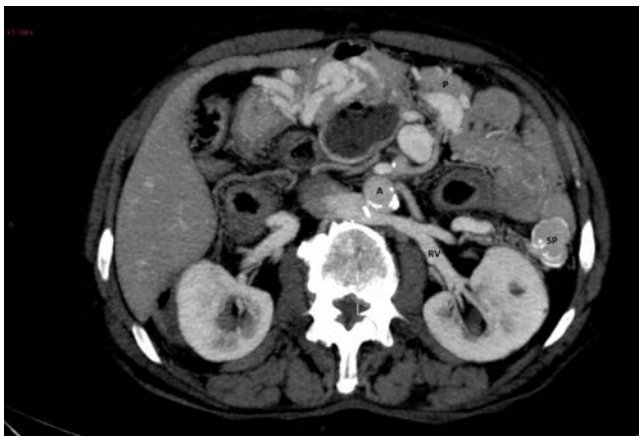

Figure 3. The abdominal computed tomography (CT) scan showing the aorta (A), the spleen (SP), the head of the pancreas (P) and the renal vein (RV).

Moreover, incomplete intestinal malrotation was seen on this CT scan. Furthermore, the stomach is in the right upper quadrant (Figure 8), whereas the liver is located on the right side, with prominent left lobe which extends up to the left hypochondrium **(Figure 9)**.

The gallbladder is located in the midline. Additionally, he was incidentally discovered to have an absent body and tail of the pancreas on CT imaging, but the head

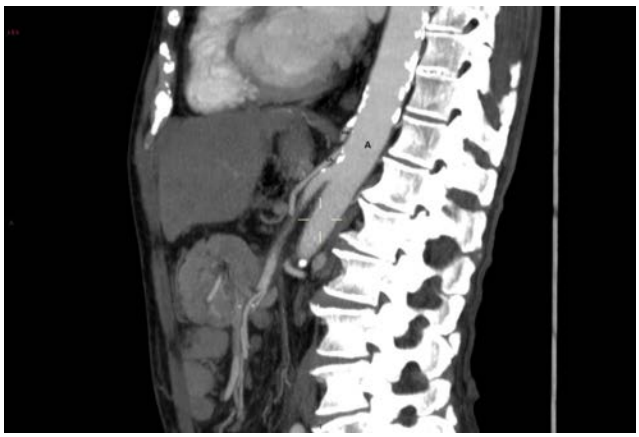

Figure 4. The abdominal computed tomography (CT) scan showing aorta (A), hepatic artery (HA) and splenic artery (LA).

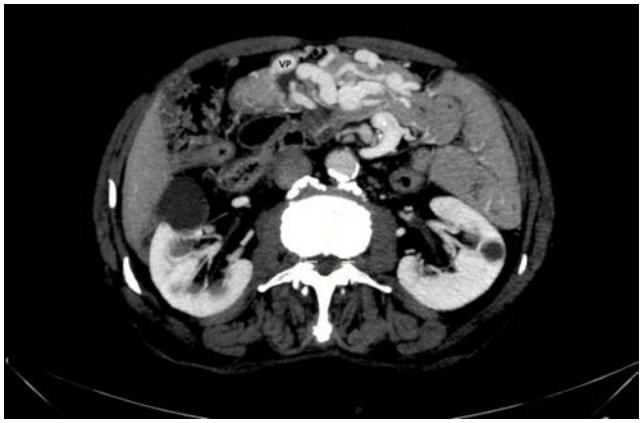

Figure 5. The abdominal computed tomography (CT) scan showing portal vein (VP).

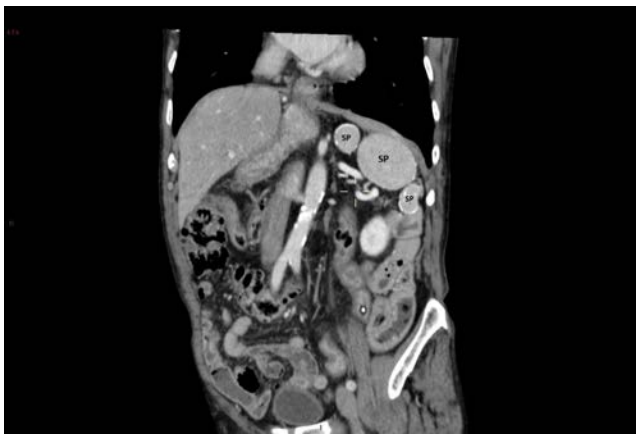

Figure 6. The abdominal computed tomography (CT) scan showing multiple spleens (SP).

of the pancreas was seen, indicating this is a congenital short pancreas defect **(Figures 3 and 7)**. Less importantly, bilateral renal cysts were described.

This morphology indicates polysplenia with agenesis of the dorsal pancreas in heterotaxy syndrome. It is important to emphasize that this patient with such important malformations has had a normal life, considering he is 85 years old. In addition, this patient doesn't have diabetes mellitus as a glycosylated hemoglobin was in normal range, in spite of agenesis of the dorsal pancreas.

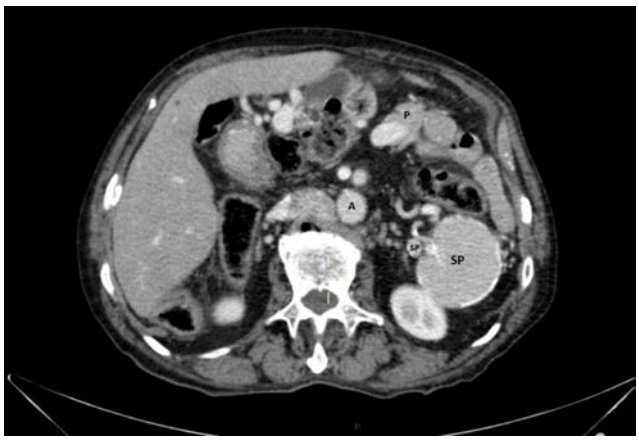

Figure 7. The abdominal computed tomography (CT) scan showing the aorta (A), the head of the pancreas (P) and spleens (SP).

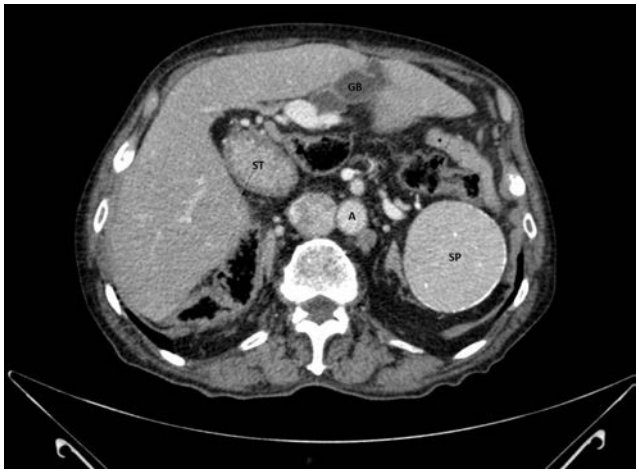

Figure 8. The abdominal computed tomography (CT) scan showing the stomach (ST), the gall bladder (GB), the aorta (A), the spleen (SP).

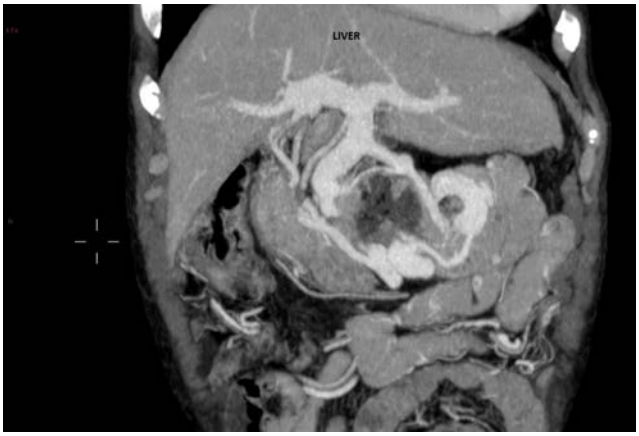

Figure 9. The abdominal computed tomography (CT) scan showing the liver.

In regard to COVID-19 infection, the patient was treated per recommendations of the National Guide for the Treatment of COVID-19 infection, which was based on the guidelines of the World Health Organization. The course of the disease was without any complications, and the patient was discharged from hospital after a few days to continue the COVID-19 treatment at home. An informed consent for this case report was obtained from the above-mentioned patient.

DISCUSSION

Although polysplenia syndrome comprises a wide range of abnormalities, there is no pathognomic abnormality that characterizes it. Abnormalities include multiple spleens and various anomalies such as visceral heterotaxia with a right-sided stomach, a left-sided or large midline liver, right-sided spleens, malrotation of the intestine, a short pancreas, and azygos or hemiazygos continuation of IVC (which is one of the most common) with the absence of the hepatic segments (3). All the spleens in this syndrome are of equal size with the main spleen in the left hypochondrium, which is vascularized by an artery with normal birth (1). What is really interesting, this syndrome can also include a single-lobed spleen or even a normal spleen (9).

The exact cause of this syndrome is still unknown, but it certainly has multifactorial inheritance. There are associations to embryonic and genetic components which are in connection with a disruption of left-right axis determination during early embryonic development and by mutations in some of the 80 genes required for normal asymmetric left-right organ development (10). There are assumptions that genetic inheritance of heterotaxy syndrome could be autosomal dominant, recessive and X-linked recessive inheritance (9).

It is described that dorsal pancreatic agenesis, the anomaly our patient has, is frequently accompanied by hyperglycemia in about 50% of cases, due to the lack of islet cells that are mainly found in the tail and body of the pancreas (11,12). Also, it brings about an increased risk of pancreatitis due to poor drainage from the remnant ventral duct (13). Splenic and pancreatic malformations develop embryonically from the dorsal bud and pancreas develops by the fusion of the ventral and dorsal pancreatic buds, so this is why polysplenia and anomalies of the pancreas, such as short pancreas or dorsal pancreas agenesis, are likely to occur together (14).

As already mentioned, this syndrome comprises multiple cardiac anomalies, but our patient has normal atrial situs and levocardia. Additionally, our patient has interruption of the IVC with azygos continuation, which is mentioned to be the most common abnormality associated with polysplenia syndrome (5). Moreover, in this present case, preduodenal portal vein was described on a CT scan, and it is another anomaly that is frequently associated with polysplenia syndrome. This anomaly can cause pressure symptoms on the duodenum and bile duct (5). It is important to emphasize that the presence of preduodenal portal vein, intestinal malrotation and vascular anomalies is very important as prior knowledge of these anomalies would help in order to avoid complications during surgical procedures and/or interventions (5).

CONCLUSION

Polysplenia syndrome is a heterotaxy disorder and it is a very rare condition. Most commonly, this syndrome is an incidental finding on an abdominal ultrasound or a CT scan performed for other reasons. It comprises multiple congenital malformations, including severe cardiovascular abnormalities and only those patients with mild anatomical abnormalities survive into adulthood. In adulthood, it is most important that both the patient and the doctor are aware of the situation in order to avoid complications during surgical procedures and/or interventions. Additionally, if dorsal pancreatic agenesis is present, we must think of very possible hyperglycemia and/or pancreatitis. This present case shows that quality of life in patients with polysplenia syndrome does not have to be seriously impaired and that these patients can experience old age.

Author Contributions: Conceptualization: Marija Branković, Tijana Gmizić, Marija Dukić, Jovana Lalatović and Davor Mrda; Investigation: Marija Branković, Tijana Gmizić, Marija Dukić, Jovana Lalatović and Davor Mrda; Writing—original draft preparation: Tijana Gmizić, Marija Dukić and Jovana Lalatović; Writing—review and editing: Marija Branković, Tijana Gmizić, Marija Dukić, Jovana Lalatović and Davor Mrda; Supervision: Marija Branković and Davor Mrda; All authors have read and agreed to the published version of the manuscript.

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AGENEZIJA DORZALNOG PANKREASA SLUČAJNO OTKRIVENA KOD 85-GODIŠNJEG PACIJENTA OBOLELOG OD KOVIDA 19: PRIKAZ SLUČAJA SA PREGLEDOM LITERATURE

Marija Branković[1,2], Tijana Gmizić[1], Marija Dukić[1], Jovana Lalatović[1], Davor Mrda[1]

Sažetak

Uvod: Sindrom polisplenije je veoma redak kongenitalni poremećaj sa multifaktorskim nasleđivanjem. Karakterizovan je malpozicijom organa u grudnom košu i trbuhu, sa ili bez abnormalnosti krvnih sudova. Uključuje više poremećaja srca, gastrointestinalnog trakta, pankreasa, jetre, slezine i bubrega. Najčešće je slučajni nalaz ehosonografije trbuha ili CT pregleda koji su bili učinjeni iz drugih razloga.

Prikaz slučaja: U ovom slučaju, prikazuje se 85 godina star muškarac, pozitivan na Kovid 19, koji je bio hospitalizovan zbog radiografski opisane obostrane upale pluća. U sklopu evaluacije bolesti, učinjen je CT pregled grudnog koša na kom je opisana obostrana pneumonija, ali uzgredno i polisplenija, nekoliko abnormalnosti krvnih sudova, malformacije gastrointestinalnog trakta i agenezija dorzalnog pankreasa, te je postavljena dijagnoza sindroma polisplenije. Uprkos ageneziji dorzalnog pankreasa, ovaj pacijent nema dijabetes melitus, niti je ikada imao pankreatitis.

Zaključak: Ovaj prikaz slučaja ukazuje na to da navedene anomalije ne moraju značajno uticati na kvalitet života pacijenata, kao i da se može doživeti duboka starost. Ipak, neophodno je dijagnostikovati ovakvog pacijenta na vreme kako bi se izbegle komplikacije tokom eventualnih hirurških procedura i/ili intervencija.

Ključne reči: pankreas, polisplenija, sindrom

Primljen: 11.01.2024. I **Revizija:** 27.03.2024. I **Prihvaćen:** 27.03.2024.

Medicinska istaživanja 2024; 57(2):137-141

Medicinska istaživanja 2024; 57(2):143-147 | DOI 10.5937/medi57-48751

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

CASE REPORT

Bilaminar dermal regeneration template for the coverage of exposed dura in a patient irradiated for tinea capitis

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Summary

Introduction: Before the discovery of antimycotics powerful enough to penetrate the hair follicle, the use of X ray radiation was a worldwide accepted method for scalp epilation as part of the treatment for tinea capitis in children. More than five decades later, the consequences of radiation in ringworm therapy became evident and included high rates of recurrent and aggressive forms of non-melanoma skin tumors, brain tumors and meningiomas, as well as osteoradionecrosis. Scalp reconstruction presents a challenge due to the unique anatomic characteristics of the scalp as well as tissue inelasticity. Cases of bilaminar dermal regeneration templates applied directly on dura in the literature are seldom described.

Case report: A male patient irradiated due to tinea capitis as a child, presented at our institution for the management of multiple recurrent infiltrative basocellular carcinomas (BCC) and osteoradionecrosis of the scalp. Severe skin atrophy affected all areas of the scalp except for the forehead, which showed moderate atrophy. The patient had multiple surgeries over the next few years due to the emergence of new and recurrent BCCs as well as zones of osteoradionecrosis of the scalp. Post-surgical defects were commonly reconstructed using local fasciocutaneous flaps and skin autografts. Surgical site complications, including partial flap necrosis or autograft loss, frequently followed the procedures. After exhausting all reconstructive options by repeated previous surgeries, a post-surgical defect following ostectomy of newly developed ORN in the occipital region was reconstructed using a dermal substitute applied directly on the dura and covered with a skin autograft, in a two-step procedure. Due to a high perioperative risk for general anesthesia, the intervention was performed under local anesthesia with anesthesiologic monitoring. The postoperative period was uneventful and the skin autograft had a 100% take.

Conclusion: The use of bilaminar dermal regeneration template Integra® can be efficiently utilized for the reconstruction of full thickness skin and calvaria defects in complex cases when all other reconstructive methods are exhausted.

Key words: Tinea capitis, skin atrophy, osteoradionecrosis, basal cell carcinoma, bilaminar dermal regeneration template

Recived: 17 January 2024

Revised: 10 March 2024

Accepted: 10 March 2024

Check for updates

Funding information:
This research received no external funding.

Competing interests:
The authors have declared that no competing interests exist

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Cite this article as: Stojičić M, Jurišić M, Nikolić Živanović M, Jovanović M, Stojanović M, Jović M, Jeremić J, Marinković M. Bilaminar dermal regeneration template for the coverage of exposed dura in a patient irradiated for tinea capitis – a case report; Medicinska istaživanja 2024; 57(2):143-147 DOI: 10.5937/medi57-48751

INTRODUCTION

In 1950s, tinea capitis, commonly known as ringworm or favus, was one of the most common fungal diseases of childhood. Before the discovery of antimycotics powerful enough to penetrate the hair follicle, hair was physically removed by radiating the scalp before the application of topical antimycotic ointments (1–4). Epilation via X-ray radiation has been widely accepted as an efficient treatment approach (1–4).

More than five decades later, the consequences of radiation therapy for ringworm became evident. Malignancies of the head and neck, including basal cell carcinoma (BCC) and squamous cell carcinoma of the skin, thyroid gland tumors, brain tumors and meningiomas, as well as high rates of aseptic osteoradionecrosis (ORN), have been associated with childhood irradiation due to ringworm infection (4–6).

In irradiated patients, a dose-dependent association has been proposed, as well as a predisposition for the occurrence of multiple BCCs at a younger age compared to general population (5). Another major issue is the poor skin and underlying bone quality in terms of radiation induced atrophy, which requires significant consideration and surgical experience for the selection of the optimal method of tissue defect reconstruction after surgical tumor removal (7). In full thickness defects of the skin and scull bones with exposed dura, the use of bilaminar dermal template Integra® placed directly on the dura is rarely described in literature but it is an efficient method of reconstruction in the challenging event when all other options are exhausted.

Here we present our experience in reconstruction of a full thickness scalp defect with exposed dura due to aggressive recurrent bone invading BCCs as well as ORN, in a patient who was irradiated due to tinea capitis as a child.

CASE REPORT

A male patient, born in 1938, irradiated due to tinea capitis as a child, presented at our institution for the management of BCC of the scalp. The dose of irradiation was unknown due to a lack of official protocols at the time. In his medical history, two acute myocardial infarctions were noted and the patient was being treated for chronic obstructive pulmonary disease with corticosteroid therapy. Severe skin atrophy affected all areas of the scalp except for the forehead, which showed moderate atrophy.

The first occurrence of infiltrative subtype of BCC localized in the right temporal region was at the age of 57. The tumor was removed down to the periosteum, and the post-surgical defect was successfully covered by a partial-thickness skin autograft. Five years later, two infiltrative BCCs emerged in the parietal region. Due to the invasion of the periosteum, the tumor was excised along with the external tabula of the parietal bone below the tumor. After sufficient granulation tissue was obtained, the defect was restored with a partial-thickness skin autograft. One year after the procedure, a significant parietal bone exposure in the anterior segment was present, with only marginal exposure in the posterior segment. Tumor recurrence was histopathologically excluded, and the diagnosis of aseptic bone necrosis was confirmed **(Figure 1)**. The patient was lost to follow-up for several years due to his poor overall health.

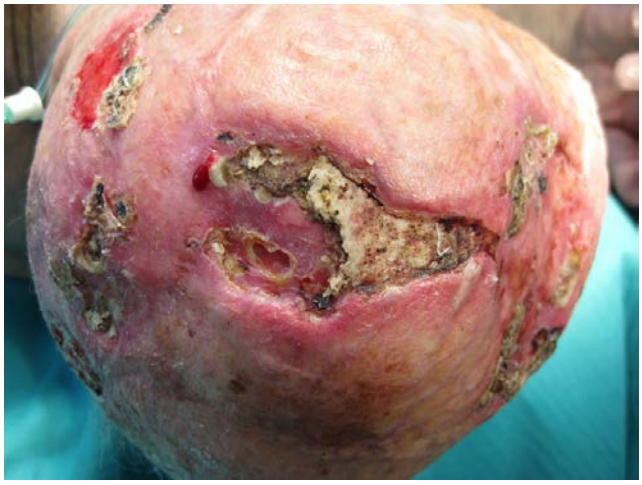

Figure 1. Patient presenting with significant bone exposure due to osteoradionecrosis.

When the patient returned for examination, BCC localized parietally, anterior to the previous tumor, was noticed as well as new zones of ORN. Due to bone invasion by the tumor, the tabula externa of the parietal bone was excised, and the defect was reconstructed with a local fasciocutaneous flap harvested from the forehead. The secondary defect from the flap donor site was covered using a Blair II skin autograft. The flap survived without complications. The aseptic bone necrosis was managed by ostectomy and reconstructed with a Blair I autograft placed on previously obtained granulation tissue. After 2 months all wounded surfaces epithelized, however, after 6 months new areas of boe exposure in terms of ORN were noted. Wound swabs showed normal bacterial skin flora. The patient was conservatively treated until the conditions for surgery were met, and a Thiersch skin autograft was used to cover the defect. Partial loss of the skin autograft was found postoperatively. Simultaneously, additional areas of ORN appeared in the occipital region.

Further occurrence of ORN in the occipital region was managed by an ostectomy of the necrotic bone with a subsequent exposure of dura. All reconstructive options were exhausted by repeated previous surgeries, and the patient's overall health limited complex reconstructive methods requiring general anesthesia. Thus, a post-surgical defect following ostectomy of newly developed ORN in the occipital region was reconstructed by placing a bil-

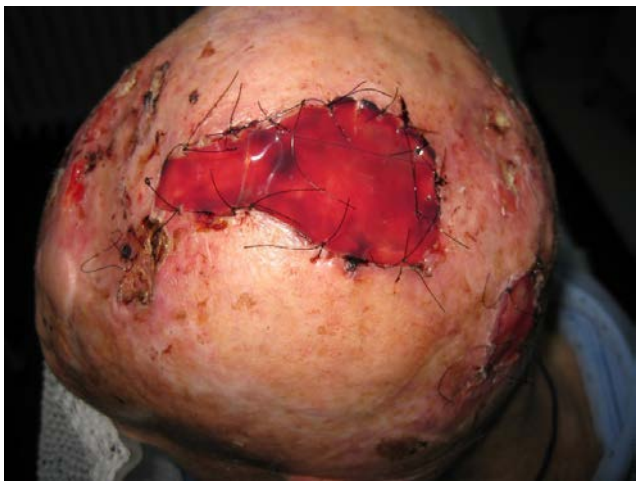

Figure 2. Integra® covering the defect after ostectomy. Severe skin atrophy is present in most regions of the scalp as well as previous surgeries, amplifying the obstacles limiting other reconstructive methods.

aminar dermal regeneration template Integra® directly on dura **(Figure 2)**. In collaboration with the anesthesiology department, the procedure was successfully done under local anesthesia with anesthesiologic monitoring. After 3 weeks, the dermal template was successfully covered with a Thiersch skin autograft **(Figure 3)**.

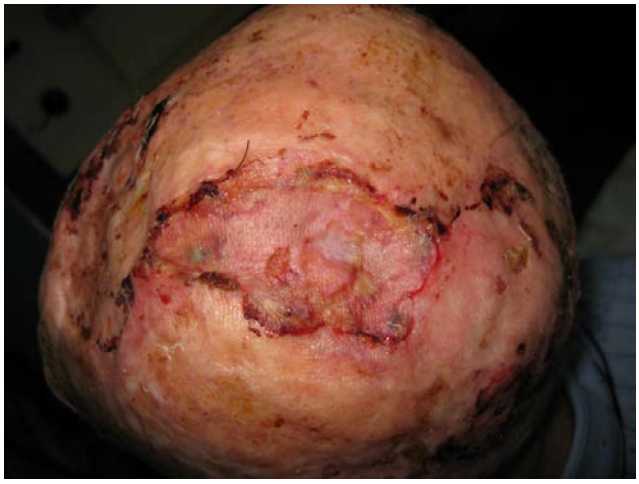

Figure 3. After 3 weeks, Integra® was covered with Thiersch skin autotransplants with a 100% successful take in further recovery.

After each surgery, all tumor samples were sent for histopathological verification with all reports verifying complete tumor removal. Additionally, all samples obtained from the surgeries of ORN were also sent for exclusion of tumor recurrence and verification of avascular necrosis.

In the following years, the patient occasionally presented with small wounds successfully managed conservatively, while BCC did not occur. The patient died due to cardiovascular disease at the age 80.

DISCUSSION

Full-thickness skin defects on irradiated scalp provide a particular challenge for both the reconstructive surgeon and the patient. These scenarios are made even more difficult when the defect involves the cranium, exposing the underlying dura. Before opting for the appropriate reconstructive approach for the patient, localization, tumor diameter, depth of invasion and type, risk of a recurrence, as well as overall patients' health should be thoroughly assessed (7,8). Radiation-damaged skin typically shows as moderate or severe skin atrophy, clinically manifesting as thinning of all skin layers, disappearance of skin appendages, hair follicles, sebaceous and sudoriferous glands, poor vascularization and insufficient oxygenation, with subsequent impairment of wound healing even after basic excisions (9–11). With this in mind, the method of defect reconstruction should be carefully chosen. Our patient's surgical therapy was followed by several complications at the surgical site, such as partial flap loss or partial skin autograft loss.

Dermal substitutes are described in literature as a suitable option for reconstruction of defects following burns, tumor resections, trauma, or radiation, particularly in full-thickness defects including underlying bony structures, when the use of a free flap is not possible (12–15). Reports of application of Integra® directly on dura is scarce in literature, still several authors promote its successful use in certain challenging cases (12,14–18). Leach et al. supported the use of Integra® for scalp skin and scull bone defects, even in cases of severe fibrotic dura (12). When compared to split-thickness skin grafting, the most evident advantage of dermal substitutes is its larger and more substantial coverage. Dermal substitutes combined with split-thickness skin grafting can offer suitable tissue coverage with better underlying structure protection and are more useful to skin grafting alone in situations where other reconstructive methods are not available (18). The two-stage process of Integra® placement allows for a controlled neodermis formation in a vascularly impaired setting such as in tinea capitis patients. Additionally, Integra® is found to tolerate well the adjuvant radiotherapy in oncologic patients (14).

Osteoradionecrosis is a severe complication of radiation therapy for head and neck cancer, or tinea capitis in this case (19,20). Radiation causes vascular damage in irradiated bones, resulting in hypoxia and tissue necrosis with clinical presentation ranging from minor, asymptomatic areas of exposed bone that heal with conservative care, to severe necrosis with pathologic fractures that require multiple surgical interventions and reconstruction, like in our patient (18–20). Multiple ORNs of the parietal and occipital bones presented an immense challenge for our patient making successful reconstruction difficult to accomplish. Severe skin atrophy affecting almost all areas of the scalp, multiple previous surgeries, as well as

the presence of multiple recurrent ORN limited the use of local and regional skin flaps, while tissue expanding in such patients is contraindicated. Additionally, an ultrasound Doppler examination demonstrated no blood vessels appropriate to serve as a recipient blood vessel for a microvascular flap. Given the challenges faced during the usage of local flaps, as well as the inability of applying distant flaps, we determined that the use of dermal substitutes would be an adequate choice.

Given the patient's poor general health due to cardiovascular disease and obstructive lung disease, which increased the patient's perioperative risk, high rate of tumor recurrence, and multiple avascular necroses, a two-stage bilaminar dermal template Integra® under local anesthesia was the reconstructive method of choice for full-thickness scalp and calvaria defect with exposed dura. The benefits of this procedure include lower perioperative risks related to general anesthesia duration, as well as reduced morbidity of donor areas. In our experience, this method of reconstruction of full-thickness scalp defects should be recommended particularly in patients who have undergone multiple surgeries and have exhausted all other reconstructive options (16,17,21). The downsides of using dermal substitutes covered by skin autografts over local flaps include decreased resistance, lack of skin appendages, and different skin color and texture. If calvaria bone reconstruction is planned in terms of artificial bone substitutes, dermal substitutes cannot be applied. Additionally, the disadvantage of this technique is the costly price of dermal substitutes such as bilaminar dermal regeneration template Integra® which remains a barrier to its common utilization.

CONCLUSION

The use of bilaminar dermal regeneration template Integra® can be efficiently utilized for reconstruction of scalp skin and calvaria defects in complex cases when all other reconstructive methods are exhausted.

Acknowledgments – None

Conflict of interest – None to declare

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UPOTREBA BILAMINARNIH ZAMENIKA DERMISA APLIKOVANIH DIREKTNO NA DURU KOD PACIJENTA KOJI JE ZRAČEN ZBOG TINEA CAPITISA – PRIKAZ SLUČAJA

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Sažetak

Uvod: Pre pronalaska antimikotika sposobnih da prodru do folikula dlake, tretman tinee capitis kod dece zasnivao se na radijacionoj epilaciji poglavine, a potom aplikaciji tada dostupnih antimikotika kao i cink paste. Tačna doza zračenja nije poznata, a posledice zračne terapije postale su vidljive nakon više od pet decenija u vidu povećane učestalosti nemelanocitnih tumora kože, tumora mozga i moždanih ovojnica kao i osteoradionekroze kod ovih pacijenata. Rekonstrukcija poglavine predstavlja izazov zbog anatomskih karakteristika regije kao i neelastičnosti tkiva poglavine. Slučajevi upotrebe bilaminarnih zamenika dermisa aplikovanih direktno na duru u literaturi retko su opisani.

Prikaz slučaja: Muškarac koji je zračen u detinjstvu zbog tinee capitis lečen je u našoj ustanovi zbog multiplih, rekurentnih bazocelularnih karcinoma kože (BCC) infiltrativnog tipa i multiplih zona osteoradionekroze na poglavini. Težak stepen atrofije kože bio je prisutan na skoro celoj poglavini izuzev na čelu gde je bila prisutna atrofija srednjeg stepena. Pacijent je u više navrata lečen u našoj ustanovi zbog de novo i recidiva BCC, novih kao i recidiva BCC, i multiplih zona osteoradionekroze na kostima poglavine. Defekti koji su nastajali nakon ekscizija lezija rekonstruisani su kombinovanjem lokalnih fasciokutanih režnjeva i kožnih autotransplantata. Učestalost komplikacija u vidu parcijalne nekroze flapa ili lize kožnih autotransplantata bila je relativno visoka. Nakon što su iscrpljene druge terapijske opcije, odlučeno je da se zaostali defekt pune debljine mekih tkiva i kosti nakon uklanjanja osteoradionekroze rekonstruiše bilaminarnim zamenikom dermisa koji je postavljen direktno na duru, a nakon tri nedelje pokrije kožnim autotransplantatima (u drugom aktu). Imajući u vidu značajne komorbiditete, operacija je izvedena u lokaloj anesteziji pod nadzorom anesteziologa. Postoperativni period je protekao bez komplikacija.

Zaključak: Upotreba bilaminarnih zamenika dermisa je efikasna metoda za rekonstrukciju defekata pune debljine poglavine i kosti lobanje, u slučajevima kada su druge opcije iscrpljene.

Ključne reči: Tinea capitis, atrofija kože, osteoradionekroza, bazocelularni karcinom kože, bilaminarni zamenici dermisa.

Primljen: 17.01.2024. I **Revizija:** 10.03.2024. I **Prihvaćen:** 10.03.2024.

Medicinska istaživanja 2024; 57(2):143-147

Medicinska istaživanja 2024; 57(2):149-161 | DOI 10.5937/medi57-49267

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Drug-drug interactions of the reserve antibiotics: a narrative review

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Received: 14 February 2024

Revised: 26 March 2024

Accepted: 26 March 2024

Check for updates

Funding information:
This paper has been supported by the Ministry of Education, Science and Technological Development of Serbia - grant Faculty of Medicine University of Belgrade.

Competing interests:
The authors have declared that no competing interests exist

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Summary

Drug interactions often cause side effects, especially in children, elderly and/or patients with chronic diseases. Antibiotics are among the most commonly used drugs, so potential impact of antibiotic-drug interactions on the ultimate outcome of therapy may be of great clinical value. Bearing in mind that antibiotic-drug interactions can lead to development of antimicrobial resistance (AMR), their identification is specifically important for reserve antibiotics. The aim of this narrative review is to analyze the drug-drug interaction potential of reserve antibiotics. The highest potential for antibiotic-drug interactions was identified with linezolid, colistin, dalfopristin/quinupristin, lefamulin and oritavancin. Special caution should be paid to concomitant administration of ceftazidime-avibactam, telavancin, colistin, polymyxin B, plazomicin with drugs that have nephrotoxic potential due to possibility of more severe renal impairment. Exceptional wariness is required when combining drugs with reserve antibiotics with limited drug-drug interactions information such as plazomicin, carumonam, iclaprim. Having in mind that antibiotic-drug interaction can lead to the changed antimicrobial efficiency and/or safety of the therapy, the antibiotic choice has to be based on data regarding interaction potential. Continuous education of clinical staff regarding the choice of antibiotics based on their interaction potential and optimizing the antibiotic dose may significantly improve pharmacotherapy and decrease the risk for AMR.

Keywords: antibiotic, drug-drug interactions, reserve antibiotics, AMR

Cite this article as: Božić Cvijan B, Labović M, Kukurić M, Bajčetić M. Drug-drug interactions of the reserve antibiotics: a narrative review Medicinska istaživanja 2024; 57(2):149-161 DOI: 10.5937/medi57-49267

INTRODUCTION

Drug-drug interactions (DDIs) are caused by the co-administration of multiple drugs and may lead to altered drug concentration, raising numerous questions regarding safety and effectiveness (1). DDIs are more often observed in vulnerable populations such as children, elderly and/or patients with chronic diseases due to polypharmacy, multi-comorbidities, frequent use of off-label drugs and special dosage regime (2-5).

Antibiotics are among the most commonly used drugs, so potential impact of antibiotic-drug interactions are of great clinical value (6, 7). Antibiotic interactions may be divided into those that affect the pharmacokinetic profile of the drug and those that affect the pharmacodynamic profile of the drug (8). Pharmacodynamic interactions can be classified into synergistic or antagonistic interactions, depending on whether the drug combination leads to increased or decreased antibiotic activity (8). So far, the most widely studied mechanism for pharmacokinetic antibiotic-drug interactions is the inhibition or induction of drug metabolizing enzymes. Besides the well- known role of cytochrome P450 (CYP450) in antibiotic-drug interaction, recently special caution has been given to the role of transmembrane proteins: P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporter (OAT) (8). Depending on which substrate antibiotics interact with, we can divide interactions into several groups: antibiotic-drug, antibiotic-redox active metal, antibiotic-food, or antibiotic-disease interactions (2, 6, 7). Bearing in mind increased antibiotic consumption in our country (9), more frequent antibiotic-drug interactions are expected. Furthermore, since antibiotic-drug interactions can lead to suboptimal antibiotic plasma concentration and to the development of antimicrobial resistance (AMR), their identification is of great value, especially for the group of reserve antibiotics (6, 7). In order to optimize antibiotic stewardship efforts at the local, national and international levels, the World Health Organization (WHO) Expert Committee developed the AWaRe Classification of antibiotics in 2017, which was later updated in 2021. According to the AWaRe Classification antibiotics are classified into three groups: Access, Watch and Reserve, based on their impact on antimicrobial resistance. The Access group includes antibiotics recommended as the first or second choice for infections caused by the most encountered susceptible pathogens, with lower resistance potential compared to the other two groups. The Watch group includes antibiotics that have higher resistance potential and are recommended for use only in a limited number of specific infectious syndromes. The Reserve group, so called "last resort", includes antibiotics that should be saved for the infections caused by multidrug-resistant microorganisms (10).

Recently published study showed a high proportion of multidrug-resistant strains of *Klebsiella pneumoniae* (*K. pneumoniae*) and *Escherichia coli* (*E. coli*) in the Republic of Serbia (RS) (9). These strains are mostly responsible for deaths attributable to AMR worldwide (9). In addition, research highlights the need for expanding the list of reserve group of antibiotics in RS. In January 2024, only 8 out of total 29 reserve antibiotics were registered in RS. Furthermore, only 5 reserve antibiotics listed on WHO Model List of Essential Medicines (EML) 2023 are registered in RS: ceftazidime/avibactam, ceftolozane/tazobactam, colistin, linezolid, meropenem/vaborbactam.

The aim of this narrative review is to analyze the drug interaction potential of the reserve group of antibiotics. By using information about potential antibiotic interactions in daily practice, we can prevent the ineffectiveness of therapy and side effects, and what is even more important, reduce the possibility of developing AMR for antibiotics that represent the last line defense for difficult-to-treat pathogens.

BETA-LACTAMS

Cephalosporins

Ceftazidime-avibactam is a combination of third-generation cephalosporin and novel, non-β-lactam β-lactamase inhibitor available in RS. It is approved for the treatment of adults with complicated urinary tract infections (cUTIs), complicated intra-abdominal infections (cIAIS), hospital-acquired pneumonia (HAP), and other infections caused by aerobic Gram-negative organisms in patients with limited treatment options (11). Avibactam does not inhibit P450 enzymes, while ceftazidime-avibactam does not inhibit the major renal or hepatic transporter nor has the potential to induce P450 enzymes (12). In *in vitro* settings, avibactam is a substrate for OAT1 and OAT3 transporters, therefore concomitant use of a potent inhibitor of OAT transporter, such as probenecid, may alter the elimination of avibactam (13). Concomitant use of ceftazidime-avibactam and drugs that have nephrotoxic potential may alter renal function (14). *In vitro,* chloramphenicol is an antagonist with ceftazidime-avibactam. Despite the absence of clinical relevance of these findings, the mentioned drug combination is not advised (12, 14). Synergistic interactions were observed when ceftazidime-avibactam was administered with colistin, tobramycin, tigecycline, aztreonam, meropenem, and imipenem (15, 16, 17). **(Table 1)**.

Ceftolozane-tazobactam

Ceftolozane-tazobactam is a combination of a novel semi-synthetic broad-spectrum fifth generation cephalosporin and well-known β-lactamase inhibitor tazobactam, available in RS (18). It is approved for treatment of cIAIs, acute pyelonephritis, cUTIs, HAP, including

Table 1. Potential drug-drug interactions involving novel antibiotics.

Drug A	Drug B	The effect
Ceftazidime-avibactam	Aminoglycosides, furosemide, high doses of cephalosporin Chloramphenicol Probenecid	Adverse effects on renal function Antagonists ↑ avibactam concentration
Ceftolozane-tazobactam	Probenecid	↑ tazobactam concentration
Cefiderocol	CYP3A4 substrates (hormonal contraceptives)	↓ systemic exposure of CYP3A4 substrates
Ceftobiprole-medocaril	Warfarin	↑ INR and PT
Meropenem-vaborbactam	Probenecid CYP2D6 substrates Valproic acid Warfarin	↑vaborbactam concentration ↓concentration of CYP2D6 substrates ↓ concentration of valproic acid ↑ anticoagulant effects
Imipenem/cilastatin-relebactam	Ganciclovir Valproic acid Warfarin	Generalized seizures ↓ concentration of valproic acid ↑ anticoagulant effects
Faropenem	Probenecid	Prolongs the exposure duration of faropenem
Aztreonam	Cefoxitin and Imipenem Ceftazidime-avibactam	Antagonists Synergism
Oritavancin	CYP2C9 and CYP2C19 substrates CYP3A4 and CYP2D6 substrates	↑ concentration of CYP2C9 and CYP2C19 substrates ↓ concentration of CYP3A4 and CYP2D6 substrates
Telavancin	Nephrotoxic agents Clotting tests	↑ Nephrotoxic potential ↑ INR and aPTT
Daptomycin	Ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin/clavulanate Oxacillin Vitamin E Drugs that reduce renal filtratiton (NSAID and COX2 inhibitors)	Synergistic interaction Synergistic interaction Synergistic interaction Synergistic interaction Immunomodulatory effect ↑concentrations of daptomycin
Fosfomycin IV	Linezolid Ciprofloxacin Ceftriaxone Metoclopramide	Synergistic interactions Synergistic interactions Synergistic interactions ↓ Fosfomycin IV
Dalfopristin/quinupristin	Cyclosporin CYP3A4 metabolized drugs	↑dalfopristin/quinupristin concentration ↑ plasma concentration that undergoesCYP3A4 metabolism (antihistamines, anti-HIV, antineoplastic drugs, benzodiazepines, calcium channel blockers, cholesterol-lowering agents, gastrointestinal tract motility agents, immunosuppressive agents and steroids)
Linezolid	Pseudoephedrine and phenylpropanolamine Serotonergic agents Rifampicin Warfarin	↑ blood pressure Serotonin syndrome ↓ linezolid concentration ↓ INR
Tedizolid	BCRP substrates	↑ concentration of BCRP substrates
Lefamulin	Amiodarone, Ia and III antiarrythimcs, antipsychotics, tricyclic antidepressants, fluoroquionolones, macrolides, verapamil, azoles, protease inhibitors Rifampicin, *Hypericum perforatum*, carbamazepine, phenytoin, primidone CYP3A inhibtors, P-gp inhibitors and CYP2C8 metabolized drugs	↑ QT interval ↓ Lefamulin concentration ↑ Lefamulin concentration
Minocycline-IV	Antacids Isotretinoin Ergot alkaloids Anticoagulants Diuretics and oral contraceptives	↓Absoroption of tetracycline Pseudotumor cerebri Acute limb ischemia and ergotism ↓ PT ↑ side effects
Tigecycline	Calcineurin inhibitors Amikacin, ampicillin/sulbactam, piperacillin/tazobactam, minocycline, rifampicin	↑ concentration of calcineurin inhibitors Synergistic interactions
Eravacycline	CYP3A4 inducers	↓ eravacycline concentration
Colistin	Non-depolarising muscle relaxants Neurotoxic agents Nephrotoxic agents Aminoglycosides, fluoroquinolones	Prolonged effects of muscle relaxants ↑ neurotoxic potential ↑ nephrotoxic effects Deterioration of Myasthenia Gravis
Polymyxin-B	Nephrotoxic agents	↑ Nephrotoxic potential
Plazomicin	Nephrotoxic agents	↑ Nephrotoxic potential

↑ - increased; ↓ - decreased; CYP – cytochrome P450; P-gp – P-glycoprotein; INR - international normalized ratio; BCRP - breast cancer resistance protein; PT- prothrombin time; aPTT – activated partial thromboplastin time;

ventilator-associated pneumonia (VAP) (19). Based on *in vitro* and *in vivo* published studies, no interactions were observed with substrates, inhibitors, and inducers of CYP450 enzymes when ceftolozane-tazobactam was administered in therapeutic doses (19). Similar to avibactam, tazobactam is a substrate for OAT1 and OAT3 and concomitant use with a potent OAT inhibitor may increase tazobactam plasma concentrations (19). So far, synergistic, or additive interactions have been observed between ceftolozane-tazobactam and fosfomycin, aztreonam, amikacin, tigecycline, colistin, and meropenem (20-22).

Ceftaroline fosamil

Ceftaroline fosamil is a "fifth-generation" cephalosporin (23). It is indicated in the treatment of complicated skin and soft tissue infections (cSSTIs) and community-acquired pneumonia (CAP) (24). *In vitro* studies showed that ceftaroline is not a substrate, inhibitor, or inducer of major CYP450 enzymes (24). Furthermore, population pharmacokinetic analysis demonstrated no interactions between ceftaroline and drugs that are inhibitors, inducers, or substrates of the cytochrome P450 system (24). As clinical studies regarding ceftaroline fosamil's interaction potential are not completed yet, the drug should be used cautiously.

Cefiderocol

Cefiderocol (S-649266), a novel cephalosporin, stands out due to its unique chemical structure featuring a chlorocatechol ring that enables it to penetrate bacteria through iron channels and is indicated for the treatment of infections caused by Gram-negative organisms in adults with limited treatment options (25). Adding clinically available serine β-lactamase inhibitors to cefiderocol might represent a significant formulation development to broaden its spectrum and therapeutic effectiveness while curbing *in vivo* resistance emergence (26). The combination of cefiderocol with β-lactamase inhibitors, especially avibactam, has a synergistic effect against resistant *Acinetobacter baumannii (A. baumanii)*. Similarly, *in vitro* synergy has been observed when cefiderocol is combined with meropenem, amikacin, tigecycline, and minocycline (27). Notably, cefiderocol has no clinically significant interference with various anion and cation organic transporters such as OAT, organic cation transporters (OCT), multidrug and toxic extrusion (MATE), organic anion transporting polypeptides (OATP) and BCRP. *In vitro*, cefiderocol induces CYP3A4 activity and to a lesser extent, CYP2C and P-gp (27). The metabolism of co-administered medicines that are substrates of CYP3A4 is increased, leading to decreased systemic exposure of these drugs, as is the case with hormonal contraceptives (27). The clinical relevance of cefiderocol's induction of CYP2C and P-gp is unknown.

Ceftobiprole-medocaril

Ceftobiprole-medocaril represents an innovative parenteral cephalosporin belonging to the fifth generation. It is indicated for the treatment of HAP (excluding VAP), and CAP (28). It is the water-soluble prodrug of ceftobiprole (28). Ceftobiprole is neither a substrate nor an inhibitor of P-gp (29). The drug undergoes minimal metabolism, does not induce CYP isoenzymes and its supratherapeutic concentrations minimally inhibits CYP isoenzymes such as CYP1A2, CYP2B6, CYP2C19, CYP3A4/5 (29). Ceftobiprole-medocaril is primarily eliminated through glomerular filtration and it seems that it is not eliminated via active tubular secretion (29). As a result, no anticipated interactions are foreseen in the renal excretion of the medication (29). There is an interaction between ceftobiprole-medocaril and warfarin, which is reflected in an increased prothrombin time and an International Normalized Ratio (INR) (30).

CARBAPENEMS

Two approved carbapenem-β-lactamase inhibitor combinations by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) are meropenem-vaborbactam and imipenem-cilastatin/relebactam (31). Meropenem-vaborbactam is among five reserve antibiotics listed on WHO Model EML registered in RS. It is indicated for the treatment of cUTIs, including pyelonephritis, cIAIs, HAP, including VAP (32). It is also indicated for the therapy of infections due to aerobic Gram-negative organisms in adults with limited therapeutic options (32). *In vitro* data demonstrated the potential of meropenem-vaborbactam to induce CYP1A2, CYP3A4, and potentially other pregnane X receptor (PXR)-regulated enzymes and transporters (32). Concomitant use of meropenem-vaborbactam and medications that are predominantly metabolised by mentioned enzymes may lead to decreased plasma concentration of the co-administered medical products (32). Meropenem-vaborbactam is a substrate of OAT3 and co-administration of OAT3 inhibitors, such as probenecid, may increase antibiotic plasma concentration (33).

Imipenem/cilastatin-relebactam is approved for the treatment of HAP, including VAP, bacteremia suspected or in association with HAP or VAP, infections due to aerobic Gram-negative organisms in adults with limited treatment options (34). No clinically significant interactions between imipenem/cilastatin-relebactam and OAT inhibitors were observed (35). Concomitant administration of carbapenems and valproic acid is associated with reductions in valproic acid concentrations, and increased risk of breakthrough seizures (36). In addition, generalized seizures have been reported in patients receiving imipenem/cilastatin and ganciclovir (35). Simultaneous

administration of antibacterial agents, such as meropenem-vaborbactam or imipenem-cilastatin/relebactam, with warfarin may lead to increased anticoagulant effects, therefore it is recommended to monitor INR during and shortly after co-administration of antibacterials with warfarin (32, 34). Meropenem-vaborbactam may decrease the efficacy of hormonal contraceptives containing estrogen and/or progesterone, and alternative contraceptive methods are generally advised during therapy with broad-spectrum antibiotics (32).

PENEMS

Faropenem represents an innovative penem antibiotic, designed for oral administration (37). Faropenem is used for the treatment of urinary tract infections (UTIs), respiratory tract infections, skin and skin structure infections, and gynecological infections (38). Its elimination primarily occurs through renal excretion (37). Several research investigations have pointed out that inorganic phosphate transporter 1(NPT1), a transport protein localized in apical membrane of proximal tubular cells, is involved in the active secretion of faropenem (39). There is a possibility of drug interaction when co-administrated with other drugs, such as probenecid (37,39). Probenecid prolongs the exposure duration of faropenem through inhibition of its renal secretion (37, 39).

MONOBACTAMS

Aztreonam

Aztreonam is a monobactam antibiotic. It is indicated for the treatment of UTIs, gonorrhoea, lower respiratory tract infections, bacteraemia/septicaemia, bone and joint infections, gynecological infections (40). Aztreonam elimination is mainly through the kidneys, involving active tubular secretion. Additionally, aztreonam undergoes hepatic metabolism and biliary secretion. Therefore, caution is advised during its administration to patients with renal or hepatic diseases, while dose adjustment is needed in patients with severe renal impairment (40). The pharmacokinetic study showed no major interactions between aztreonam and several antibiotics, such as cephradine, clindamycin, gentamicin, metronidazole or nafcillin (41). On the other hand, antagonism between aztreonam and cefoxitin or imipenem has been described (40). A recently published study described a synergistic interaction between aztreonam and ceftazidime-avibactam. This combination could be an effective strategy for treating infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE), especially in those with high-level resistance levels against carbapenems and/or ceftazidime-avibactam (42). No clinically significant interactions have been observed during concomitant administration of probenecid or furosemide with aztreonam (40). Continuous monitoring and dose adjustment is necessary during concomitant administration of anticoagulants and aztreonam (40).

Carumonam

Carumonam is a new monocyclic beta-lactam antibiotic. It is indicated in the treatment of Gram-negative UTIs. So far, data of interaction potential are scarce. Since the pharmacokinetics of carumonam depend on renal function, in patients with reduced renal function dose adjustment is needed (43).

LIPOGLYCOPEPTIDES

Oritavancin

Oritavancin is a new lipoglycopeptide, approved for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) (8). Oritavancin is a weak inhibitor of CYP2C9 and CYP2C19, and an inducer of CYP3A4 and CYP2D6. Coadministration of oritavancin leads to increased plasma concentration of CYP2C9 (warfarin) and CYP2C19 (omeprazole) substrates and to decreased plasma concentration of CYP3A4 (midazolam) and CYP2D6 (dextromethorphan) substrates (8). Based on this information, caution should be used when administering oritavancin and drugs with a narrow therapeutic index that are metabolized by one of the affected CYP450 enzymes. In addition, oritavancin also has drug-laboratory interactions and may produce falsely elevated results, for example, prothrombin time, INR, activated partial thromboplastin time, and activated clotting time. Therefore, the anticoagulation effect of heparin and warfarin is hard to predict, and alternative anticoagulants should be considered (44).

Dalbavancin

Dalbavancin is also a new representative of lipoglycopeptides, approved for ABSSSIs. Dalbavancin is not metabolized by CYP enzymes, therefore co-administration of CYP substrates, inducers, or inhibitors has no clinically significant effects (8, 44). It is not known if dalbavancin is an inhibitor of transporters or a substrate for hepatic and efflux transporters (8). So far, increased dalbavancin concentration in cases of co-administration with transport inhibitors (verapamil, itraconazole, protease inhibitors) cannot be ruled out. Similarly, increased exposure to transport substrates (statins, digoxin) may be observed after co-administration with dalbavancin (45).

Telavancin

Telavancin is a semisynthetic lipoglycopeptide derivative of vancomycin, approved for treatment of ABSSSIs caused by Gram positive organisms (46). Notably, telavancin is nephrotoxic drug, predominantly eliminated by the kidneys, heightening the risk of interactions with other nephrotoxic drugs and potential accumulation in individuals with renal impairment. It is extensively bound to plasma proteins, so it is possible that DDIs may occur due to the displacement of other highly plasma protein-bound drugs. Additionally, telavancin has been reported to interfere with clotting tests (47). Co-administration of telavancin with aztreonam and piperacillin/tazobactam has shown no observed interactions, indicating that they can be safely used together. Furthermore, CYP450 inducers or inhibitors do not appear to significantly affect telavancin's metabolism (46).

CYCLIC-LIPOPEPTIDE

Daptomycin

Daptomycin is a novel cyclic lipopeptide antibiotic. The co-administration of daptomycin and rifampicin could be a valuable alternative in the treatment of vancomycin-resistant *Enterococcus* (VRE) infections (48). It is approved for the treatment of cSSTIs, infective endocarditis and bacteraemia due to *Staphylococcus aureus* (*S. aureus*) (49). Synergistic interaction is seen with ampicillin/sulbactam, piperacillin/tazobactam, ticarcillin/clavulanate and oxacillin (50). Study represented that daptomycin has a minimal immunomodulatory effect on natural-killer (NK) cells, and synergistic interaction with other immunomodulators such as vitamin E (51). Daptomycin is neither inhibitor nor inducer of CYP450 enzymes, so interactions with drugs metabolized by these enzymes are not expected. No clinically significant interactions were observed between daptomycin and aztreonam, tobramycin nor probenecid. Caution and frequent monitoring are advised during concomitant administration of warfarin and β-hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (50). Daptomycin is primarily cleared by renal filtration, so its increased plasma concentration is observed during concomitant administration with drugs that reduce renal filtration such as non-steroidal anti-inflammatory drugs (NSAID) and cyclooxygenase (COX) 2 inhibitors (50).

PHOSPHONIC ANTIBIOTIC

Fosfomycin IV

Fosfomycin is a bactericidal antibiotic. It is indicated as a therapy for osteomyelitis, cUTIs, nosocomial lower respiratory tract infections, bacterial meningitis, bacteremia and shows qualities in combination with other antibiotics (52). Synergistic interactions were observed during concomitant administration of fosfomycin with linezolid (53), ciprofloxacin (54) and ceftriaxone (55). Potential synergistic activity may occur with daptomycin against methicillin-resistant *S.aureus* (MRSA) infections (56). Clinically significant decreased fosfomycin concentration was observed during concomitant administration of metoclopramide and food (56).

STREPTOGRAMINS

Dalfopristin/quinupristin

Dalfopristin/quinupristin is a broad-spectrum Gram-positive antibacterial belonging to streptogramin class (57). Dalfopristin/quinupristinis indicated for the treatment of severe or life-threatening infections of *staphylococci* or vancomycin-resistant*Enterococcus faecium* (VREF), and skin infections caused by methicillin susceptible *S. aureus* or *Streptococcus pyogenes* (57). It is excreted mainly through feces and lesser through renal excretion as metabolites (57). Dalfopristin/quinupristin inhibits CYP3A4, leading to potential drug interactions (58). Its co-administration with cyclosporine increases dalfopristin/quinupristin plasma concentration (58). It is advisable to closely monitor patients taking drugs metabolized by CYP3A4 concomitantly with dalfopristin/quinupristin (58). *In vitro* studies have shown quinupristin/dalfopristin inhibits the CYP3A4 metabolism of cyclosporin, nifedipine, tamoxifen, midazolam, docetaxel and terfenadine (59). Increased plasma concentration of drugs such as antihistamines, anti-human immunodeficiency virus (HIV) drugs, antineoplastic agents, benzodiazepines, calcium channel blockers, cholesterol-lowering agents, gastrointestinal tract motility agents, immunosuppressive agents, and steroids were observed during concomitant administration with dalfopristin/quinupristin (59).

OXAZOLIDINONE ANTIBIOTICS

Linezolid

Linezolid, an antibiotic belonging to the oxazolidinone class, is registered in RS. Linezolid is indicated for the treatment of VREF infections, complicated and uncomplicated skin and skin structure infections, CAP and nosocomial pneumonia (60). It is a potent OAT1 and OAT3 inhibitor (61). Unlike many other drugs, linezolid does not interfere with the CYP450 system which means that linezolid does not have any interactions with drugs that are metabolized by the CYP system (60). However, recent research has discovered that linezolid's metabolism

can be influenced by two enzymes, cytochrome P450 2J2 and cytochrome P450 4F2, which are not typically associated with drug metabolism (62). Linezolid can be safely combined with aztreonam, ceftazidime, ciprofloxacin, meropenem, gentamicin, amphotericin B, azoles and antiviral drugs (63). However, the *in vitro* study published in 2015 indicated that the bactericidal potential of meropenem was hindered in co-administration with linezolid, diminishing the combined impact of the two drugs on the linezolid-induced bacteriostasis. The authors postulated that as linezolid inhibits protein synthesis and meropenem acts against actively replicating bacteria, the former might halt bacterial growth and thereby neutralize the effect of the latter (64). These findings need further validation, considering that the meropenem–linezolid combination is often employed as empirical antibiotic therapy in healthcare-associated infections. Another *in vitro* study that examined various antibiotic combinations against human macrophage cell lines infected with *Mycobacterium tuberculosis (M. tuberculosis)* demonstrated an antagonistic interaction between linezolid and moxifloxacin. The reasons behind this phenomenon remained unclear (65). Linezolid's blood concentrations are diminished when used alongside rifampicin, and this has garnered increased attention lately due to the risk of antibiotic-resistant bacteria (66, 67). Results from an in *vitro* study showed that linezolid antagonized vancomycin and daptomycin activity (68). A pharmacokinetic interaction study pointed to increased linezolid concentration after co-administration of clarithromycin (69). Linezolid has lipophilic features and excellent tissue penetration, especially into the central nervous system, where it has inhibitory effects on monoamino oxidase (MAO). Therefore, the co-administration of linezolid with drugs that may put patients at risk from MAO inhibition should be closely monitored. Having in mind that linezolid use with serotonergic drugs may lead to serotonergic syndrome, their co-administration is contraindicated (70). Linezolid also interacts with warfarin, leading to decreased INR values (71). Co-administration of linezolid with pseudoephedrine or phenylpropanolamine increases blood pressure (72).

Tedizolid

The history of oxazolidinones provides a vivid illustration of the growing challenges in developing new antimicrobials. Tedizolid phosphate, also known as tedizolid, has joined the ranks of a few antimicrobials that have successfully navigated preclinical and clinical assessments to gain approval in treatment of acute skin and skin structure infections in recent years (73). Tedizolid undergoes minimal hepatic oxidation, and it exerts no detectable influence on the metabolism of CYP450 enzyme substrates, either through inhibition or induction. Moreover, tedizolid did not demonstrate significant hindrance to drug uptake transporters such as OAT1, OAT3, OATP1B1, OATP1B3, OCT1, and OCT2 (74). Orally administered tedizolid inhibits BCRP at the intestine level, which may lead to increased concentration of BCRP substrates such as rosuvastatin, imatinib, lapatinib, methotrexate, pitavastatin, sulfasalazine, and topotecan (75). Similar to linezolid, tedizolid is an inhibitor of MAO, however, no clinically significant interactions were observed when co-administered with pseudoephedrine (75). So far, the potential for serotonergic interactions has not been studied.

PLEUROMUTILIN

Lefamulin

Lefamulin is the inaugural systemic antibiotic in the pleuromutilin class (76). Lefamulin exhibits activity against gram-positive and atypical organisms commonly associated with CAP (76, 77). Lefamulin is both a substrate and inhibitor of CYP3A and a substrate of P-glycoprotein *in vitro*(76) and it acts as an inhibitor of OATP1B1, OATP1B3, BCRP, OCT1, and MATE1 transporters. Concomitant administration of lefamulin with amiodarone, Ia and III class antiarrhythmics, antipsychotics, tricyclic antidepressants, fluoroquinolones, macrolides, verapamil, azoles, protease inhibitors and CYP3A4 substrates that prolong the QT interval, is not recommended and should be avoided (78). Concomitant use of lefamulin with moderate or strong CYP3A inducers such as rifampicin, *Hypericum perforatum*, carbamazepine, phenytoin, primidone, can significantly decrease lefamulin plasma concentration (79) and lead to subtherapeutic concentration. On the other hand, concomitant use of strong CYP3a and P-gp inhibitors may lead to increased lefamulin plasma concentration. Therefore, these drug combinations are contraindicated. Co-administration of oral lefamulin with agents metabolised by CYP3A such as alprazolam, alfentanil, ibrutinib, lovastatin, simvastatin, triazolam, vardenafil, and verapamil may result in increased plasma concentrations of these medicinal products (79). Concomitant use of lefamulin with drugs metabolized by CYP2C8 may lead to increased plasma concentration of these medicines (79).

TETRACYCLINE ANTIBIOTICS

Minocycline-IV

Intravenous minocycline, a second-generation semi-synthetic tetracycline, is approved for use in patients with infections due to susceptible strains of Gram-positive and Gram-negative pathogens, such as *A. baumanii* (80). It's important to note that antacids may reduce tetracycline absorption, and concomitant use of isotretinoin and

minocycline should be avoided due to an increased risk of pseudotumor cerebri (80). Additionally, combining tetracycline derivatives with ergot alkaloids heightens the risk of ergotism and acute limb ischemia (81). Concomitant administration of minocycline with penicillin should be avoided. Having in mind that plasma prothrombin activity is depressed by tetracyclines, it is necessary to reduce the dose of concomitant anticoagulants (81). Special caution is needed when minocycline is administered with diuretics or oral contraceptives due to the possibility to aggravate side effects (81).

Omadacycline

Omadacycline is an aminomethylcycline antibiotic designed for the treatment of ABSSSIs and CAP with antibacterial activity against a range of bacteria, including Gram-positive and Gram-negative aerobes, anaerobes, and atypical bacteria (82). In line with other members of the tetracycline family, omadacycline forms non-absorbable chelation complexes by binding with cations (82, 83). It was demonstrated that omadacycline does not act as a substrate, inducer, or inhibitor of the major CYP enzymes (84). Limited data suggest that certain tetracycline antibiotics, including omadacycline, might interfere with warfarin. Therefore, it is recommended to monitor the coagulation profile of patients concomitantly using these medications (82).

Tigecycline

Tigecycline is the first glycylcycline and the first new tetracycline analog marketed after a period of over 40 years (85). Tigecycline is approved for the treatment of cSSTIs with the exclusion of diabetes foot infections, cIAIs, and CAP, while recent studies indicate it may be effective in the treatment of severe *Clostridioides difficille* infection (85). Tigecycline does not inhibit or induce the hepatic CYP450 enzyme system, and it is unlikely to modify the metabolism of drugs metabolized by this system (86). Similarly, substances that induce or inhibit these enzymes are not expected to alter tigecycline clearance. However, tigecycline should be used cautiously in patients with diabetes and patients on oral contraceptive therapy (86). Pharmacokinetics interaction between tigecycline and calcineurin inhibitors, such as cyclosporin A, leads to increased concentration of immunosuppressive agents (87). Co-administration of tigecycline with other antibiotics exhibited either synergy or no interaction effects against the panel of Gram-negative bacteria. No antagonism was observed when tigecycline was co-administrated with piperacillin/tazobactam, ampicillin, amikacin, vancomycin, rifampicin, colistin, polymyxin B, sulbactam, imipenem, ciprofloxacin and levofloxacin. Synergistic interactions were noted with amikacin, ampicillin/sulbactam, piperacillin/tazobactam, minocycline and rifampicin (88). Tigecycline is a substrate for P-gp. Co-administration with P-gp inhibitors, such as cyclosporine and ketoconazole, or P-gp inducers, such as rifampicin, may impact tigecycline's pharmacokinetics (89).

Eravacycline

Eravacycline, a synthetic fluorocycline, shares similarities with tigecycline in terms of mechanism of action, structure and antibacterial spectrum (90). It is approved in several countries for the treatment of cIAIs for adult patients (91). Co-administration of eravacycline and CYP3A4 inducers such as rifampin, phenobarbital, carbamazepine, and phenytoin, led to a reduction in total eravacycline exposure. This data indicates that the dose of eravacycline should be increased when administered concomitantly with potent CYP inducers. On the other hand, the described increased concentration of eravacycline when administered with CYP inhibitors, such as itraconazole, is still clinically unclear (92).

TRIMETHOPRIM-DERIVATIVES

Iclaprim

Iclaprim is a potent and selective inhibitor of bacterial dihydrofolate reductase, and it has exhibited both *in vitro* and *in vivo* efficacy against a range of Gram-positive pathogens, including MRSA, beta-hemolytic *Streptococci* as well as Gram-negative respiratory pathogens (93, 94). Iclaprim is metabolized by CYP3A4 and CYP2C19 enzymes. The interaction potential of iclaprim is not fully investigated. It is not an inhibitor nor inducer of CYP3A4 or CYP2C19 enzymes, suggesting a low potential for clinically significant drug-drug interactions involving these enzymes (93, 94). On the other hand, iclaprim inhibits renal transporters such as OCT1, OCT2 and MATE2-K (93-95).

POLYMYXINS

Colistin

Colistin (polymyxin E) is a lipopeptide antibiotic, registered in RS. It is indicated for the therapy of serious infections caused by aerobic Gram-negative bacteria such as, *Pseudomonas aeruginosa* (*P. aeruginosa*). *A. baumannii*, *K. pneumoniae*, CRE, in patients with limited treatment options (96). *In vitro* studies showed that colistin did not induce the CYP enzymes (97). Co-administration of colistin with other medicines that are potentially neurotoxic (including non-depolarizing neuromuscular blocking agents, aminoglycosides) or nephrotoxic (vancomycin, aminoglycosides, furosemide, calcineurin inhibitors, cephalosporins) should be avoided due to possibility of

summative toxicity (98). *In vitro,* studies have shown that co-administration of colistin and drugs such as macrolides (azithromycin, clarithromycin) and fluoroquinolones (norfloxacin, ciprofloxacin) should be avoided in patients with myasthenia gravis (99). Colistin use may lead to muscle weakness due to reduced acetylcholine release or due to postsynaptic blockade of the acetylcholine receptor (100). Considering colistin's ability to cause muscle toxicity, its co-administration with non-depolarizing muscle relaxants should be used with great caution (98). Several studies have analyzed the synergistic impact of colistin in combination with other antibiotics for the treatment of infectious diseases. For example, colistin and azithromycin have been found to synergistically eliminate Gram-negative bacteria. Simultaneous administration of both drugs could potentially enhance neutrophils' bactericidal capabilities (101). Combinations of colistin with rifampicin and azithromycin have been shown *in vitro* to provide a more potent therapeutic regimen than monotherapy or double combinations against *E. coli* (102). Research has indicated no evidence of antagonism between colistin and antimicrobial agents like gentamicin, ciprofloxacin, piperacillin, and meropenem. Synergy was observed when combining colistin with ceftazidime or aztreonam against *P. aeruginosa* (103). In the context of CRE, combining colistin with another effective *in vitro* antibiotic has proven beneficial. This combination has shown a significant reduction in mortality, particularly in patients with septic shock, high mortality scores, or rapidly fatal underlying conditions (104).

Polymyxin-B

Polymyxin-B, an antibiotic belonging to the polymyxin class, exhibits potent activity against multidrug-resistant Gram-negative bacteria (105). Limited data are available on the specific drug interactions of polymyxin B (105, 106). Coadministration of polymyxin-B with nephrotoxic agents such as aminoglycosides or vancomycin may lead to additive toxicity. Concomitant use of polymyxin B with medications that may potentiate neurotoxicity, such as neuromuscular blocking agents or certain anesthetics, should be used with caution (106).

AMINOGLYCOSIDES

Plazomicin

Plazomicin is a semisynthetic aminoglycoside, with enhanced *in vitro* activity against *Enterobacteriaceae,* encompassing extended-spectrum β-lactamase-producing and carbapenem-resistant isolates (107, 108). It gained approval for the treatment of cUTI, including pyelonephritis caused by susceptible bacteria *E. coli*, *K. pneumoniae*, *Proteus mirabilis* (*P. mirabilis*), *Enterobacter cloacae* (*E. cloacae*) in adult patients (107). Limited information is available on plazomicin's drug interactions with specific agents. Notably, plazomicin is primarily excreted by the kidneys, and there is no evidence of drug metabolism in plasma, liver microsomes, or hepatocytes. *In vitro* studies reveal that plazomicin neither inhibits nor induces the CYP enzymes and is not a substrate for P-gp or BCRP transporters. The concomitant administration of plazomicin with other nephrotoxins may result in additive toxicity (107).

To the best of our knowledge this is the first study that provides prescribers with information about DDIs of reserve antibiotics. Regarding severity level DDIs may be divided into minor, moderate, major or unknown. It is important to emphasize that most DDIs are minor but still can result in health problems and lead to economic burden. The most important sources of information for DDIs are Summary of Product Characteristics (SmPC), DDIs clinical studies, as well as web databases (lexicomp.com, rxlist.com, drugbank.com, medscape.com, drugs.com). Providing prescribers with information regarding antibiotic-drug interactions and education on how to obtain it and how to act on it may be of great impact in the era of combating AMR.

CONCLUSION

The drugs that have high potential for drug-drug interactions are linezolid, colistin, dalfopristin/quinuprstin, lefamulin and oritavancin, all belonging to the reserve group of antibiotics. Special attention should be paid to concomitant use of ceftazidime-avibactam, telavancin, colistin, polymyxin B, plazomicin and drugs that have nephrotoxic potential due to possibility to cause more severe renal impairment. In cases of the reserve antibiotics with limited information regarding interaction potential such as plazomicin, carumonam, iclaprim, further DDIs studies as well as the updating data in the SmPC are needed. Educating clinical staff and supporting the role of clinical pharmacologists regarding the choice of antibiotics based on their interaction potential and optimizing the antibiotic dose may significantly improve the safety and efficacy of pharmacotherapy which is especially important for infections caused by bacterial strains mostly responsible for deaths attributable to AMR.

ACKNOWLEDGMENTS

This paper has been supported by the Ministry of Education, Science and Technological Development of Serbia - grant Faculty of Medicine University of Belgrade.

CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

Design of the work, B.B.C. and M.B.; preparing the draft of the manuscript and literature review, B.B.C., M.L, M.K.; revision of the manuscript M.B. All authors have read and agreed to the published version of the manuscript.

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UPOTREBA BILAMINARNIH ZAMENIKA DERMISA APLIKOVANIH DIREKTNO NA DURU KOD PACIJENTA KOJI JE ZRAČEN ZBOG TINEA CAPITISA – PRIKAZ SLUČAJA

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Sažetak

Interakcije lekova su veoma česti razlog za pojavu neželjenih efekata naročito kod dece, starijihi/ili pacijenata sa hroničnim oboljenjima. S obzirom da su antibiotici najčešće korišćeni lekovi potencijalni uticaj interakcija antibiotika sa drugim lekovima na krajnji ishod terapije može biti od kliničkog značaja. Osim toga, interakcije antibiotika sa drugim lekovima mogu uticati i na razvoj antimikrobne rezistencije (AMR) pa je njihovo prepoznavanje od velikog značaja naročito za rezervne antibiotike. Cilj našeg pregleda je analiza potencijalnih interakcija rezervnih antibiotika. Najveći potencijal za stupanje u interakcije imaju linezolid, kolistin, dalfopristin/kvinupristin, lefamulin i oritavancin. Poseban oprez je potreban prilikom istovremene primene antibiotika: ceftazidim-avibaktama, telavancina, kolistina, polimiksina B i plazomicina sa lekovima koji mogu oštetiti bubrežnu funkciju i posledično potencirati nefrotoksične efekte. Takođe, neophodno je obratiti pažnju na rezervne antibiotike za koje nemamo dovoljno informacija o potencijalu za stupanje u interakcije: plazomicin, karumonam, iklaprim. Imajući u vidu da antibiotik-lek interakcije mogu dovesti do izmenjene antimikrobne efikasnosti i/ili bezbednosti, izbor antibiotika mora biti zasnovan na podacima o potencijalnim interakcijama. Kontinuirana edukacija lekara o interakcijama i pravilnom doziranju antibiotika može značajno unaprediti farmakoterapiju i smanjiti rizik za razvoj AMR.

Ključne reči: antibiotik, lek-lek interakcije, rezervni antibiotici, AMR

Primljen: 14.02.2024. I **Revizija:** 26.03.2024. I **Prihvaćen:** 26.03.2024.

Medicinska istaživanja 2024; 57(2):149-161

Medicinska istaživanja 2024; 57(2):163-171 | DOI 10.5937/medi57-49676

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

Type 1 diabetes: prevention and screening in focus

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Received: 06 March 2024

Revised: 17 April 2024

Accepted: 22 April 2024

Check for updates

Funding information:
This paper has been supported by the Ministry of Science, Technological Development and Innovations Republic of Serbia - grant Faculty of Medicine University of Belgrade.

Competing interests:
The authors have declared that no competing interests exist

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Summary

It has been reported that the prevalence of type 1 diabetes (T1D) is increasing worldwide. Moreover, patients with T1D have a significant reduction in life expectancy, compared to their nondiabetic peers. In that context, prevention of T1D is a burning issue, having in mind multiple unsuccessful attempts in the past 50 years. However, recently there has been a turning point in this investigational area when it was shown that it is possible to delay T1D with immunotherapy in people with a high risk for T1D, in stage 2 of prediabetes. Teplizumab, a humanized IgG1 kappa CD3-directed monoclonal antibody modifies disease progression from stage 2 to overt T1D by preserving β-cell function. In future, T1D prevention studies should include combining immunomodulatory methods through the depletion of diabetogenic cells, strengthening regulatory cells, and islet regeneration, with a focus on the time of the onset of therapy and the duration of treatment. Primary prevention studies should start earlier, and secondary prevention studies should include more people at risk, which implies screening for T1D in the general population. People with immune markers of risk for T1D can now live without diabetes or with low metabolic risk for many years, which will allow for a reduction in acute and chronic complications of T1D and potentially a final cure. This review presents data from the newest primary, secondary, and tertiary prevention of T1D, as well as novelties in diagnostics, predominantly screening, and therapy of T1D.

Key words: type 1 diabetes, prevention, screening, therapy

Cite this article as: Miličić T, Jotić A, Lukić Lj, Maćešić M, Stanarčić J, Stoiljković M, Milovančević M, Rafailović Đ, Božović A, Radisavljević N, Lalić N. Type 1 diabetes: prevention and screening in focus Medicinska istaživanja 2024; 57(2):163-171 DOI: 10.5937/medi57-49676

INTRODUCTION

The prevalence of type 1 diabetes (T1D) is increasing worldwide, and in the Europe and Central Asia region, it will increase by as much as 49% in the next 20 years. At the same time, the analysis of the prevalence by age category indicates that T1D is no longer a disease related exclusively to the pediatric age. In that context, data published in the latest International Diabetes Federation (IDF) atlas show that more than half of the people with newly diagnosed T1D in 2022 are over 20 years of age (1, 2). On the other hand, in patients who developed T1D before the age of 10, a significant reduction in life expectancy has been recently reported, up to 18 years compared to their nondiabetic peers. Moreover, reduced life expectancy up to 10 years, was registered in patients who developed T1D after 26 years of age (3). It is suggested that significant advancements in automated insulin delivery, along with the development of innovative software solutions for diabetes management and continuous glucose monitoring devices, will form the cornerstone of efforts to prevent and treat T1 D. Additionally, the integration of novel therapeutic approaches for immunomodulation and the preservation of β cells are expected to complement these technological advancements, further enhancing the efficacy of preventive and therapeutic strategies against T1D (4,5,6,7).

PATHOGENESIS OF T1D: BACKGROUND FOR PREVENTION STRATEGIES

It is a well-known fact that T1D is an autoimmune disease, a consequence of selective destruction of pancreatic β cells that secrete insulin (8). Previously, it was shown that diabetogenic, autodestructive T cells were not eliminated in the thymus due to negative selection, so they migrated into the circulation (9). Most likely, the initial meeting between the autoantigen and the autoreactive diabetogenic T cell, in genetically susceptible individuals, takes place in the pancreatic lymph node, from where, after differentiation and proliferation, the diabetogenic T cells migrate to the pancreatic islet and renew the encounters with autoantigens presented by antigen-presenting cells. They secreted numerous cytokines and chemokines that further attract macrophages, B, and other T cells, and destroy β cell mass. The destruction of the β cell mass is not linear, but rather a wavy line, with periods of relapse and remission (8). The immunological parameters of the intensity of immune response might be associated with residual β cell function as well as predictors of the clinical course of T1D (10,11, 12).

In 2015, the Juvenile Diabetes Research Foundation (JDRF), the Endocrine Society, and the American Diabetes Association (ADA) recommended a new classification of prediabetes that integrated aspects of beta cell mass destruction and clinical aspects of T1D progression. In this sense, there are 3 stages in the progression of T1D. In the first presymptomatic stage, in genetically predisposed individuals exposed to a triggering event, an autoimmune response cascade is triggered and the destruction of the mass of beta cells begins, while the level of glycemia is normal. In the second presymptomatic phase, the autoimmune destruction of beta cells occurs in a series of waves, marked by cycles of relapse and remission. These fluctuations lead to gradual changes in the beta cell mass, initially subtle and then progressively pronounced. Consequently, glycemic levels oscillate, initially remaining within the normal range. However, as the immune response escalates and extends, there is a sharp decline in beta cell mass, causing a sudden surge in glycemia beyond normal limits. This transition heralds the onset of the third symptomatic phase, marking the clinical manifestation of the disease (13,14).

In this context, the course of T1D was defined through stages. Stage 0 includes subjects with genetic/familiar predisposition for T1D. Stage 1 is defined by the presence of two or more islet autoantibodies and euglycemia, stage 2 is marked with multiple islet autoantibodies and dysglycemia, and stage 3 is clinically manifested T1D (15).

PREVENTION STUDIES IN TYPE 1 DIABETES UNTIL NOW: FRUSTRATION

In the prediabetes phase, which can last for months or even years, it is possible to detect immunological markers of T1D prediction, in peripheral circulation, in the form of 5 autoantibodies, but also disorders of cellular immunity, as well as in metabolic disturbances that reflect impaired insulin secretion and sensitivity (15,16). In that sense, it is possible to identify people with at risk of developing T1D, due to genetic, immunogenic, and metabolic risk markers.

However, individuals with a lifetime risk exceeding 75% for developing type 1 diabetes (T1D) account for less than 0.01% of the population. This means that for every 10,000 individuals screened within the general population, only one person with an exceptionally high risk for T1D would likely be identified (17).

On the other hand, first-degree relatives (FDRs) of patients with T1D are the largest healthy subpopulation with a familial risk of developing T1D and have 10-20 times higher relative risk of T1D compared to the general population (18).

Simultaneously, last year's ADA recommendations for T1D suggest screening for prediabetes using tests that detect autoantibodies to insulin, glutamic acid decarboxylase (GAD), islet antigen 2, or zinc transporter 8. However, the ADA suggests that screening for T1D should be performed only in FDRs of patients with T1D or for research purposes (19).

Nevertheless, the long-standing strategy of involving predominantly FDRs in interventional studies of T1D prevention has not been successful. Completed T1D prevention studies have been ineffective so far, and the fact is that 85% of T1D patients have no relatives with T1D (17). Immunotherapy interventions have been focused on multiple levels and have included virtually all participants in the activation of autoimmune response, including both cellular and humoral response, i.e. diabetogenic pro-inflammatory T cells, regulatory or anti-inflammatory T cells, B cells, and dendritic cells (20,21). Overall, preventive interventions are divided into studies of primary, secondary, and tertiary prevention. Primary prevention studies focus on intervening in individuals with genetic risk only, to prevent the onset of an autoimmune response. Moreover, secondary prevention studies are done in individuals with additional immunological risk (one or more antibodies to β cell antigens), to slow or block activated autoimmune process. Finally, tertiary prevention studies are conducted in patients with recent-onset T1D (RT1D), aiming to preserve impaired endogenous insulin reserve (14).

Generally, primary prevention trials evaluated the effect of different environmental risk factors on islet autoimmunity. The study BABYDIET showed no benefit from delaying exposure to gluten in early childhood in 150 at-risk children (22). The FINDIA study, including more than 1000 babies with genetic risk of T1D, showed that cow milk formula free of bovine insulin did not reduce the cumulative incidence of islet autoantibodies by the age of 3 (23). TRIGR was a randomized, placebo-controlled study, that included 2160 genetic-risk children and showed no benefit of using highly hydrolyzed milk instead of conventional milk formula on the development of islet antibodies by 6 years of age nor the development of T1D by 11 years of age (24). Studies of primary prevention are ongoing: INGR1D2 study in Belgium, Germany, UK, Poland, and Sweden, where newborns can be tested for an increased genetic risk of T1D. The SINT1A study (**S**upplementation with B. **IN**fantis for Mitigation of **T**ype **1** Diabetes **A**utoimmunity) is a study for infants up to the age of six weeks with an increased genetic risk of T1D, aiming to evaluate whether giving the probiotic B. infantis might modulate immune response (25). The Freder1k study includes newborns up to seven days old in Germany, and the risk of T1D developing is determined by testing a few drops of blood obtained from the umbilical cord. The last 3 studies are under the auspices of the GPPAD (Global Platform for the Prevention of autoimmune diabetes) platform, which brings together experts from Europe and America, to prevent T1D. They calculate genetic risk score (GRS) from blood to identify children at 10% risk for multiple autoantibodies by 6 years of age. They offer those 4–7-month-old children to be included in a primary prevention study Primary Oral Insulin Trial (POInT), in 5 European countries. They will be treated with oral insulin as immunomodulator, until the age of 3 and followed for 7 years (26).

Secondary prevention trials include intervention at stages 1 and 2 of T1D, and some of them used insulin for immunomodulation and induction of anti-inflammatory Th2 or regulatory immune response, which might protect β-cells. The National Institute of Health Diabetes Prevention Trials (DPT-1) consisted of two clinical trials and demonstrated that low-dose subcutaneous or oral insulin therapy did not prevent T1D in FDRs irrespective of prediabetes stage. However, post-hoc analysis of subgroups of FDRs with high IAA titers reported delayed progression to T1D with oral insulin (27,28). The European Nicotinamide Diabetes Intervention Trial (ENDIT) showed that nicotinamide previously demonstrated a protective effect on β-cells in animal models, and did not delay or prevent T1D in high-risk FDRs (29).

Finally, tertiary prevention trials include interventions in stage 3, clinically manifested T1D, aiming to preserve β-cell function and mass to achieve better metabolic control of T1D (lower incidence of hypoglycemia, lower HbA1c) and delay microvascular complications (30). Historically, cyclosporin transiently preserved β- cell function, but it was related to renal toxicity (31). Later on, several immunomodulatory drugs did not succeed in protecting β-cells in the long term, did not induce insulin independence, and were associated with adverse events. Along this line, trials used anti-CD3 monoclonal antibodies teplizumab (32) and otelixizumab, abatacept (CTLA4-Ig) (33), alefacept (34), anti-CD20 monoclonal antibody, rituximab (35), as well as anti-inflammatory agents (36) and mycophenolate mofetil with or without daclizumab (37).

NOVELTIES IN TYPE 1 DIABETES THERAPY: PREVENTION IN FOCUS

Recently, there was a turning point in this investigational area when it was shown that it is possible to delay T1D with immunotherapy in people with a high risk for T1D, in stage 2 of prediabetes (38). In that context, in November 2022, teplizumab was approved in the USA to delay the onset of overt T1D in adults and children ≥ 8 years of age with stage 2 T1D. It can be said that this event was the most important for the community of T1D patients in the last 100 years and since the discovery of insulin: a drug that can slow the progression of T1D, appeared on the market under the tradename Tzield®.

Teplizumab, a humanized IgG1 kappa CD3-directed monoclonal antibody (Anti-CD3 mAb) modifies disease progression from stage 2 to stage 3 T1D by preserving β-cell function. The effect of teplizumab is based on blocking Th 1 proinflammatory autoimmune response, and inducing T regulatory, protective, anti-inflammatory response (38). Research in this field has been published

in the last 20 years, first in patients with RT1D (32). In this multinational, randomized, placebo-controlled 2-year trial, in a large sample of RT1D up to 12 weeks from diagnosis, a 14-day treatment with different doses of teplizumab was implemented. The primary outcome was composite, the percentage of patients on insulin therapy <0.5 U/kg per day and $HbA_{1C<}$ 6.5% at 1 year, and it was not achieved. Despite this, post-hock analyses suggest that teplizumab could protect β-cells and might lower the daily insulin dose (32). Subsequently, recognizing the partial success achieved in certain cases along with the limited duration of response, further investigations including teplizumab are undertaken. ABATE study aimed to evaluate the efficiency and safety of two doses of teplizumab, to slow the decline in C-peptide levels in patients with RT1D within 2 weeks of diagnosis, and to identify characteristics of responders on the study drug (39). The results pointed out that patients on teplizumab had a higher level of C-peptide at 2 years, which was a 75% improvement. It has been reported that subjects on teplizumab have a delay of decline in C-peptide by 15.9 months, but responses to the drug varied, and the authors identified responders and nonresponders to the drug. Moreover, responders to the drug were identified by metabolic (lower HbA_{1c} levels of and insulin use at baseline) and immunologic (lower level of Th1-like IFN-γ-producing CD8+ T cells) features (39). The most frequent adverse events were rash, transient upper respiratory infections, headache, and nausea.

Surprisingly, after 7 years of follow-up, interesting findings have been published, suggesting there is still a slower decline in C-peptide and sustained beneficial immunological responses up to 7 years after diagnosis of T1D in drug responders, although they did not differ significantly according to insulin use and HbA1c level (40).

Finally, the results from the most successful prevention study in the area of T1D prevention in the last 50 years were reported in 2019. This was a phase 2, randomized, placebo-controlled, double-blind trial of teplizumab involving FDRs of patients with T1D who were nondiabetics but had a high risk for T1D, stage 2 of prediabetes (2 autoantibodies and dysglycemia). Patients were randomized to a single 14-day course of teplizumab or placebo, and follow-up for progression to overt T1D with the use of OGTT every 6 months (38).

The study included 76 participants, mainly children and adolescents, and there were 44 on teplizumab and 32 on placebo. It was reported that the average time to the diagnosis of T1D was 48.4 months in subjects on teplizumab and 24.4 months on placebo. Moreover, in overt T1D progressed 43% of subjects on teplizumab and 72% of subjects on placebo.

Furthermore, a sustainable effect on the progression of T1D was detected after 923 days of follow-up. In that sense, the average time to overt T1D was 27.1 months in placebo and 59.6 months in the teplizumab group. After this period, 22% and 50% respectively were not diagnosed with T1D (41). Moreover, besides metabolic changes (increased C peptide level), immunological changes in responders were detected. In that sense, a higher percentage of just one subset of T cells, $KLRG1^{+-}$ $TIGIT^{+}EOMES^{+}CD8^{+}$ T cells, associated with T-cell unresponsiveness, was reported, suggesting selectivity in the effect of teplizumab (41). Simultaneously, changes in T cells correlate with improved metabolic function β cells, and the frequency of T cells that produce proinflammatory cytokines IFNγ and TNFα, was reduced in subjects on teplizumab (41).

NOVELTIES IN TYPE 1 DIABETES DIAGNOSIS: SCREENING IN FOCUS

The great success achieved within this study breathed new life into research in this area. It is suggested that limitations in prevention studies relate mainly to the study population: dominantly FDRs in stage 2, a small number of subjects, and age, because so far teplizumab has been adminitered only to children over 8 years of age.

So, the idea of screening for T1D in the general population, and not only in the population of FDRs, has arisen and become popular. In that context, it has been shown that children who progress from stage 2 to stage 3 T1D, make this progress at the same rate (50% risk by 2 years), regardless of whether they are children-FDRs of patients with T1D or children from the general population (42). Furthermore, the advantages of screening for T1D in the general population are identifying children at risk, offering them education and metabolic monitoring, and lowering the rate of diabetic ketoacidosis (DKA) at the clinical manifestation of T1D. Simultaneously, it was reported that children identified with prediabetes in public health screening compared to children with incident T1D, had a lower prevalence of DKA, lower rates of hospitalization in emergency departments, and higher levels of residual β cell function (43).

In that context, in 2015, the Fr1da study was initiated in Bavaria, Germany, designed to evaluate screening in the general population for multiple islet autoantibodies for early detection of T1D in children (44,45). The study was conducted in collaboration with primary care physicians and included over 165,000 children until now. The authors also created a predictive score that took into account the level of HbA1c, the level of glycemia in the 90^{th} minute of 2h OGTT, and the titter of IA2 antibodies, by which it is possible to identify the normoglycemic group of children, in stage 1, which rapidly, with a high risk of 50% over 2 years, progress to clinically manifest T1D (stage 1b children). The main findings of this study were: that screening in the general population is feasible, public health screening for islet autoantibodies detected 0.027% of children with undiagnosed overt T1D and 0.038% with

undiagnosed stage 2 or stage 1b T1D, with 50% risk to develop clinical T1D within 2 years. Moreover, identifying people in stage 1b prediabetes will double the number of people who may benefit from disease-modifying drugs, and there is a huge social benefit (lower DKA rate, better course of T1D, education, less distress) (42).

Ongoing screening programs in FDRs of patients with T1D – TrialNet (a U.S.-based consortium) and INNODIA (a European private/public partnership) – began by screening FDRs to increase efficiency for enrolment in preventive clinical studies. The Type 1 Diabetes TrialNet Pathway to Prevention Study, started in 2004, has screened more than 220,000 FDRs. Initially, assays for ICA, IAA, IA2A, and GADA (by RBA) were performed, and from 2019, screening was modified to GADA and IAA only, and then they might undergo testing for other available antibodies. Generally, TrialNet identified 5% of FDRs with at least one autoantibody, and half of these had multiple autoantibodies. INNODIA screens for four autoantibodies by RBA and has screened more than 4,400 FDRs, with similar results regarding the detection of FDRs with prediabetes (46).

On the other hand, screening in the general population might be divided into two categories: birth cohorts or autoantibody-based screening programs. Birth cohorts use genetic screening and those who have higher risk undergo autoantibody screening. The Type 1 Diabetes Prediction and Prevention Study (DIPP) has started in Finland, the country with the highest incidence of T1D in the world, with more than 250,000 infants screened until now. Moreover, the Newborn Screening for Genetic Susceptibility to Type 1 Diabetes and Celiac Disease and Prospective Follow-up Study (BABYSCREEN), in Finland also, screens for genetic risk for T1D and celiac disease. Furthermore, GPPAD screened more than 279,000 infants as of July 2021 and detected 1.1% of those with increased genetic risk. Additionally, in the USA there are further programs: the Combined Antibody Screening for Celiac and Diabetes Evaluation (CASCADE) program, the Sanford Population-Level Estimation of T1D Risk Genes in Children (PLEDGE) project, and the Precision Individualized Medicine for Diabetes (PRiMeD) project, and they also use calculating GRS from blood spots or saliva.

On the other hand, screening in the general population using autoantibodies include the following studies: ASK (Autoimmunity Screening for Kids, Colorado), T1Detect (USA), Early Detection of Type 1 Diabetes (Fr1da), and Early Detection of Type 1 Diabetes and Hypercholesterolemia in Lower Saxony (Fr1dolin) (Germany) (46).

Having all this in mind, with the expectation that screening in the general population will be accepted worldwide, there are some recommendations for clinical practice. In that context, it is suggested that the best time for screening would be the age of 2 and 5–7 years of age. Moreover, monitoring of subjects with immune markers includes discussion of results and implications; and education about the signs and symptoms of diabetes. The recommendations for metabolic monitoring include OGTT, HbA1c levels, random glycemic levels, or continuous glycemic monitoring (46). In that sense, a 10% increase in HbA1c level during 3-12 months or two consecutive values of HbA1c≥5.9% are markers of progression into stage 3 T1D. Moreover, it has been shown that spending ≥10% with glucose levels ≥7.8 mmol/l, the risk for progression to overt T1D is up to 80% within the next 12 months, and if it is more than 5%, the risk for progression will be 40% in the next 2 years (47,48).

YEAR 2023: DISAPPOINTMENTS AND SUCCESSES

Finally, last year began with disappointments in the area of prevention of T1D.

Abatacept, which stops the activation and proliferation of diabetogenic T cells, due to costimulation blockade, given to individuals in stage 1 T1D during 12 months, disappointingly, did not result in a delay from stage 1 to stage 2. However, abatacept preserved C peptide as well as it was previously shown in R T1D and it implies the possibility of modifying the course of T1D (49). Hydroxychloroquine, an immunomodulatory drug acts on alterations in insulin metabolism through cellular receptors (50), but the study in relatives at risk for T1D was stopped in July 2023, due to unsatisfying results at interim analysis.

On the other hand, low doses of anti-thymocyte globulin preserved C peptide and decreased HbA1 in R T1D (51). Furthermore, a randomized, double-blind, placebo-controlled, phase 2 trial used anti-interleukin-21 antibody and liraglutide for the protection of β cells in adults with RT1D, showed that both drugs act synergistically on C peptide and HbA1c levels, and better than each of them alone (52). Furthermore, verapamil has inhibitory effect on β cell apoptosis by inhibiting thioredoxin interacting protein (overexpressed in diabetes, promoting oxidative stress). It was reported that in children with RT1D, verapamil preserved C peptide for 30% more than placebo after 52 weeks of follow-up (53).

Recently, the results of the T1GER study have been published, about the effect of golimumab (a human monoclonal antibody specific for tumor necrosis factor α) in individuals with RT1D. This was phase 2, a multicentre, placebo-controlled, double-blind, parallel-group trial, that included 56 children and young adults with RT1D on subcutaneous golimumab or placebo for 52 weeks. The authors demonstrated that individuals on golimumab had significantly higher levels of C peptide, lower daily doses of insulin, and a higher incidence of partial clinical remission (54). Moreover, the results from the PROTECT study were published. Teplizumab was given to individuals with RT1D in a new study design, two times, at the beginning of the disease and 6 months

later, and it protected the C peptide, but it did not decrease significantly daily insulin dose (55). Additionally, baricitinib, a JAK kinase inhibitor, that blocks cytokine signaling, previously used for treatments of autoimmune diseases in rheumatology and dermatology, was investigated in RT1D. The study was a phase 2, double-blind, randomized, placebo-controlled trial, that included 60 children and young adults in RT1D on baricitinib or 30 individuals on placebo, orally for 48 weeks. The results showed a protective effect on C peptide level, with no difference concerning daily insulin dose and HbA1c, compared to placebo. However, baricitinib reduced glucose variability and improved time in range (56). The oral formulation of the drug will certainly improve the adherence of patients to the medication and the safety profile of all of these drugs is acceptable. Finally, this year, the FDA granted fast track to intralymphatic injection of anti-GAD vaccine. A previous trial of intralymphatic injections of aluminum-formulated glutamic acid decarboxylase showed preservation of β-cell function in patients with HLA DR3-DQ2 (57), and a correlation between the C-peptide level and time in the target glucose range (58). In that context, the phase 3 DIAGNODE-3 trial is assessing the safety and efficacy of the therapy among 330 adolescents and young adults up to 29 years with RT1D, and DR3-DQ2 genotype. The co-primary endpoints of DIAGNODE-3 will be the preservation of endogenous insulin-producing capacity and improved HbA1c.

FUTURE THERAPY OF T1D: COMBINED APPROACH OF IMMUNOMODULATION AND β CELL PROTECTION

Finally, a meta-analysis of 21 trials of disease-modifying interventions in RT1D comprising 1315 adults and 1396 children, was published. The results showed that a 24.8% higher C-peptide level was accompanied by a 0.55% lower HbA1c, after 6 months of treatment. Moreover, improvements in HbA1c are proportional to the degree of C-peptide preservation, suggesting the use of C-peptide as a surrogate endpoint in clinical trials (59). In addition, the immune interventions aimed to protect β cell function and/or mass may soon be offered to patients with RT1D but must be proven to be safe in the short as well as long term (60).

At the same time, the future of studies of T1D prevention should include combining immunomodulatory methods through the depletion of diabetogenic cells, strengthening regulatory cells, and islet regeneration, with a focus on the time of start of therapy and the duration of treatment (59,60).

CONCLUSION

In conclusion, T1D is a predictable autoimmune disease, with clearly defined stages preceding the clinical manifestation of the disease. Primary prevention studies should start earlier, and secondary prevention studies should include more people at risk, which implies screening for T1D in the general population. For the first time, it is possible to postpone the clinical manifestation of T1D in individuals at risk for T1D. People with immune markers of risk for T1D can now live without diabetes or with low metabolic risk for many years, which will allow for a reduction in acute and chronic complications of T1D and potentially a final cure.

Acknowledgments

This work was funded by Project 451-03-66/2024-03/200110 from the Ministry of Science, Technological Development and Innovations Republic of Serbia.

Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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TIP 1 DIJABETESA: PREVENCIJA I SKRINING U FOKUSU

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Sažetak

Poznato je da je broj obolelih od tipa 1 dijabetesa (T1D) u porastu u celom svetu. Istovremeno, utvrđeno je da u pacijenata sa T1D postoji značajno smanjenje očekivanog životnog veka, u poređenju sa vršnjacima bez dijabetesa. U tom smislu, prevencija T1D je goruće pitanje, imajući u vidu više neuspešnih pokušaja u poslednjih 50 godina. Međutim, nedavno je došlo do prekretnice u ovoj naučnoj oblasti, kada je pokazano da je moguće odložiti T1D imunoterapijom kod osoba sa visokim rizikom za T1D, u fazi 2 predijabetesa. Teplizumab, humanizovano IgG1 kappa anti CD3 monoklonsko antitelo modifikuje progresiju bolesti od faze 2 do klinički manifestnog T1D protekcijom β-ćelija. Istovremeno, smatra se da bi u budućnosti, studije prevencije T1D-a trebalo da uključuju kombinovanje imunomodulatornih metoda kroz iscrpljivanje dijabetogenih ćelija, jačanje regulatornih ćelija i regeneraciju β ćelija, sa fokusom na vreme početka terapije i trajanje lečenja. Studije primarne prevencije trebalo bi da počnu ranije, a studije sekundarne prevencije trebalo bi da uključuju više osoba sa visokim rizikom za ispoljavanje T1D, što podrazumeva skrining za T1D u opštoj populaciji. Osobe sa imunološkim markerima rizika za T1D sada mogu da žive bez dijabetesa ili sa niskim metaboličkim rizikom dugi niz godina, što će omogućiti smanjenje akutnih i hroničnih komplikacija T1D i potencijalno konačno izlečenje. Ovaj pregledni članak predstavlja podatke iz nedavno završenih studija primarne, sekundarne i tercijarne prevencije T1D, kao i novitete u dijagnostici, pretežno skriningu, i terapiji T1D.

Ključne reči: tip 1 dijabetesa, prevencija, skrining, terapija

Primljen: 06.03.2024. I **Revizija:** 17.04.2024. I **Prihvaćen:** 22.04.2024.

Medicinska istaživanja 2024; 57(2):163-171

Medicinska istaživanja 2024; 57(2):173-182 | DOI 10.5937/medi57-48405

OPEN ACCESS

Medical Research | Published by Faculty of Medicine University of Belgrade

REVIEW ARTICLE

The use of tocilizumab in severe COVID-19: a comprehensive review

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Received: 25 December 2023

Revised: 22 February 2024

Accepted: 29 February 2024

Check for updates

Funding information:
This study received financial support from the Ministry of Science of the Republic of Serbia (grant no. 200110).

Competing interests:
The authors have declared that no competing interests exist

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Summary

This review focuses on the therapeutic application of Tocilizumab (TCZ) in the treatment of COVID-19, specifically exploring its mechanisms, safety aspects, clinical efficacy, dosing strategies, and outcomes in the Serbian context. TCZ, acting as an IL-6 receptor inhibitor, mitigates the cytokine storm observed in severe cases, leveraging its structure and pharmacokinetics. While the overall safety profile indicates good tolerability, there are subtle concerns regarding the occurrence of rare complications in critically ill patients. Clinical trials, with certain variations, emphasize the need for careful interpretation of indications and patient selection for TCZ therapy. Current protocols in place in the Republic of Serbia recommend the use of TCZ at a dose of 8 mg/kg body weight based on clinical parameters and inflammation markers, primarily IL-6 levels. Literature review suggests that during TCZ shortages, dosing may be adjusted to 400 mg as a single dose in the treatment of severe COVID-19. The optimal timing for initiating therapy coincides with the phase of increased inflammation (7-10 days after symptom onset), with an emphasis on patient selection based on biomarkers, disease severity, and the need for respiratory support. Combining TCZ with corticosteroids shows reduced mortality, necessitating cautious dosing. Potential benefits arise from combining TCZ with remdesivir, NSAIDs, and anticoagulants, requiring careful dosing and monitoring. Retrospective studies in Serbia report positive outcomes, highlighting the potential of TCZ in treating severe cases. In summary, TCZ shows promising results in the treatment of COVID-19, necessitating further research and careful patient monitoring, especially in resource-limited settings.

Key words: Tocilizumab, severe COVID-19, effectiveness, safety, experiences

Cite this article as: Milošević I, Beronja B. The use of tocilizumab in severe covid-19: a comprehensive review Medicinska istaživanja 2024; 57(2):173-182 DOI: 10.5937/medi57-48405

INTRODUCTION

The emergence of coronavirus disease 2019 (COVID-19) represents a global health crisis of unprecedented proportions (1). The World Health Organization (WHO) declared the COVID-19 pandemic on March 11, 2020, and the first case in Serbia was recorded on March 6, 2020 (2-4). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) attaches to angiotensin-converting enzyme 2 (ACE-2) receptors, triggering the activation of Th1 lymphocytes and the subsequent generation of proinflammatory cytokines, such as interleukin-6 (IL-6) and granulocyte colony-stimulating factor (GM-CSF) (5-7). Considering the significant role of IL-6 in cytokine release syndrome (CRS), it has been identified as a potential target for therapeutic interventions in severe patients (8).

Tocilizumab (TCZ) is a genetically engineered humanized monoclonal antibody specifically crafted to inhibit IL-6 receptors, thereby reducing its impact on the pathogenesis of COVID-19 (9-12). As per the guidelines set by the European Medicines Agency, TCZ was authorized for use in adults experiencing severe COVID-19 (13, 14). TCZ, as one of the utilized modalities of biological therapy started to be employed in Serbia (15, 16).

Prior to its therapeutic application in COVID-19, TCZ had been utilized in the treatment of connective tissue disorders such as rheumatoid arthritis, giant cell arteritis, polyarticular juvenile idiopathic arthritis, and systemic juvenile idiopathic arthritis [17, 18]. Additionally, it has been employed for the treatment of cytokine release syndrome induced by T cells with chimeric antigen receptors in both adult and pediatric patients aged over 2 years (19). The utilization of TCZ is indicated in patients exhibiting severe COVID-19 pneumonia, characterized by heightened inflammatory markers: CRP and IL-6 (15, 16, 20). Furthermore, TCZ is deemed appropriate for individuals experiencing a rapidly progressing respiratory failure, extensive lung infiltration, and systemic inflammation (15, 16, 20).

STRATEGIC TARGETING: TCZ IN IL-6 SIGNALING

IL-6 functions as a multifaceted regulator in various biological processes associated with inflammation, metabolism, and tumorigenesis, operating in an autocrine, paracrine, and "hormone-like" manner (21). In COVID-19, a meta-analysis of 1302 cases revealed a threefold increase in IL-6 levels in severe compared to mild/moderate cases (22). Elevated IL-6 concentrations correlated with bilateral lung damage, fever, and increased mortality. The risk of severe COVID-19 and death escalated at IL-6 concentrations >55 pg/ml and >80 pg/ml, respectively (22).

Its unique signaling system involves IL-6 receptors (IL-6R) and downstream molecules. IL-6R comprises IL6-binding chain (IL6-Rα) and transmembrane protein gp130 (IL6-Rß), initiating the IL6-induced signaling cascade upon binding to IL-6.IL6-Rα is expressed in specific cell types, while gp130 is present ubiquitously (23-25). The signaling cascade involves the dimerization of IL-6 and IL-6R complex with gp130, activating Janus kinases 1 and 2, leading to phosphorylation of gp130 [23-25]. Soluble IL6-Rα in the bloodstream, formed by proteolytic cleavage and alternative splicing, facilitates IL-6 circulation, enabling "trans-signaling" where IL-6 and sIL-6Rα bind to gp130 in cells lacking mIL-6Rα [23-25]. The pathogenic effects of IL-6 are primarily determined by trans-signaling, while classical (cis) signaling contributes to acute-phase response, Th17 and Th22 cell production, and T regulatory cell suppression (23-25). "Trans-presentation" on dendritic cells further expands IL-6 signaling, influencing the differentiation of pathogenic Th17 cells (23-25). **(Figure 1)**

TCZ, a monoclonal antibody with the capacity to antagonize the IL-6 receptor **(Figure 2)**. TCZ exhibits a molecular structure comprising two heavy and two light chains, encompassing 12 intrachain and 4 interchain disulphide bonds, resulting in a global molecular weight of 149 kDa (26). The pharmacokinetics of TCZ are nonlinear, characterized by a relatively long half-life ranging from 5 to 12 days (26). Numerous randomized controlled trials demonstrate TCZ's significant and sustained improvement in structural joint damage, health-related quality of life, and reduction in C-reactive protein levels in rheumatoid arthritis patients, as well as improvement in the quality of life in individuals with specific hematological malignancies and immunological deficits (27-30). Additionally, TCZ serves as an adjuvant therapy for the uncontrolled inflammatory state associated with hemophagocytic lymphohistiocytosis in visceral leishmaniasis (31). These specific indications should be taken into consideration for TCZ, given the described atypical clinical courses of COVID-19 in patients from Serbia with severe hematological and immunological deficits (32-34).

After intravenous administration, TCZ undergoes biphasic elimination from circulation (35). The total clearance of TCZ is concentration-dependent and represents the sum of linear and nonlinear clearance (35). Linear clearance, measured as a parameter in population pharmacokinetic analysis, was 9.5 mL/h. Nonlinear clearance, dependent on concentration, plays a crucial role at low TCZ concentrations (36). When the nonlinear clearance pathway saturates, at higher TCZ concentrations, further clearance is predominantly determined by linear clearance (36). The elimination half-life (t1/2) of TCZ is concentration-dependent. In a steady state after an 8 mg/kg dose every 4 weeks, the effective t1/2 decreases with decreasing concentrations in the dosing interval, ranging from 18 days to 6 days (36).

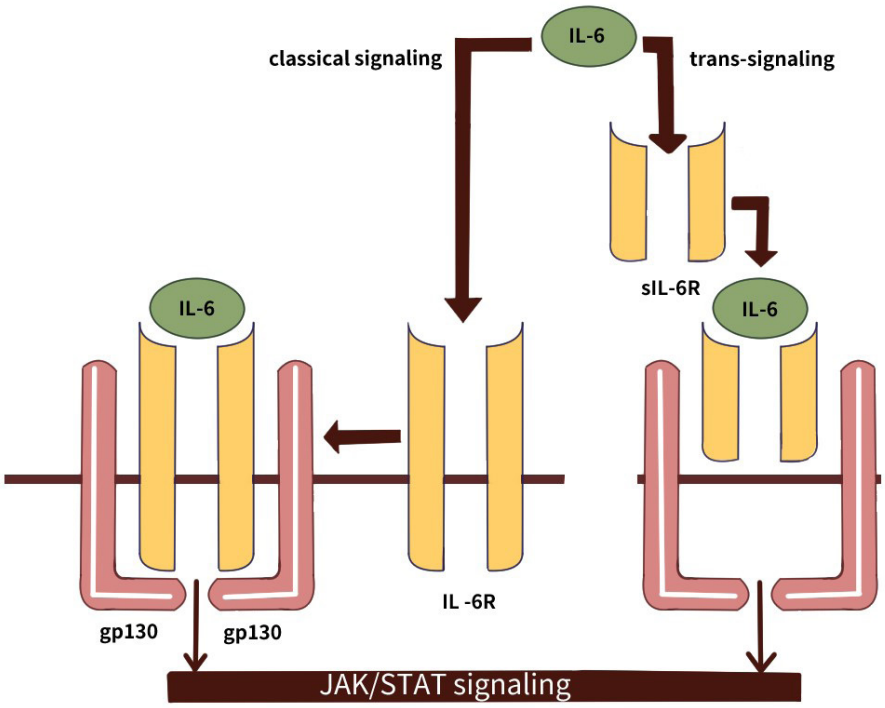

Figure 1. The image illustrates the IL-6 signaling pathway. IL-6 is transmitted either through the cell surface IL-6R or in the form of soluble IL-6R. This is followed by the dimerization of the signal transducer gp-130, which binds to the IL-6/IL-6R complex. Subsequently, activation of the JAK/STAT kinase pathway occurs.

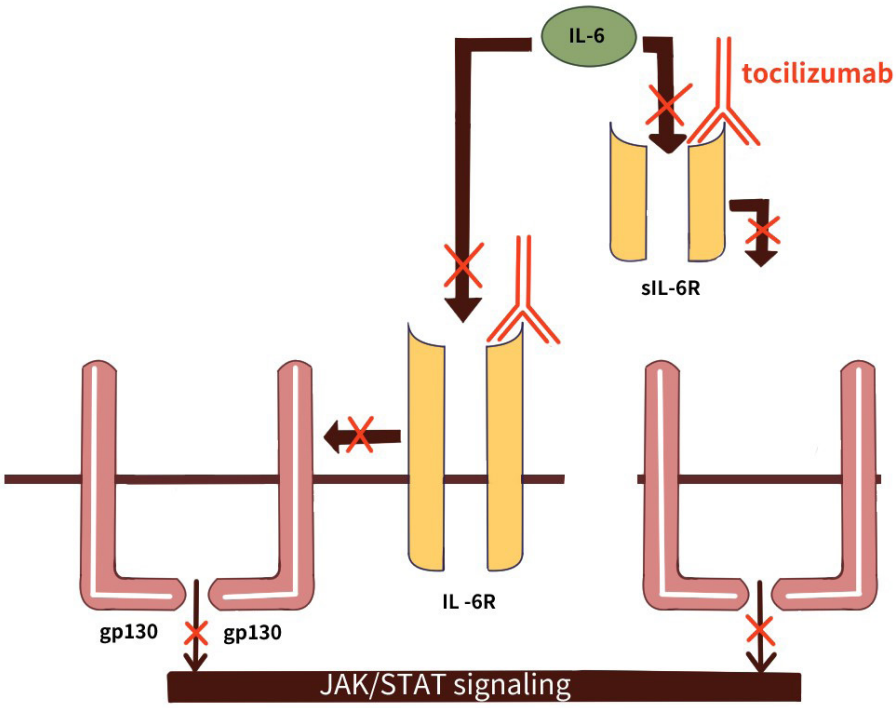

Figure 2. IL-6 classic and trans-signaling pathways, with a model of tocilizumab-mediated therapeutic receptor antagonism. Tocilizumab inhibits the binding of IL-6 to IL-6R or sIL-6R.

SAFETY PROFILE IN COVID-19 TREATMENT

Relevant literature data specific to the adverse effects of TCZ treatment in patients with COVID-19 have been analyzed. In terms of safety, our review suggests that TCZ is generally well-tolerated with a comparable rate of secondary infections in COVID-19 patients. The analysis of available literature did not reveal a significantly increased risk of secondary infections among individuals treated with TCZ (37-41). It's crucial to note that TCZ administration often involves patients with severe COVID-19, many of whom are in the intensive care

unit (ICU) or require ICU admission during follow-up (37,39). The anticipated higher incidence of infections in this subgroup can be attributed to various factors, notably the increased number of invasive procedures, including mechanical ventilation (MV), in severely ill patients (39).

In six-month controlled studies, the overall infection rate with TCZ 8 mg/kg plus disease-modifying antirheumatic drugs (DMARD) therapy was 127 events per 100 patient-years, compared to 112 events per 100 patient-years in the placebo plus DMARD group (42, 43). In the long-term exposure of patients, the overall infection rate in the TCZ group was 108 events per 100 patient-years (42, 43). In six-month controlled clinical trials, the rate of severe infections with TCZ 8 mg/kg plus DMARD therapy was 5.3 events per 100 patient-years, compared to 3.9 events per 100 patient-years in the placebo plus DMARD group (42, 43). In monotherapy trials, the rate of severe infections was 3.6 events per 100 patient-years in the TCZ group and 1.5 events per 100 patient-years in the MTX group (42, 43).

In the long-term exposure population, the overall rate of severe infections (bacterial, viral, and fungal) was 4.7 events per 100 patient-years. Recorded severe infections (some fatal) included active tuberculosis, invasive pulmonary infections (such as candidiasis, aspergillosis, Pneumocystis jirovecii pneumonia), cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis, and bacterial arthritis (42). Cases of opportunistic infections were also documented (43).

Two cases of critically ill COVID-19 patients developing hypertriglyceridemia following TCZ administration have been reported (44, 45). This suggests that TCZ may induce a metabolic response in critically ill patients (44). IL-6, acting as an immunomodulator, has direct effects on metabolism, triggering the release of free fatty acids (FFA) from adipocytes and promoting glucose and FFA uptake by skeletal muscles (44). Another concern is the risk of intestinal perforation associated with TCZ use in critical COVID-19 patients, particularly those with preceding diverticulitis (45). The high expression of ACE 2 in the intestines, a key player in SARS-CoV-2 infection, provides a suitable medium for viral replication, leading to gastrointestinal symptoms such as abdominal pain (45). Changes in hemodynamics, induced by TCZ weakening the acute phase response, may cause low blood flow to the intestines, potentially resulting in intestinal perforation (45). This adverse effect may present without a significant escalation of CRP levels and might be overlooked in sedated and ventilated patients. In light of these findings, TCZ administration to critically ill COVID-19 patients has shown significant potential adverse events (45). Close monitoring of critical parameters, evaluation of therapeutic outcomes, and vigilance towards possible adverse effects influenced by concomitant drug activities are essential when employing TCZ therapy (44, 45).

TCZ administration in pregnant women with COVID-19 has demonstrated an absence of adverse effects on both the mothers and their newborns (46, 47). While a minimal risk of secondary infections was noted, it is advisable to maintain vigilant monitoring for infections, particularly when employing other immunosuppressive agents (47).

Interactions were only studied in adults. Simultaneous administration of a 10 mg/kg dose of TCZ with 10-25 mg MTX weekly has no clinically significant effect on MTX exposure (48). Population pharmacokinetic analyses found no impact of MTX, nonsteroidal anti-inflammatory drugs (NSAIDs), or corticosteroids on TCZ clearance. Cytokines like IL-6, which stimulate chronic inflammation, suppress the expression of hepatic CYP450 enzymes (49). Hence, cytokine-inhibiting therapies like TCZ may enhance CYP450 expression. In vitro studies on human hepatocytes showed IL-6 decreases the expression of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 enzymes. TCZ normalizes their expression (50).

In an RA patient study, simvastatin levels (CYP3A4) dropped by 57% a week after a TCZ dose, reaching levels similar to or slightly higher than in healthy subjects (26). Initiating or discontinuing TCZ therapy requires careful monitoring for patients taking individually dosed drugs metabolized via CYP450 3A4, 1A2, or 2C9 (e.g., methylprednisolone, dexamethasone, atorvastatin). Doses may need adjustment to maintain therapeutic effects (42). Due to its long elimination half-life (t1/2), TCZ's impact on CYP450 enzyme activity can persist for several weeks after therapy cessation (47).

TCZ EFFECTIVENESS IN COVID-19

In the COVACTA trial, a multicenter Randomized Controlled Trial (RCT) involving 438 hospitalized patients, the impact of a single 8 mg/kg dose of TCZ was assessed (51). Notably, 36% of the TCZ group received concurrent dexamethasone, in contrast to 55% in the control group (50). Day 28 mortality and clinical status did not exhibit significant differences, but potential benefits in time to discharge and ICU stay duration were noted (51). Limitations of this trial include the absence of patient stratification for hyperinflammation signs and unequal corticosteroid use between groups (51).

The CORIMUNO-TOCI-1 trial, encompassing 131 patients with moderate or severe pneumonia, revealed no significant difference in day 28 mortality (52). However, there were indications of potential benefits, including a suggested reduction in ventilation or death by day 14, and no discernible increase in adverse events (52). Unequal steroid administration and a relatively small sample size may influence the perceived benefits of TCZ in this context (52).

In the EMPACTA trial, 389 hypoxemic patients received either one or two doses of TCZ (53). A statistically

significant reduction in progression to mechanical ventilation or death by day 28 was observed. However, overall clinical failure did not demonstrate a significant difference (53). The study's strengths include equal steroid use, adequate statistical power, and a focus on high-risk populations (53). Nevertheless, limitations are evident, particularly concerning the definition of the composite outcome.

Contrastingly, the RECOVERY trial, with its substantial cohort of 4116 patients, reported a 28-days mortality benefit associated with TCZ, especially when combined with systemic corticosteroids (54). TCZ recipients were more likely to be discharged alive, less likely to reach the composite endpoint, and exhibited a reduction in the use of hemodialysis or hemofiltration (54). The trial's robustness lies in its large sample size, diverse patient criteria, and the consistency of benefits observed across subgroups (54).

There is a lack of clear data on the impact of TCZ on the quality of life in COVID-19 convalescents. TCZ of exacerbations in rheumatoid diseases might provide a rough parallel.Patients treated with TCZ reported improvements in all patient-assessed outcomes, including the Health Assessment Questionnaire Disability Index (HAQ-DI) and the Short Form-36 questionnaire for functional assessment of chronic disease therapy (55). Statistically significant improvement in HAQ-DI scores was observed in patients receiving TCZ compared to those treated with DMARD (55). During the open-label period of Study II, improvement in physical function was sustained over 2 years. After 52 weeks, the mean change in HAQ-DI index was -0.58 in the TCZ group at a dose of 8 mg/kg (55, 56).

These trials collectively contribute to a nuanced understanding of TCZ's efficacy in COVID-19 treatment, emphasizing the importance of considering patient characteristics, treatment combinations, and outcome definitions in the interpretation of results.

TCZ DOSAGE STRATEGIES

The current treatment protocol for COVID-19 patients in Serbia, in accordance with the recommendations of the World Health Organization, involves the administration of TCZ when the IL-6 value exceeds 40 pg/ml, or when a threefold increase in inflammatory markers occurs within a one-day interval or the development of respiratory insufficiency (16, 20). The anticipated duration of TCZ therapy for COVID-19 treatment is one day, with a 12-hour interval between two daily doses (13, 16, 20). The recommended therapeutic dose of TCZ is 8 mg/kg of body weight, with a maximum individual dose of 800 mg (13, 16, 20).

The significant surge in COVID-19 cases has resulted in a shortage of TCZ, prompting healthcare practitioners to explore alternative options. Study findings indicate a comparable mortality benefit, although the 8 mg/kg dosing strategy may pose an elevated risk of fungal and viral infections (57). However, this investigation suggests a practical solution—employing the 400 mg dose—which could potentially lead to substantial cost savings, up to 50% (57). This cost-effective alternative not only addresses financial considerations but also preserves critical resources, enabling the broader administration of the medication to a greater number of patients (57). In the context of limited resources, such a strategy becomes crucial for optimizing healthcare delivery and ensuring the widespread availability of essential treatments during these challenging times.

TCZ TREATMENT TIMING

The consideration of optimal timing for initiating IL-6 blocking treatment requires a closer examination of specific days during the course of COVID-19. Existing studies have predominantly focused on patients in the advanced stages of the disease. To enhance precision in therapeutic decisions, it is suggested to postpone the administration of TCZ until the onset of the inflammatory phase, typically observed around days 7 to 10 after the initial symptoms manifest (58-61).

This proposed delay is grounded in the hypothesis that allowing the natural release of IL-6 during the acute infection stage may exert positive effects by hindering the proliferation of SARS-CoV-2 (60). The intricate dynamics of the immune response in COVID-19 further emphasize the importance of selecting an opportune time for intervention (61). Notably, amid the ongoing discourse among researchers, a consensus emerges on the potential efficacy of targeted immune suppressants like TCZ (58-61). The suggested window for initiating such therapies aligns with the period when patients begin to exhibit a noticeable trend towards hypoxia and inflammation, typically occurring around days 7 to 14 post-symptom onset (58-61).

OPTIMIZING PATIENT SELECTION FOR TCZ

Selection of patients for TCZ therapy in the context of COVID-19 involves a meticulous analysis of various biomarkers and clinical parameters. Studies like COVACTA and RECOVERY have provided deeper insights into these aspects (51, 54). In the COVACTA study, the analysis of biomarker values such as CRP, IL-6, and ferritin played a crucial role in identifying patient subsets most likely to benefit from TCZ (51). For instance, post hoc analysis revealed that high ferritin levels were predictive of a positive response to TCZ (62). Although there were no clinically significant differences in time to death by day 60 among subgroups based on CRP, IL-6, or ferritin

values, these biomarkers continued to play a pivotal role in identifying patients with a hyperinflammatory response (62).

In the REMAP-CAP study, which included critically ill patients, TCZ demonstrated efficacy in reducing the need for ongoing organ support and improving survival compared to standard therapy (63). This study further emphasized the importance of considering disease severity and the need for intensive support when selecting patients (63). RECOVERY, a large randomized trial, provided robust support for the use of TCZ in hospitalized COVID-19 patients. TCZ significantly reduced the risk of death by day 28 compared to standard therapy, regardless of the type of respiratory support (54). This study underscored the importance of interpreting results in the context of respiratory support and highlighted that the greatest benefit of TCZ might be seen in patients already receiving corticosteroids (54). In addition to the mentioned studies, trials like BACC Bay TCZ Trial and MARIPOSA also contributed to understanding patient selection for TCZ therapy (64, 65). BACC Bay TCZ Trial focused on patients with severe COVID-19 pneumonia and showed a significant reduction in the risk of progression to severe respiratory failure or death (64). MARIPOSA, a randomized, double-blind, placebo-controlled study, investigated the efficacy of TCZ in patients with non-critical COVID-19 pneumonia and did not show significant clinical improvement compared to placebo (65).

These studies emphasize the need for personalized and careful patient selection for TCZ therapy, considering the specificities of the disease, severity of the condition, inflammatory levels, and other relevant factors (51, 54, 62-65). The combination of biomarkers, clinical parameters, and respiratory support plays a crucial role in decision-making to achieve optimal clinical outcomes (51, 54, 62-65).

TCZ AND CORTICOSTEROID SYNERGY

The mechanisms of synergy are intricate and involve the immunosuppressive effects of corticosteroids, such as dexamethasone, inhibiting the immune response (66-70). The combination of these characteristics with the specific IL-6 inhibitor, such as TCZ, leads to a deeper impact in controlling the cytokine storm and reducing hyperinflammation (67, 68). A coordinated approach to these mechanisms becomes crucial for successfully addressing severe forms of COVID-19. Corticosteroid dosage plays a pivotal role in achieving synergy (70). Dose individualization, especially of dexamethasone, enables precise control of inflammation without a significant risk of adverse effects (69). Preliminary data indicate that optimal dexamethasone dosage, in combination with TCZ, may result in a reduction in mortality of up to 30% compared to monotherapy (69).

Research results also suggest a similar mortality benefit between patients treated exclusively with TCZ and those receiving combination therapy (66-70). For instance, patients who received a combination of these medications had a 15% lower mortality rate compared to those who received TCZ alone (68). However, simultaneously, an increased risk of infections, especially fungal and viral, is observed. Preliminary data show that the risk of these infections increases by 20% in patients receiving combination therapy compared to those using TCZ alone (68). Hence, careful risk-benefit evaluation becomes crucial in making dosage decisions.

TCZ AND OTHER MEDICATIONS

One crucial combination involves pairing TCZ with remdesivir, an antiviral medication targeting virus replication (71). TCZ, as an IL-6 inhibitor, intervenes in the proinflammatory response, while remdesivir directly targets the virus. This combination has the potential to act selectively on both the virus and the pathological host response (71-73).

In combination with nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or diclofenac, TCZ may enhance anti-inflammatory effects, reducing inflammation symptoms (74). This is particularly significant in controlling the cytokine storm characteristic of severe COVID-19 cases (74). Moreover, the use of anticoagulants in combination with TCZ can significantly reduce the risk of thrombosis, a common occurrence in severe cases of the disease (75-77). The interaction between these drugs can effectively target the coagulation system, mitigating hypercoagulability (75). Combining it with remdesivir can further block vital steps in viral replication, while combining it with NSAIDs can intervene in inflammation-related signaling pathways.

TCZ has also demonstrated interactions with other drugs through liver enzyme systems (49, 50). For instance, studies have shown no clinically significant impact on the exposure of methotrexate, but caution is needed when combining it with statins like simvastatin, as it may affect the blood levels of these drugs (50). It is important to note that drug combinations come with certain risks, including potential interactions and adverse effects (49). Therefore, precise dosing, monitoring of laboratory parameters, and individualized therapy are crucial elements for the successful implementation of such therapeutic strategies (50). These findings provide a foundation for further consideration and adaptation of therapeutic protocols in the treatment of COVID-19, particularly considering the individual characteristics of patients and the specifics of the disease (49, 50).

TCZ IN COVID-19: PATIENTS FROM SERBIA

In the context of TCZ therapy for COVID-19 in Serbia, the available evidence presents a nuanced picture of its efficacy and outcomes. In a retrospective study evaluating TCZ in COVID-19 treatment, distinct observations emerge. The study, involving 205 patients with severe pneumonia, highlights positive outcomes (78). TCZ, administered alongside corticosteroids, led to a significant decrease in CRP and IL-6 levels, with notable improvements in oxygen support requirements. However, a mortality rate of 18.5% among severely ill patients suggests ongoing challenges (78).

A closer examination reveals that the first study, involving 205 patients with severe pneumonia, offers distinct advantages over the second, particularly when considering the unique context of experiences in Serbia. A notable strength of the first study lies in its substantial sample size, involving 205 patients. This larger cohort, especially relevant in the Serbian context, provides a more robust dataset for analysis, potentially offering insights into how TCZ may impact a diverse patient population.

In the second study, the focus was on complications in COVID-19, specifically ARDS and CRS (79). The study conducted a retrospective observational analysis of 92 severe COVID-19 pneumonia patients in Serbia who received tocilizumab in addition to standard therapy (80). The results indicated that patients receiving conventional oxygen therapy before tocilizumab showed a significant decrease in respiratory support after treatment, suggesting a potential beneficial effect (80). Notably, patients requiring high-flow oxygen therapy before tocilizumab treatment had a higher mortality risk compared to those on conventional oxygen therapy (79).

As for the third study, a case presentation involved a male patient with COVID-19 and bilateral pneumonia, who also had psoriasis vulgaris (80). Tocilizumab was administered due to disease progression, and a notable observation was the retreat of psoriatic lesions after tocilizumab administration (80). Despite reports of tocilizumab-induced psoriasis, the study suggested that disrupting IL-6 signaling could be a treatment option for psoriasis (80). This case, although singular, adds an intriguing perspective to the potential broader applications of tocilizumab beyond COVID-19 treatment (80).

In summarizing these studies collectively, while the first study offers insights into the general efficacy and challenges of tocilizumab in severe COVID-19 cases in Serbia, the second and third studies provide additional layers of understanding. The second study hints at the potential benefits of tocilizumab in reducing respiratory support requirements, especially in the earlier stages of ARDS and CRS. Meanwhile, the third study introduces a unique case where tocilizumab appears to have had a positive impact not only on COVID-19 but also on a pre-existing dermatological condition.

CONCLUSION

In conclusion, TCZ shows promising results in reducing inflammatory markers and improving oxygen needs in COVID-19 treatment globally. Despite generally good tolerance, caution is warranted, especially in critically ill patients prone to specific side effects. TCZ's effectiveness varies across clinical trials, emphasizing the importance of timely initiation during the inflammatory phase (days 7-10 post-symptoms). A personalized approach, considering biomarkers like CRP, IL-6, and ferritin, is crucial for patient selection. In the broader context, including Serbia, retrospective studies indicate positive outcomes but also challenges in managing severely ill patients. Amidst the TCZ shortage, investigating alternative dosage strategies, such as a pragmatic 400 mg dose, potentially provides a cost-effective option with comparable mortality benefits, despite the absence of a pharmacoeconomic study to support this. The need for further research and continuous patient monitoring remains essential in managing pandemic challenges.

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UPOTREBA TOCILIZUMABA U LEČENJU TEŠKE FORME KOVIDA 19: PREGLED

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Sažetak

Ovaj pregled se bavi terapijskom primenom Tocilizumaba (TCZ) u lečenju Kovida 19, posebno istražujući njegove mehanizme, bezbednosne aspekte, kliničku efikasnost, strategije doziranja i rezultate u srpskom kontekstu. TCZ, koji deluje kao inhibitor IL-6 receptora, suzbija citokinsku oluju koja se javlja u teškim slučajevima, koristeći svoju strukturu i farmakokinetiku. Dok ukupni profil bezbednosti ukazuje na dobru podnošljivost, postoje suptilne zabrinutosti kod kritično obolelih pacijenata u pogledu javljanja retkih komplikacija. Klinička ispitivanja, sa određenim varijacijama, naglašavaju potrebu za pažljivom interpretacijom indikacija i selekcijom pacijenata za terapiju TCZ-om. Trenutni protokoli koji su na snazi u Republici Srbiji preporučuju primenu TCZ-a u dozi od 8 mg/kg telesne težine na osnovu kliničkih parametara i parametara inflamacije, prvenstveno nivoa IL-6. Uvidom u literaturu uočeno je da se doza tokom nestašice TCZ može korigovati na 400 mg u jednoj dozi tokom lečenja teškog Kovida 19. Optimalno vreme za početak terapije podudara se sa fazom povećane inflamacije (7-10 dana nakon pojave simptoma), uz naglasak na izboru pacijenata prema biomarkerima, težini bolesti i potrebi za respiratornom podrškom. Kombinacija TCZ-a sa kortikosteroidima pokazuje smanjenje smrtnosti, uz potrebu za opreznim doziranjem. Potencijalne koristi proizilaze iz kombinacija TCZ-a sa remdesivirom, NSAIL lekovima i antikoagulansima, što zahteva pažljivo doziranje i nadzor. Retrospektivna istraživanja u Srbiji beleže pozitivne rezultate, ističući potencijal TCZ-a u lečenju ozbiljnih slučajeva. Ukratko, TCZ pokazuje obećavajuće rezultate u lečenju Kovida 19, zahtevajući dalja istraživanja i pažljivo praćenje pacijenata, posebno u uslovima ograničenih resursa.

Ključne reči: Tocilizumab, teška forma Kovida 19, efikasnost, bezbednost, iskustva

Primljen: 25.12.2024. I **Revizija:** 22.02.2024. I **Prihvaćen:** 29.02.2024.

Medicinska istaživanja 2024; 57(2):173-182

Izdavač i vlasnik | Publisher and owner
Medicinski fakultet Univerziteta u Beogradu

Uredništvo i administracija | Editorial board and administration
11105 Beograd, Dr Subotića br. 8, soba 311

Tehnički urednik | Technical editor
Radević Vladimir

Lektor za engleski jezik | English language editor
doc. dr Danka Sinadinović

Tehnički sekretar | Technical secretary
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Fakultet pedagoških nauka u Jagodini Univerziteta u Kragujevcu