THE UNIVERSITY OF MEDICINE AND PHARMACY
„CAROL DAVILA”, BUCHAREST
DOCTORAL SCHOOL
GENERAL MEDICINE
GASTROENTEROLOGY

PHD THESIS

SUMMARY

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PREDICTIVE FACTORS OF SPONTANEOUS BACTERIAL PERITONITIS DIAGNOSIS AND MORTALITY

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Introduction

I chose spontaneous bacterial peritonitis (SBP) as a research topic considering that it represents one of the most widespread infections in patients diagnosed with liver cirrhosis (LC), has a different frequency and a significant mortality rate, although there are guidelines for the diagnosis and treatment of this condition. Thus, the current gold standard for the diagnosis of PBS involves performing a paracentesis with the detection of the number of polymorphonuclear neutrophils (PMN) in the ascites fluid. This technique is an invasive method and can be burdened by complications.

I believe that one of the most important factors in treating this disease is early detection, for this reason the identification of non-invasive, accessible and easy to implement parameters that have a predictive role for the diagnosis of patients with SBP is essential. Therefore, recently, special attention has been paid to non-invasive techniques for PBS prediction, especially prediction models that are simple, available and reliable, involving parameters that are easy to collect in hospitals and help doctors to start treatment without delay to avoid complications. However, data in the literature have not demonstrated whether these non-invasive methods are sufficiently accurate to identify the development of SBP in patients with LC, and we believe that these initiatives and areas of uncertainty should be the focus of future research.

This work aims to identify predictive factors of the diagnosis and mortality of patients with PBS, which can be incorporated into a non-invasive prediction algorithm and demonstrate its applicability in clinical practice.

I. GENERAL PART

1. Liver cirrhosis

1.1 Incidence and prevalence.

According to data provided by the World Health Organization (WHO), in the countries of Eastern Europe in the early 1950s, the highest mortality rate was recorded in Hungary (57.5/100,000 men and 22.0/100,000 women), Romania (34.8/100 000 men and 17.1/100 000 women), Czechoslovakia and the former Yugoslavia [1]. Although mortality has decreased in most countries of the world, in Great Britain and in the countries of Central and Eastern Europe there is still an upward trend due to the increase in the prevalence of alcohol consumption [2].

Between 1970 and 2015, countries in Southern and Western Europe had a low mortality rate, while countries in Eastern and Northern Europe experienced a significant increase [3].

1.2. Etiology

Alcohol consumption, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection remain among the leading etiologies of LC and liver disease.

According to the WHO, alcohol is a risk factor involved in the occurrence of more than 200 diseases and globally causes 5.9% of deaths and in the 20-39 age group it causes more than 25% of deaths [4].

HBV and HCV are recognized as being involved in the occurrence of acute and chronic hepatitis that can progress to the stage of LC, liver cancer and even death [5]. In the period 2000-2015, the countries with the highest prevalence of viral hepatitis B (VHB) were Romania and Greece (3.3%) whereas most countries had a prevalence of approximately 1% or less [6].

Nonalcoholic hepatic steatosis includes a series of conditions that can evolve from the stage of simple hepatic steatosis to the stage of steatohepatitis where apoptosis, inflammation and fibrosis appear with a progressive evolution towards cryptogenetic cirrhosis [7].

Autoimmune hepatitis (HAI) manifests itself as a chronic inflammatory disease of the liver and the detection of serum autoantibodies, hypergammaglobulinemia and specific histological changes is necessary for diagnosis. The correct diagnosis must exclude the other diseases that can mimic autoimmune hepatitis, namely: viral, medicinal, alcoholic, hereditary (Wilson's disease, hemochromatosis), metabolic and immune-mediated cholestatic diseases [8].

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by cholestasis and chronic inflammation whose diagnosis requires the presence of 2 of the following 3 criteria: positive mitochondrial antibodies (AMA), unexplained persistent cholestasis manifested by elevated alkaline phosphatase over 24 weeks and/or characteristic histological changes. Liver biopsy is indicated when CBP-specific antibodies are absent or when HAI or NAFLD is suspected [9].

Primary sclerosing cholangitis is a chronic, autoimmune disease affecting the large, intra- and extra-hepatic bile ducts. Compared to PBC where the involvement is predominant in the small bile ducts and occurs more frequently in women, CSP occurs more frequently in men with an average age of 40 years. The diagnosis is based on the cholangiographic changes of the

bile ducts (multifocal strictures and segmental dilations) with the exclusion of secondary sclerosing cholangitis [10].

Hereditary hemochromatosis is an autosomal-recessive condition, manifested by iron overload and associated with a mutation of the HFE gene [11]. Indirect markers of iron storage, total iron-binding capacity and serum ferritin, are used initially in the suspected diagnosis of hemochromatosis.

Wilson's disease is an autosomal recessive condition that occurs as a result of mutation of the ATP7B gene located on chromosome 13 and encoding the copper-transporting ATPase. All this leads to an excess of copper in the liver and extrahepatic tissue with the appearance of clinical manifestations of the disease [12]. They can vary from the asymptomatic state to the appearance of chronic liver manifestations, neuropsychiatric or even fulminant liver failure.

Chronic liver disease produced by drug toxicity is uncommon, usually showing mild changes in liver tests, except for a small number of cases that develop LC from onset. Among the risk factors associated with chronic evolution, are advanced age, dyslipidemia and the severity of the acute episode [13].

1.3 Diagnostic methods.

Invasive methods

Although liver biopsy is still considered the "gold standard" because it provides additional information in selected cases, it also has certain limitations related to sampling error or high interobserver variability [14].

Portal hypertension (PHT) is responsible for the majority of LC complications and its measurement by hepatic venous pressure gradient (HVPG) may yield more valuable prognostic information than liver biopsy in certain situations.

Portal pressure can be determined by measuring HVPG, defined as the difference between the pressure in the portal vein and the inferior vena cava, with a normal value in the range of 3-5 mm Hg. Thus a value >5 mm Hg is suggestive of compensated advanced chronic disease (cACLD). A value > 10 mm Hg indicates the occurrence of complications (clinical decompensation, appearance of varices) [15].

Upper digestive endoscopy (EGD) is a method by which both the presence of eso-gastric varices and signs indicating an increased risk of bleeding (large varices or "red dots") can be

identified. Currently, the Baveno VI consensus conference recommends avoiding EGD in patients with cACLD who have an elastographically measured liver stiffness < 20 kPa and a platelet count > 150,000 mm3 due to the low risk of having "high-risk" varices [16].

Non - Invasive methods

Hyaluronic acid in combination with indirect tests such as bilirubin (BT), gamma-glutamyl-transferase (GGT), alpha 2- macroglobulin, age and sex form the Hepascore.

The enhanced liver fibrosis (ELF) score comprises a combination of direct tests (hyaluronic acid, TIMP-1 and P3NP) and age.

Among indirect non-invasive tests, the platelet value is used quite frequently in practice, but in combination with other tests because it represents the first and most frequent change that patients with CH present [17]

The AST - platelet ratio index (APRI) score is based on a formula containing the AST value and the platelet value, whose utility in the diagnosis of fibrosis has been validated especially in patients with HCV [18].

Fibrotest is a patented formula that uses two clinical parameters (age and sex) and 5 biological parameters (BT, GGT, alpha 2- macroglobulin, haptoglobin, apolipoprotein), the results of which are correlated with the stages of fibrosis in the METAVIR score, originally developed on HCV patients [19].

The FIB4 score is based on a formula containing age, serum transaminase and platelet count. It was initially developed and validated in HIV patients coinfected with HCV [20] and subsequently validated in patients with C viral liver cirrhosis.

The Fibro Meter score includes age and a series of biological parameters: platelets, prothrombin index, AST, macroglobulin G2, hyaluronic acid, urea nitrogen (BUN).

FibroScan is an easy-to-perform method that requires a short procedure time and can be performed even at the patient's bedside. However, in obese patients it has a reduced applicability even with the XL probe which, although it reduces the failure rate, increases the rate of unreliable results and is not recommended in patients with ascites [21].

Transient elastography (TE) has the ability, with high accuracy, to identify patients with clinically significant portal hypertension (CSPH), but it is not an optimal method in identifying gastroesophageal varices [22].

The Acoustic Radiation Force Impulse (ARFI) technique allows the measurement of a region of interest to estimate liver elasticity, it can also be used in obese patients and the Two Dimensional Shear Wave Elastography (2D-SWE) technique allows the measurement of liver elasticity in real time and can be used and in patients with ascites [23].

Magnetic Resonance Elastography (MRE) has some advantages compared to other methods of elastography by ultrasonography, namely: it allows measuring the elasticity of a wider area, it is less operator-dependent and has a lower rate of technical errors. [24]

Imaging investigations in the diagnosis of LC

Ultrasound can provide signs related to liver morphology as well as signs of PHT. Signs related to morphology include: nodular appearance of the liver, left or caudate lobe hypertrophy and among the fundamental signs of CSPH are: porto-systemic abdominal collaterals, splenomegaly, dilatation of the porto-splenic-mesenteric venous axis, minimal ascites, hepatofugal flow in the system portal venous, decreased flow velocity in the portal venous system and lack of variability in inspiration/expiration of the splenic and mesenteric veins [25].

Liver morphologic changes may not appear in the early stages of cirrhosis on cross-sections, so evaluation by computed tomography and nuclear magnetic resonance is not useful at this stage. In the more advanced stages, a series of signs can be observed such as: nodular liver surface, hypertrophy of the caudate lobe, hypertrophy of segments II, III and atrophy of segments IV, VI, VII at the level of the right liver lobe, enlargement of the hilar periportal space, enlarged gallbladder fossa , widening of interlobular fissures. The sign with high diagnostic accuracy is represented by the ratio between the transverse width of the caudate lobe and the width of the right lobe greater than or equal to 0.65 [26].

1.4 Complications of liver cirrhosis

1.4.1 Hepatic encephalopathy

HE is a complication of liver dysfunction, determined by acute liver failure and CH and/or the presence of porto-systemic shunts. It manifests as a spectrum of neuropsychiatric manifestations that can range from minimal cognitive impairment to the stage of coma [27]. The main recommendations in the treatment of HE according to the European and American guidelines are represented by the identification and treatment of precipitating factors, the use of lactulose as the first therapeutic option, the association of rifaximin to prevent recurrence, the

use of BCAA (branched chain amino acids) and LOLA (L-ornithine-L-aspartate), neomycin or metronidazole as an alternative in patients unresponsive to conventional therapy.

1.4.2 Variceal hemorrhage

Varicella hemorrhage is a gastroenterological emergency that requires the patient to be hospitalized in the intensive care unit. Management of variceal hemorrhage includes: volume resuscitation, prophylactic antibiotic therapy, vasoactive drug therapy, and performing upper digestive endoscopy within the first 12 hours of presentation.

Blood transfusions should be performed conservatively with the goal of maintaining a hemoglobin (Hb) value between 7 and 9 g/dl. Ceftriaxone administered in a dose of 1g/24 h for a short duration (maximum 7 days) is the effective antibiotic in infectious prophylaxis. Among the vasoactive drugs, somatostatin or its analogue octeotride, vasopressin or its analogue terlipressin can be used [15].

1.4.3 Hepato-renal syndrome

Hepatorenal syndrome (HRS) is a severe complication that can occur in patients with liver cirrhosis and ascites, in patients with severe acute liver failure or severe acute alcoholic hepatitis. The treatment of patients who meet the criteria for HRS-AKI consists in the administration of vasoconstrictors associated with albumin [28].

1.4.4. Hepato-pulmonary syndrome

Hepatopulmonary syndrome (HPS) is characterized by the triad of abnormal arterial oxygenation caused by intrapulmonary vascular dilatations in the setting of advanced liver disease, PHT, or congenital portosystemic shunts. Contrast-enhanced echocardiography is the gold standard for the diagnosis of pulmonary vasodilatation. Liver transplantation is the only effective treatment, which can achieve a survival of 88% at 5 years post-transplant in patients with HPS [29].

1.4.5. Portopulmonary hypertension

Portopulmonary hypertension (POPH) is characterized by pulmonary arterial hypertension occurring in the context of portal hypertension, with or without advanced liver

disease. The diagnosis requires the exclusion of other causes of pulmonary hypertension such as: chronic thromboembolism, chronic lung diseases/hypoxia, chronic left heart failure [28]. According to the AASLD guideline, POPH screening in patients who are candidates for liver transplantation is performed with the help of echocardiography. At a value of right ventricular systolic pressure ≥ 45 mmHg, right heart catheterization is indicated [30].

1.4.6. Hepatic hydrothorax

Hepatic hydrothorax (HH) is defined as an excessive accumulation (> 500 ml) of transudate in the pleural cavity in patients with decompensated liver cirrhosis, in the absence of cardiac and pulmonary disease . Diagnosis requires performing a thoracentesis with pleural fluid analysis to exclude the presence of infection or other pulmonary pathologies. First-line treatment of HH is similar to that of ascites and consists of sodium restriction and diuretic therapy. Despite this therapy, approximately 21% to 26% of patients develop refractory hydrothorax and the only effective treatment is liver transplantation [31].

1.4.7. Cirrhotic cardiomyopathy

Cirrhotic cardiomyopathy is defined as a chronic cardiac dysfunction characterized by impaired contractile sensitivity to stress stimuli and/or impaired diastolic relaxation and electrophysiological abnormalities in the absence of known cardiac disease. There is currently no specific treatment for this condition. The cardiac decompensation episode may follow the same principles as in non-cirrhotic patients including salt and fluid restriction, diuretics, and preload-reducing medications [32].

1.4.8 Ascites

The development of ascites in patients with CH is associated with a poor prognosis, as mortality at one year is approximately 40% and at 2 years approximately 50% [33]. For this reason, patients with grade 2 and 3 ascites should be evaluated as possible candidates for liver transplantation.

Treatment of uncomplicated ascites depends on the degree of severity. Thus, grade 1 ascites does not require treatment. In contrast, grade 2 ascites requires sodium restriction and

diuretic therapy. Large-volume paracentesis combined with administration of human albumin is the first-line therapy for patients with grade 3 ascites [34].

Refractory ascites occurs in approximately 5% - 10% of all ascites cases and is associated with an increased mortality rate. The median 1-year survival rate is approximately 50% [35].

1.4.9. Hyponatremia

Hyponatremia is one of the most common dyselectrolytemias in patients with advanced liver cirrhosis and is defined as a serum Na concentration <130 mmol/l, but a value <135 mmol/l should also be considered hyponatremia in the general patient population. Cirrhotic patients may develop hypervolemic hyponatremia to a greater extent due to increased extracellular volume and to a lesser extent hypovolemic hyponatremia due to loss of extracellular fluid (overtreatment with diuretics or laxatives). Treatment in hypervolemic hyponatremia consists of fluid restriction and measures to enhance renal excretion of free water [36].

1.4.10. Hepatocellular carcinoma

HCC is a leading cause of death, estimated to be the fourth most common cause of cancer death worldwide. Definitive diagnosis is made by CT with contrast substance and/or MRI. Typical signs are hypervascularity in the late arterial phase and rapid contrast washout in the portal venous phase and/or delayed phases. Histological diagnosis by liver biopsy may be necessary if HCC develops in the non-cirrhotic patient and if imaging tests are inconclusive [37].

The Barcelona - Clinic Liver Cancer (BCLC) staging system has been repeatedly validated and is recommended for prognostic prediction and treatment allocation. Liver transplantation is the most definitive treatment option, as it removes not only the tumor but also the liver, which has a limited functional capacity [37].

2. Spontaneous bacterial peritonitis

2.1 Incidence and prevalence

The incidence of SBP varies depending on the population studied. Thus, it is estimated that the incidence reaches 3.5% at 1 year in outpatients with decompensated LC and varies

between 7%-30% in hospitalized patients [198]. A higher prevalence of pneumonia and UTI and a lower prevalence of SBP was detected in Asian centers compared to American and European centers [40].

2.2. Clinical features

Patients with SBP may have the following symptoms: abdominal pain, abdominal tenderness, vomiting, diarrhea, ileus, hyper or hypothermia, chills, leukocyte change, tachycardia/tachypnea, worsening liver function, HE, renal failure, gastrointestinal bleeding [28].

2.3. Diagnostic methods

According to the European guideline, the diagnosis of SBP is based on the performance of a paracentesis for diagnostic purposes, which must be performed without delay, ideally within 6 hours of admission or deterioration of the patient's condition and before the administration of antibiotic treatment Thus, the analysis of ascites fluid with a value of PMN> 250 mm3 confirms the diagnosis. There are several variants of SBP, namely: neutrocytic ascites in which PMN≥250 mm3 and culture is negative and bacterascites in which PMN<250 mm3 and culture is present [28]. Definite diagnosis requires the exclusion of secondary bacterial peritonitis.

Therefore, the gold standard in the diagnosis of SBP is the PMN value in the ascites fluid, but different laboratory markers have been studied that could be useful in prompt diagnosis and prediction of response to initial treatment. However, the data are limited and require further studies to confirm the results.

2.4. SBP treatment

In patients with community infection, in areas with a low degree of bacterial resistance, the antibiotic treatment of choice is the third generation cephalosporins. Conversely, in areas with a high degree of bacterial resistance, piperacillin/tazobactam or a carbapenem are appropriate therapeutic options. For healthcare-acquired or nosocomial infections, in areas with a high prevalence of ESBL, carbapenems are the antibiotic of choice and should be used in combination with glycopeptides or daptomycin or linezolid in areas with a high prevalence of MDR gram-positive bacteria [28].

Patients who show a real benefit from treatment with albumin are patients who have had a history of renal failure (BUN>30 mg/dl, creatinine>1mg/dl) or severe hepatic decompensation (BT>5mg/dl) and the standard recommended dose it is 1.5 g/kg at the time of diagnosis and 1 g/kg on the third day of treatment [41].

Patients who are indicated for prophylactic treatment are: patients with upper gastrointestinal bleeding (UGIB), patients with a low value of total protein in the ascites fluid associated with severe liver failure or renal failure and the absence of a previous episode of SBP (primary prophylaxis) and patients with previous episodes of SBP (secondary prophylaxis) [41].

The use of treatment with norfloxacin 400 mg/day or ciprofloxacin 500 mg/day or, alternatively, co-trimoxazole (160 mg trimethoprim and 800 mg sulfamethoxazole)/day during hospitalization has been shown to be useful in reducing the incidence of SBP as well as the incidence of extraperitoneal infections and of short-term mortality [42]. Patients who have recovered from an episode of SBP should receive long-term prophylactic treatment with norfloxacin (400 mg/day) and in situations where norfloxacin treatment is unavailable, oral ciprofloxacin may be an acceptable alternative [28].

2.5 Prognosis

The one-, two-, and three-year mortality rates for patients hospitalized for SBP were 53.9%, 61.4%, and 66.5%, respectively. Also, patients with LC and SBP had a 2.5-fold risk of 3-year mortality compared to those without ascites [43].

II. PERSONAL CONTRIBUTIONS

3. Working hypothesis and general objectives

Working hypothesis

SBP is a complication of patients with liver cirrhosis, with increased morbidity and mortality. Prompt diagnosis and treatment can reduce the risk of progression to an adverse event.

General objectives :

- correlation of the occurrence of SBP with epidemiological, clinical and paraclinical data
- identification of a prediction model of the diagnosis
- correlation of SBP mortality with epidemiological, clinical and paraclinical data
- identification of a mortality prediction model.

4. General research methodology

The data obtained from the study protocols were entered into a database and processed using SPSS 23.0 and Microsoft Excel 2010 programs.

For the descriptive statistics part, the mean and standard deviation were calculated, respectively the medians and quartiles for the quantitative variables, and for the qualitative ones, frequencies and percentages. To compare the quantitative data, depending on the normality of the data, the Student t-test (for two groups with normally distributed data) and Mann-Whitney (for data that did not have a normal distribution) were used.

To verify the existence of correlations between the categorical variables (synthesized as frequencies and percentages), the Fisher exact (binary data) and Pearson Chi-square tests as well as the Likelihood Ratio (if 20% of the expected frequencies were lower than 5) were used.

Probability of error less than 5% ($p< 0.05$) was considered the significance threshold.

Also included in the thesis were graphical representations such as pie, simple and layered bar charts for summarized data as frequencies and percentages.

The ROC curve was used to determine an optimal cut-off point for the test, as well as to assess the per-total accuracy of the test. The validity of the tests was estimated using the area under the curve (AUC) with 95% confidence interval.

5. Study 1.

Predictive factors of the diagnosis of spontaneous bacterial peritonitis in patients with liver cirrhosis in the last decade in Constanța county

5.1 Introduction

SBP is one of the most common infections that patients with liver cirrhosis can develop and despite the diagnostic and treatment recommendations presented in the European guidelines, there are inconsistencies in current practice. The diagnostic challenge lies in the fact that patients do not always present obvious symptoms and for this reason a paracentesis for diagnostic purposes is necessary. According to data from the literature, paracentesis is performed late, leading to a delay in diagnosis and implicitly the appearance of complications.

Numerous non-invasive methods have been studied for a faster diagnosis and to avoid paracentesis, but the data are contradictory. The present study has as its main purpose the evaluation of the factors that could be used for the prediction of SBP and the realization of a prediction model of the diagnosis, which would be non-invasive and accessible.

5.2 Patients and methods

We conducted a retrospective observational study that included 216 patients previously diagnosed with liver cirrhosis of various etiologies and ascites, in the Gastroenterology clinic of the Emergency County Hospital "Sfantul Apostol Andrei", Constanța, between January 2010 and December 2019. The study was carried out with the approval of the ethics committee of the County Emergency Hospital "Sfântul Apostol Andrei" in Constanța, preserving the confidentiality of patient data.

Criteria for inclusion in the batch :

- patients over 18 years of age with an established diagnosis of LC (clinical, biological, ultrasound, endoscopic or histological according to the criteria of the diagnostic guidelines) and ascites with a subset of patients over 18 years of age with an established

diagnosis of SBP (according to the criteria of the diagnostic guidelines) diagnosis) with or without positive culture

Exclusion criteria :

- patients diagnosed with hemorrhagic ascites or peritonitis due to surgery
- patients diagnosed with another diagnosed infection than SBP such as upper and lower respiratory tract infection, urinary tract infection, otitis media
- patients with evidence of intra or extrahepatic malignancy
- patients with hematological disorders, non-infectious inflammatory diseases such as ankylosing spondylitis and rheumatoid arthritis
- patients with current or recent pregnancy.

The group of patients was divided into two subgroups, namely: 72 cirrhotic patients diagnosed with SBP and 144 cirrhotic patients who did not present this diagnosis. When the group of patients was formed, an analysis of the observation sheet was carried out, which included: epidemiological factors, the severity of liver cirrhosis assessed by the Child-Pugh score, the presence of antecedents of UGID and HE, the clinical examination, the presence in the personal pathological antecedents of cardiac and pulmonary comorbidities , the paraclinical factors: values of serum leukocytes, hemoglobin (Hb), platelets, BT, albumin, creatinine, INR, erythrocyte sedimentation rate (ESR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), the ratio between neutrophils and lymphocytes (NLR), sodium (Na) and alkaline reserve (RA), values of leukocytes and polymorphonuclear neutrophils (PMN) in ascites fluid and imaging methods. Diagnosis of SBP was based on PMN count in ascites fluid ≥ 250 cells/mm3 and/or positive culture.

Statistical analysis

The experimental data were processed using the statistical processing program IBM SPSS Statistics 23.

Subchapters 5.3 and 5.4. Results and discussion

216 cirrhotic patients were included in the study for which clinical, biological and paraclinical data are recorded and processed, and then their impact on SBP detection is calculated. In the studied group, the male gender predominates and the predominant etiology is alcoholic liver cirrhosis.

In the present study, no significant differences were found in terms of age and occurrence of SBP between the group of patients diagnosed with SBP (mean age was 59.29 years) and those without PBS (mean age was 62.23 years), ($p=0.061$, Independent Samples Test).

Regarding the Child-Pugh score, there were statistically significant differences in class B and C in patients with SBP compared to those without SBP. A percentage of 71.5% of patients without SBP were assigned to class B vs. 29.1% of patients with SBP and 70.8% of patients with SBP were assigned to class C vs. 28.5% of patients without SBP ($p<0.001$). However, the Child Pugh score was not identified as a predictor of SBP.

In the study conducted, no correlation was found in terms of mean values of transaminases and hemoglobin and the occurrence of SBP.

Ascites fluid analysis showed statistically significantly higher levels of leukocytes and PMNs in patients with SBP compared to those without SBP ($p<0.001$, Mann Whitney Test). These results are consistent with those of the study by Mohammed et al. [44] who reported a higher value of leukocytes and PMNs in patients with SBP ($p=0.001$). In contrast, univariate analysis in the present study did not identify a correlation between leukocyte and PMN values in ascites fluid and the occurrence of SBP.

Univariate analysis (logistic regression) confirms that the changed values of serum leukocytes, platelets, BT, serum albumin, INR, creatinine, ESR, serum Na, RA, NLR as well as the presence of digestive hemorrhage can be used as factors of prediction in the occurrence of SBP in the group of patients studied (table number 1).

Table number 1. Univariate analysis (logistic regression) regarding predictors in the occurrence of SBP

Parametres	P-value	OR value	95% CI
Serum leukocytes (10^3/μl)	< 0.001	1.61	1.42-1.83
Trombocites (10^3/μl)	< 0.001	0.98	0.98-0.99
BT (mg/dl)	< 0.001	1.79	1.49-2.16
Serum albumin (g/dl)	< 0.001	0.46	0.25-0.82
INR	< 0.001	6.82	3.50-13.29
Creatinine (mg/dl)	< 0.001	1.52	1.13-2.05
VSH (mm/h)	< 0.001	1.30	1,20-1,40
Na (mmol/L)	< 0.001	0.89	0.85-0.93
RA (mmol/L)	0.001	0.89	0.83-0.95
NLR	< 0.001	138.2	26.4-724.2
UGID	< 0.001	6.72	3.59-12.57
Cardiac comorbidities	0.013	0.45	0.24-0.85

Regarding the role of serum leukocytes in the occurrence of SBP, in the conducted study it was observed that there are statistically significant differences between the mean value of leukocytes in patients with SBP (10.61 10^3/μL) compared to the mean value in patients without SBP (6.36 10^3/μL). Also, for each unit added beyond the normal value, the risk of predicting SBP increases 1.61-fold.

The results of the present study show that patients with SBP have lower mean platelet values compared to patients without SBP. Thus, for each unit that decreases from the normal value, the chance of SBP increases by 0.98 times. Therefore, recent data [45] have shown that thrombocytopenia can be used as a non-invasive marker to predict SBP, being more often used alongside other parameters in various prediction scores.

The increase in total bilirubin reflects the degree of liver damage and the decline of the liver synthesis function, which can lead to a significant decrease in the monocyte-macrophage system with the migration of bacteria and an increase in the risk of SBP. The study by Xiang et al.[46] confirms this, namely the value of total bilirubin (OR =1.003; p<0.001) was associated with a higher incidence of PBS. Similar to the results of the present study, where for each

additional unit added (1 mg/dl) over the normal value, the risk of SBP increases 1.79-fold ($p<0.001$).

In literature studies [47], there is a statistically significant difference between serum albumin values in patients with PBS and those without in the sense that patients with SBP presented lower values compared to patients without SBP. At the same time, they do not identify this parameter as a predictor in the occurrence of SBP. Contrary to these data, in the study carried out, serum albumin was identified as a predictive factor of SBP and for each unit (1 g/dl) that decreases from the normal value of serum albumin, the risk of SBP increases by 0.46 times ($p<0.001$).

INR measurement has a good ability to predict SBP, so for each unit added above the normal value, the risk increases 6.82 times. The study by Metwally et al [45] shows that patients with SBP have a mean INR of 2.2 compared to 1.8 in patients without SBP ($p<0.001$). In the present study the mean value of INR in patients with SBP was 2.24 compared to 1.6 in patients without SBP ($p<0.001$).

In the study performed, measurement of creatinine value is a good non-invasive marker for predicting SBP. Thus, for each unit (1 mg/dl) added in addition to the maximum allowed normal value, the chance of PBS increases 1.52 times ($p<0.001$). On the other hand, data from the literature have shown that an increased creatinine value is a risk factor in the occurrence of kidney damage in patients with PBS, one of the complications of this disease. For this reason, albumin is administered prophylactically to decrease the incidence of kidney disease and thereby the mortality rate in these patients.

Also, in the results of this study [46] as in the conducted study, the value of potassium was not identified as a predictive factor of SBP. In contrast, the present study shows that RA value can be used in predicting SBP (OR=0.89; $p=0.001$). However, in the specialized literature there is no correlation between the value of RA and the appearance of SBP.

Biologically, the measurement of serum sodium level reflects the state of liver function and hyponatremia can be a precursor to increased intestinal permeability and the appearance of SBP. In this study, it was found that hyponatremia can be used as a useful parameter in predicting SBP (OR=0.89; $p<0.001$). Another study conducted by Wang et al.[48] also identify hyponatremia as an independent risk factor for SBP (OR=3.54; $p=0.001$).

Data from the present study show that the presence of UGID has a good ability to predict SBP (OR=6.72; p<0.001) and the study by Ali et al [49] concludes that the overall prevalence of SBP in patients who had UGID is high (29%).

In terms of comorbidities, 42.4% of patients without SBP were observed to have cardiac comorbidities. Thus, the present study identified cardiac comorbidities as a predictive protective factor in the occurrence of SBP (OR=0.45; p=0.013). These results are in discord with the study of Thiele et al. [50] in which there were no statistically significant differences regarding the presence of arterial hypertension between the group of patients with SBP and the control group.

Following the multivariate analysis, in the conducted study it was observed that the value of the NLR and the ESR can be used as independent predictors of PBS. It is found that the ESR value has a very good ability to predict PBS (AUC=0.916, p<0.001) with a sensitivity of 80.56% and a specificity of 87.50% at a cut-off value >33 mm /h (figure no. 1). In other words, we can hypothesize the use of ESR as an independent non-invasive marker with a role in the diagnosis of SBP. It should also be taken into account that although it is a cheap, quick and simple test to perform, it can be affected by a variety of factors and is not sensitive enough for screening.

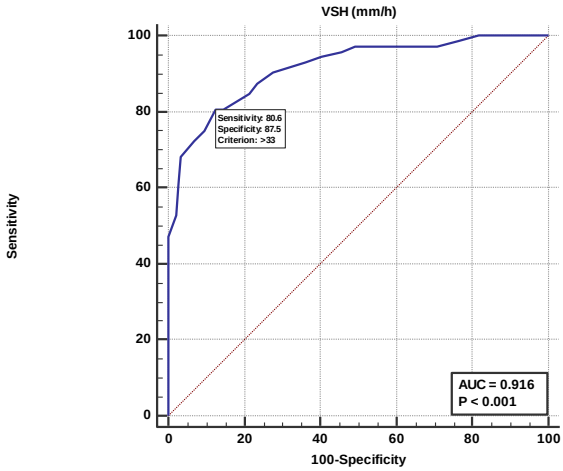

Figure number 1. ROC curve on the significance of ESR in predicting PBS

The evaluation of the diagnostic value of NLR in SBP has gained particular interest, but the data are contradictory regarding the cut-off value. In the study conducted, it was observed that the NLR values were significantly higher in patients with PBS compared to patients without SBP (p<0.001). At a cut-off value >2.4 the NLR value had a sensitivity of 98.61% and

specificity of 81.94% to predict SBP, with excellent diagnostic accuracy (AUC=0.963, p<0.001) (figure number 2).

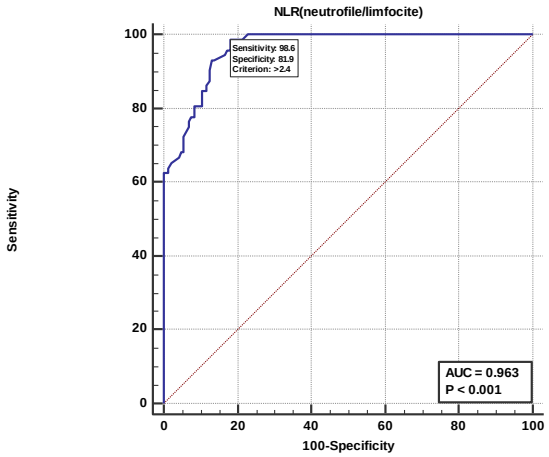

Figure number 2. ROC curve on the significance of NLR in SBP prediction

An attempt was made to develop a non-invasive algorithm for predicting SBP, involving the value of ESR and NLR. The area of the ROC curve regarding the predictive ability of this algorithm was 0.990, with a 95% confidence interval from 0.965 to 0.999, with an excellent score for predicting SBP in cirrhotic patients (figure number 3).

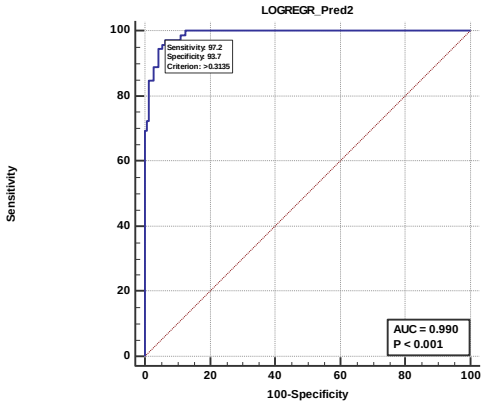

Figure number 3. ROC curve for the PBS prediction model constructed from VSH and NLR variables

5.5 Conclusions

1. The presence of SBP, one of the most severe complications of liver cirrhosis, can be predicted with the help of non-invasive markers such as an increase in the number of serum leukocytes, BT, INR, creatinine, ESR and a decrease in the number of platelets, albumin serum, Na, RA or NLR value.
2. Also, another predictive factor of SBP is the presence of UGID. In the logistic regression model, a 6.72-fold increase in the occurrence of SBP is observed ($p<0.001$).
3. Cardiac comorbidities were identified as a protective factor in the occurrence of SBP (OR=0.45; p=0.013).
4. The increase in the number of serum leukocytes has proven to be an effective non-invasive marker for confirming the diagnosis of SBP. In addition, the chance of developing SBP increases 1.61-fold ($p<0.001$) for each additional unit added to the normal value of serum leukocytes.
5. Determination of the BT value is another useful marker for predicting SBP and for each unit added to the normal value, the chance of occurrence is added 1.79 times ($p<0.001$).
6. Another effective non-invasive parameter in the diagnosis of SBP is the determination of the INR value, and adding an extra unit to its value increases the chance of occurrence of SBP by 6.82 times ($p<0.001$).
7. The chance of SBP can also be predicted by determining the creatinine value. In addition, analyzing the logistic regression model, a 1.52-fold increase in chance is found ($p<0.001$).
8. Elevated values of the ESR represent a non-invasive marker with a role in the diagnosis of SBP. In the present study, a specificity of 80.50% was observed at a cut-off value >33 mm/h and with a very good performance (AUC=0.916, 95% CI 0.870-0.949).
9. Thrombocytopenia can be used as a non-invasive marker to predict SBP increasing the chance of occurrence by 0.95 times ($p<0.001$).
10. The determination of the value of serum albumin is an easy parameter to use in predicting SBP and for each unit that decreases from the normal value, the chance of occurrence increases by 0.46 times ($p<0.001$).

11. The low values of Na and RA represent other non-invasive markers with a role in the diagnosis of SBP considering that following the logistic regression these parameters had a 0.89 times chance of prediction ($p<0.001$ and respectively $p= 0.001$).
12. The NLR value is a relatively recent non-invasive parameter with a role in the diagnosis of PBS. In the study performed, a specificity of 81.94% is observed at a cut-off value > 2.4 and an excellent performance (AUC=0.963) in predicting SBP.
13. The present study highlighted that, although after univariate analysis several parameters were significantly associated with the diagnosis of SBP, their incorporation in the multivariate logistic regression model showed that only certain biological parameters were independent predictive factors in the occurrence of SBP, namely ESR and NLR. Therefore, their inclusion in a non-invasive algorithm determined a predictive model with an excellent performance (AUC=0.990) for establishing the diagnosis of SBP.

6. Study 2

Predictive factors of spontaneous bacterial peritonitis mortality in patients with liver cirrhosis in the last decade in Constanța county

6.1 Introduction

The mortality trend of patients with SBP has seen a decrease in the recent period compared to the time when this condition was discovered, but it remains on an upward slope despite the efforts of a prompt diagnosis and an effective treatment administered according to the recommendations of the great experts.

The use of the optimal treatment is often difficult, knowing that recently there has been a change in the microbial epidemiology involved in the emergence of SBP, in the sense of the increase of multi-drug resistant bacteria. These being a cause of mortality in patients with SBP. Also, most patients with liver cirrhosis and SBP develop renal failure and elevated creatinine values are a predictor of mortality. The main aim of the present study is to evaluate the factors that could be used as predictors of mortality.

6.2 Pacients and methods

We included in this observational retrospective study 72 patients previously diagnosed with liver cirrhosis of various etiologies and SBP, in the Gastroenterology clinic of the Emergency County Hospital "Sfantul Apostol Andrei", Constanța, between January 2010 and December 2019. The study was carried out with approval of the hospital's ethics committee, preserving the confidentiality of patient data.

Criteria for inclusion in the batch :

- patients over 18 years of age diagnosed with liver cirrhosis (clinically, biologically or histologically according to the criteria of the diagnostic guidelines)
- patients over 18 years of age diagnosed with SBP (with or without positive culture)

Exclusion criteria :

- patients with secondary cause peritonitis
- patients diagnosed with HCC or other neoplasms
- patients diagnosed with severe comorbidities
- patients with active UGID

The patients were divided into two groups, namely: 52 curable SBP patients and 20 deceased SBP patients. Patient data were collected from observation sheets.

The diagnosis of CH was made based on clinical, biological and ultrasound data and the diagnosis of SBP based on a number of PMN ≥250 cells/mm^3 and/or positive culture in the ascites fluid. Epidemiological data, etiology of liver cirrhosis, severity of LC assessed by Child Pugh score, number of episodes of SBP and symptomatology, duration and cost of hospitalization of patients were analyzed. Paraclinical data such as serum leukocyte count, ESR, serum Na, and ascites fluid leukocyte count and PMN percentage were recorded. Also the data related to complications developed by patients during hospitalization. The types of microorganisms involved in the occurrence of SBP in patients with a positive culture, the duration of treatment and the type of antibiotic chosen to treat this condition were studied.

Statistical analysis

The experimental data were processed using the statistical processing program IBM SPSS Statistics 23.

Subchapters 6.3 and 6.4. Results and discussion

In the present study, in-hospital mortality between December 2019 and January 2010 was 27.8%. In the data from the literature there is a variation in the mortality rate, which can be caused, in large part, by the guidelines and local practice, the heterogeneity of the patients' characteristics, the variability in the onset of the diagnosis, the time of the initiation of antibiotic therapy, as well as antibiotic resistance.

In the study conducted, no correlation was observed between the age and gender of patients with SBP and mortality. The average age of the patients was 59.29 years and the male gender was predominant (66.7%). Regarding the Child Pugh score, a significantly higher proportion of deceased patients compared to curable patients were assigned to class C (100% versus 59.6%, $p<0.001$). However, univariate analysis in the present study did not identify Child Pugh score as a predictor of mortality in patients with SBP.

From the point of view of the culture and type of microorganism identified, no significant differences were recorded between the two groups of patients with SBP. In a percentage of 58.3% the culture was positive and Escherichia coli was identified in 33.3% of cases. In the present study, five cases of positive culture with Staphylococcus were identified (11.9%) and three of them (4.2%) were resistant to methicillin and treated with vancomycin. Of these cases, only one patient survived. Because of the relatively low proportion of cases, the study data showed that the type of microorganisms involved in the occurrence of SBP is not a risk factor for in-hospital mortality in these patients.

The results of the study conducted are similar to those of the study by Thuluvath et al [51] in that there were no statistically significant differences in the mean length of hospital stay between deceased and curable patients. Conversely, contrary to the present study, the authors observed an increase in average costs due to increased resource use by a few patients.

Regarding the etiology of liver cirrhosis, the most common was alcoholic in a percentage of 61.1% followed by viral C etiology in a proportion of 30.6%. In particular, it was observed

that a higher proportion of curable patients were diagnosed with viral liver cirrhosis C compared to deceased patients (38.5% versus 10%, p=0.001) (table no.1). Thus, it was found that the presence of C viral etiology is a protective factor (OR=0.17; p=0.001). One hypothesis for this correlation could be related to new antiviral treatments for which there is evidence that they can improve histological regression of liver cirrhosis and this improvement is associated with decreased morbidity [52].

Table number 1. Univariate logistic regression analysis of risk factors associated with mortality in patients with SBP.

Parametres	**Coefficients**	**Value p**	**OR**	**95%CI**
Fever	0.000492	0.000205	7.77	2.45-24.65
Chills	0.000036	0.000004	14.90	4.13-53.72
Hepatic encephalopathy	0.028503	0.000205	3.31	1.13- 9.70
Serum leukocytes ($10^3\mu L$)	0.000875	0.000825	1.16	11.06-1.27
Ascites leukocytes (/mm3)	0.008195	0.012568	1.00	1.00-1.01
% PMN from ascites	0.014296	0.002023	1.04	1.00-1.08
ESR (mm/h)	0.016362	0.004098	1.05	1.00-1.09
Previous episodes of PBS	0.007682	0.004479	3.27	1.36-7.83
IRA	0.000831	0.000408	6.92	2.22-21.52
Sepsis	0.001461	0.000079	34.00	3.87-298.34
Sirsi	0.000001	0.000000	36.00	8.55-151.48
Viral etiology C	0.030431	0.018862	0.17	0.03- 0.84

In terms of symptomatology, it has been observed that there is a correlation between the occurrence of fever and chills and in-hospital mortality in patients with SBP. Therefore, univariate analysis in the present study identified fever and chills as predictors of mortality. It is found that for each additional unit of fever (1 degree), the risk of mortality increases 7.77 times ($p<0.001$). Also, the presence of chills increases the risk of mortality by 14.90 times ($p<0.001$). Similarly, in the study by Zakareya et al [53] it was observed that the presence of fever was an independent clinical factor predicting mortality at 1 month (OR=1.49; 95%CI=1.058-2.182, p=0.041) and at 3 months (OR=1.67; 95%CI=1.230-2.351, p=0.025).

Hepatic encephalopathy was another predictor of in-hospital mortality identified in the study (OR=3.31; p=0.002). Similar results were also observed in the study by Melcarne et al

[54] in which hepatic encephalopathy was one of the independent risk factors for 30-day mortality (OR=4.34; p=0.001).

An increased percentage of deceased patients had a previous episode of SBP compared to curable patients (65% versus 26.9%, p=0.004). Thus, it is found that the presence of a previous episode of SBP increases the risk of in-hospital mortality by 3.27 times (OR) with a confidence interval between 1.36 and 7.83; which is why it is a predictor of mortality in the study conducted. Contrary to these results, no correlation was observed between previous episodes of SBP and mortality in the study by Morsy et al [55].

In the study by Bal et al [56] it was observed that AKI increases the risk of mortality by 2.16 times (95%CI=1.36-3.42) and septic shock by 1.73 times (95%=1.05 -2.83). These data are consistent with the study conducted, in which it was found that the diagnosis of AKI in patients with SBP increases the risk of in-hospital mortality by 6.92 times and sepsis by 34 times, being predictive factors of mortality (p<0.001). In addition, SIRS diagnosis was observed to be another good predictor of mortality (OR=36, p<0.001).

In the conducted study it was observed that the average value of Na in deceased patients was significantly lower compared to curable patients (p<0.001). Although the Na value had an excellent ability to predict mortality, with an ROC curve area of 0.998, logistic regression did not identify it as a predictor of mortality. In contrast, the results of Morsy et al [55] identified the value of Na as a predictive factor of in-hospital mortality in patients with PBS with a higher cut-off value (mean value was ≤126 mmol/L) than in the study performed (mean value was ≤120 mmol/L) and lower discrimination accuracy (AUROC=0.840).

The results of the present study show that deceased patients have significantly higher mean values of serum leukocytes compared to curable ones (18.5 10^3µl versus 12.5 10^3µl; p<0.001). Furthermore, the study demonstrated that a cut-off value > 14.39 10^3µl of serum leukocytes has a good ability to predict the mortality of patients with SBP, having an area of the ROC curve of 0.756. Poca et al [57], demonstrated that incorporating the value of serum leukocytes alongside other parameters in a predictive model has a good accuracy of predicting mortality (AUC=0.850).

In the study carried out, it was demonstrated that there was a correlation between the values of leukocytes and the percentage of PMN in the ascites fluid and in-hospital mortality in patients with SBP. Thus, for each additional unit of leukocytes in ascites, the risk increases 1.00

times (OR) and may be a predictor of mortality, but with low accuracy, having an ROC curve of 0.691. Also, for each additional added unit of PMNs in ascites fluid, there is a 1.04-fold increase in mortality risk (OR) with good predictive ability (AUC=0.736). The data are consistent with the study by Ahmed et al [58] in which ascitic fluid leukocyte and PMN values were significantly associated with mortality ($p<0.001$).

The value of ESR can be used as a predictor of mortality (OR=1.09) with greater accuracy than the value of leukocytes and the percentage of PMN in ascites fluid or the value of serum leukocytes, having the area of the ROC curve of 0.777 (figure no. 1). Moreover, the study performed demonstrated that a cut-off value greater than 40 mm/h has a sensitivity of 84.6% and a specificity of 71.0% in predicting mortality.

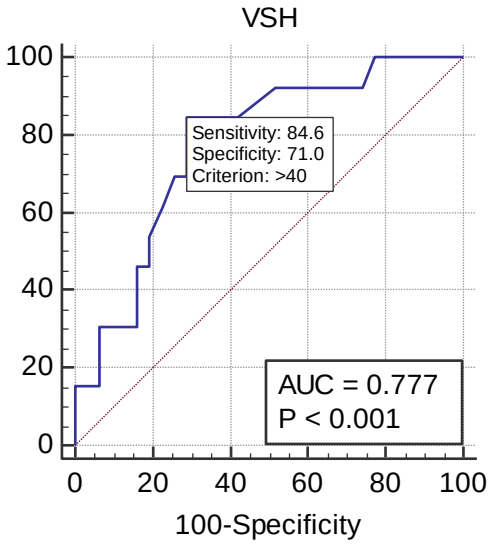

Figure number 1. ROC curve on the value of ESR in predicting mortality.

Comparatively, it is observed that for the assessment of mortality risk, the ROC curve for the variable serum Na differs significantly from the ROC curves of the variables: serum leukocytes ($p=0.0135$), ESR ($p=0.0032$), leukocytes from ascites ($p= 0.0048$), PMN percentage from ascites ($p=0.0026$), meaning that the use of serum Na has a very good ability to predict mortality. It cannot be ignored that the other parameters can also be used as non-invasive markers for predicting mortality, but they must be used with caution (figure no. 2).

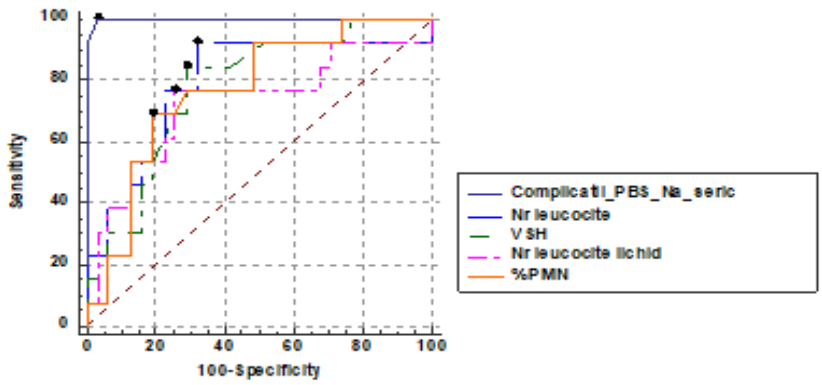

Figure number 2. AUROC comparison of the significance of serum Na, serum and ascites leukocytes, ESR, and percentage of ascites PMN in predicting mortality.

An attempt was made to develop a model for predicting mortality in SBP patients using multivariate logistic regression, and thus SIRS (OR=103.68) and fever (OR=18.71) remained the only independent predictors. The association of these factors had an excellent ability to predict in-hospital mortality with an area of the ROC curve of 0.940.

ROC curve analysis

Area under the ROC curve (AUC)	0.940
Standard Error	0.0546
95% Confidence interval	0.825 to 0.989

6.5 Conclusions

1. Mortality of cirrhotic patients diagnosed with SBP is statistically significantly correlated with Child Pugh C Score and hyponatremia ($p<0.001$).
2. The presence of viral C etiology of liver cirrhosis represents an independent protective factor of mortality in patients with SBP ($p=0.01$).
3. The presence of a previous episode of SBP is a predictor of in-hospital mortality ($p=0.004$).

4. The determination of the number of serum leukocytes proved to be a non-invasive marker with a good ability to predict the mortality of patients with PBS. In the study performed, a sensitivity of 80% is observed, at a cut-off value >14.39 10^3μl and with a good performance (AUC=0.756).

5. Mortality in patients with SBP can also be predicted by the leukocyte count in the ascites fluid. In the present study, a specificity of 90.4% is observed at a threshold value >2598, but with poor performance (AUC=0.691).

6. The value of ESR is another predictive factor of in-hospital mortality in patients diagnosed with SBP (OR=1.05; p=0.016). Using the ROC curve, a sensitivity of 84.6% was revealed at a cut-off value >40 mm/h and a good discriminatory performance (AUC=0.777).

7. Also, determining the percentage of PMN in ascites fluid can be used as an independent predictor of mortality in patients with SBP (OR=1.04; p=0.014). In the conducted study, a specificity of 78.8% at a cut-off value >87.6% and a good performance (AUC=0.736) is observed.

8. The onset of fever and chills in patients with SBP are other predictors of in-hospital mortality. It is found that for each additional unit of fever (1 degree), the risk of mortality increases 7.77 times (p<0.001) and the presence of chills leads to an increase in the risk of mortality 14.90 times (p<0.001).

9. The presence of HE is an independent predictor of mortality in patients with SBP. In addition, the risk of mortality increases 3.31 times (p<0.001) for each additional degree added.

10. Another predictor of mortality is the diagnosis of AKI in patients with SBP, which increases the risk of mortality 6.92 times (p<0.001).

11. The occurrence of sepsis and SIRS may predict mortality in patients with SBP. In the logistic regression model, a 34-fold and 36-fold increase in mortality risk is observed respectively (p<0.001).

12. The present study revealed that a model for predicting the mortality of patients with SBP that includes the presence of fever and SIRS shows an excellent performance (AUC= 0.940).

7. Personal contribution

In the first stage, I carried out a research of the scientific literature by consulting over 200 bibliographic references and the work entitled "Spontaneous bacterial peritonitis: update on diagnosis and treatment" appeared, where I made a brief review of the current methods of diagnosis and treatment of this condition. Also, my personal contribution materialized by creating non-invasive models useful both in terms of the diagnosis of patients with SBP and regarding the stratification of patients at increased risk of adverse events, who would benefit from new therapeutic strategies in addition to the standard current care.

The studies carried out in the framework of the doctoral thesis presented several limitations, namely: the limitations inherent in a retrospective analysis (missing data in the observation sheets or the correct adjudication of the clinical results), the retrieval of data from a single center, the relatively small number of patients involved and the study short-term (in-hospital) mortality.

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ANNEXES

List of abbreviations and symbols

AMA– Anti- Mitochondrial Antibody

AKI– Acute Kidney Injury

APRI– AST to Platelet Ratio Index

ARFI– Acoustic Radiation Force Impulse Imaging

BT– Total Bilirubin

BCAA– Branched-Chain Amino Acids

BUN– Blood urea nitrogen

cACLD– Compensated Advanced Chronic Liver Disease

2D SWE– Two Dimensional Shear Wave Elastography

ELF– Enhanced Liver Fibrosis

ESBL– Extended Spectrum Beta-Lactamase

HAI– Autoimmune Hepatitis

HIV– Human immunodeficiency virus

HVB– Hepatitis B virus

HVC– Hepatitis C virus

HE– Hepatic Encephalopathy

HVPG– Hepatic venous-portal gradient

HRS– Hepatorenal syndrome

HPS– Hepatopulmonary syndrome

HCC– Hepatocellular carcinoma

HH– Hepatic hydrothorax

INR– International normalized ratio

LC- Liver Cirrhosis

LOLA– L-ornithine L-aspartate

MRE– Magnetic Resonance Elastography

NAFLD– Non-Alcoholic fatty liver disease

Na– Sodium

NLR– Neutrophil-Lymphocyte Ratio

PMN– Polymorphonuclear neutrophils

POPH– Portopulmonary hipertension

PHT- Portal hypertension

PBC– Primary Biliary Cholangitis

RA– Alkaline reserve

SIRS– Systemic Inflammatory Response Syndrome

SBP– Spontaneous bacterial peritonitis

TE- Transient elastography

UGID– Upper Gastrointestinal Bleeding

UTI- Urinary tract infection

ESR– Erythrocyte Sedimentation Rate

WHO– World Health Organization

List of published scientific papers

- From the topic of the thesis

1. **Popoiag Roxana-Emanuela**, Panaitescu E, Suceveanu AI, Suceveanu AP, Micu SI, Mazilu L, Parepa I, Voinea F, Costea DO, Enache F, Fierbințeanu-Braticevici C. Spontaneous bacterial peritonitis mortality trends of cirrhotic patients in the last decade in Constanta County. Exp Ther Med. 2021 Jul;22(1):732. DOI: 10.3892/etm.2021.10164. **ISI-IF: 1,448** (capitolul II.6.2- p.104,105, subcapitolul 6.3- p.121-137)
https://www.spandidos-publications.com/10.3892/etm.2021.10164

2. **Popoiag Roxana-Emanuela**, Suceveanu AI, Suceveanu AP, Micu SI, Voinea F, Mazilu L, Petcu LC, Panaitescu E, Cozaru G, Fierbințeanu-Braticevici C. Predictors of spontaneous bacterial peritonitis in Romanian adults with liver cirrhosis: Focus on the neutrophil-to-lymphocyte ratio. Exp Ther Med. 2021 Sep;22(3):983. DOI: 10.3892/etm.2021.10415. **ISI-IF: 1,448** (capitolul II.5.2- p.56-58, subcapitolul 5.3- p.83-93)
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1. Suceveanu AI, **Popoiag R**, Mazilu L, Parepa IR, Gheorghe A, Stoian A, et al. Management of Ascites Associated with Severe Hyponatremia. Management of Chronic Liver Diseases - Recent Advances. InTech; 2018.
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- Posters

1. **Roxana-Emanuela Popoiag**, Andrada Florinela Dumitru, Sergiu-Ioan Micu, Andra-Iulia Suceveanu, Adrian-Paul Suceveanu, Felix Voinea, Laura Mazilu, Lucian Cristian Petcu, Carmen Braticevici-Fierbinteanu. Erytrocyte sedimentation rate and neutrophil-to-lymphocyte ratio can be a simple and useful test for the diagnosis of spontaneous bacterial peritonitis. Journal of Gastrointestinal and Liver Diseases, volume 30, supplement 1, May 2021.