DOCTORAL DISSERTATION SUMMARY

HEPATO-RENAL DYSFUNCTION IN SEPSIS: ETIOLOGICAL, CLINICAL, PARACLINICAL AND THERAPEUTIC CORRELATIONS BENEFICIAL IN ETIOLOGICAL THERAPY

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I. Introduction
Sepsis is the host’s response to a microbial agent and its toxins, while severe sepsis and septic shock are most often the causes of anomalies in the infectious process. The concept of *sepsis* has been the subject of intense debate over time (1, 2), so a huge effort has been made over the past 20 years to find new therapies to reduce mortality, but unfortunately, leaving aside the progress made, dissatisfaction is still present (2, 3).

**Epidemiological data in sepsis**
The major impact of sepsis with multiple organ disorder on the nursing units was estimated, in the European Union, at 90.4 cases per 100,000 inhabitants, as compared to 58 per 100,000 in breast cancer (4). It is estimated that there are 1.8 million cases annually, worldwide, and this in terms of a weak identification rate or under diagnosis. Recent estimates showed an incidence of sepsis in intensive care units between 0.25 and 0.38 per 1,000 inhabitants, which suggests two million admissions to intensive care units (5, 6).

III. Sepsis pathogenesis
Sepsis comprises a great heterogeneity within patients with different conditions, infection sites, a multitude of microbial agents with high variation of microbial virulence and concentration, with differences in relation to the inflammatory response and immunological context.
The response from the host is connected to either the secretion products of the pathogen agent or to one of its fragments. For Gram positive or negative bacteria, teichoic acid and lipopolysaccharides together with pieces of cell wall constitute components able to induce inflammatory response, sepsis and septic shock. Thus, in the face of this aggression, the hostess triggers, successively, a series of defensive mechanisms such as: natural barriers (epithelium, mucous membranes), innate immunity and adaptive immunity. The imbalance of these different means of defence participates in the evolution toward a misfit response, characterized clinically by septic shock (7, 8), Figure 1.
The inflammatory response is often adapted to the etiologic agent or toxic aggression, or, on the contrary, a series of micro-organisms may lead to high levels of mediators from infected body, having increased expression in patients with multiple organ dysfunction associated. Currently it is not possible to provide a quick assessment of organisms’ capacity to have an adequate inflammatory response, and it is considered that the mechanisms related to organ dysfunction as those which are carried out in the evolution towards death would be similar in the context of different etiologic agents (7, 9).

Bacterial toxins such as endotoxins and superantigens are commonly involved in the pathogenesis of sepsis with Gram-positive and Gram-negative, as they determine signals through cellular mechanisms and induce inflammation through different routes (10, 11).

The affected organ plays an important role, being known for example that the defence mechanisms of the lung and those of the peritoneum are different, leading to different possibilities for evaluation of inflammation (12). These antigenic structures and toxins determine the inflammatory process through the connection with the CD14 receptor of the mononuclear phagocytes in circulation. Tumour necrosis factor (TNF), interleukin 1 (IL-1), IL-6, IL-8 and platelet activating factor (PAF) are released by monocytes. IL-1 and IL-6 activate the T-cells, resulting in the formation of interferon-gamma, IL-2, IL-4, the activation factor of granulocytes monocytes (GM-CSF) (13, 14).
XIII. Hepatorenal syndrome (HRS) in sepsis

Clinical and physiopathogenical aspects in HRS

- Hepatorenal syndrome may occur spontaneously, by altering liver function or secondarily by precipitation of an event such as infection (15).
- Hepatorenal syndrome is caused by intrarenal vasoconstriction that occurs in patients with terminal liver disease status and associated circulatory dysfunction.
- Circulatory dysfunction is characterized by vasodilatation in splanchnic circulation with relative and insufficient decrease of carbon monoxide, leading to hypovolemia (16).

HRS: short history

At the end of the 19th century, assessments made by Frerichs (1861) and Flint (1863) noted an association between advanced liver disease, ascites and renal insufficiency with oliguria in the absence of renal histological changes (17). Almost 100 years later, in an article signed by Hecker and Sherlock, it was described the pathogenesis of HRS (18). Detailed studies carried out by Epstein et al. have shown that splanchnic circulation and systemic vasodilatation together with the intense renal vasoconstriction are the physiopathogenic mechanism of renal HRS (19). However, despite the advances in knowledge, HRS prognosis remained gloomy at that time, and in the 1970’s the concept of "functional terminal renal failure" was the equivalent of hepatorenal syndrome (20).

In 2006, the definitions for HRS showed that this is a functional reversible kidney damage that occurs in patients with advanced liver cirrhosis or fulminant liver failure and is characterized by reduction in glomerular and plasma filtration rate in the absence of other causes of renal dysfunction (21). With the rise of knowledge, it has been proved that the original definition was very narrow as to a large number of exclusion criteria such as sepsis (22). Until it was reviewed, guidelines proposed in 2007 that bacterial infections are considered an exclusion criterion for diagnosis of HRS (23).

Mechanism of the hepatorenal syndrome (HRS)

HRS mainly consists of an extreme renal vasoconstriction caused by activation of sodium retention mechanism and vasoconstriction systems, resulting in a major decline in the glomerular filtration rate (24). The cascade of events is determined by vasodilatation in the splanchnic circulation and blood volume decrease.

When data were available regarding HRS type II, a number of studies showed that terlipressin increases kidney function by more than 65% in patients with HRS type I, with a significant increase in survival (25,26,27). None of these
studies included patients with sepsis, which at that time was in agreement with the original definitions of the HRS (20). More recently, Rodriguez E et al. presented the results of a study that explored the role of terlipressin and albumin in patients with HRS type I and sepsis, the authors pointing out that there are similar results to those in studies where patients with sepsis were excluded (20).

The condition of hepatorenal syndrome diagnosis is kidneys’ integrity and this has been repeatedly demonstrated both in terms of morphology and normal function of the kidneys. The physiopathologic principle of mechanisms includes increased renal artery resistance, particularly affecting the renal cortex, which occurs in renal hypoperfusion (28).

Renal vasoconstriction *per se* is not sufficient for the development of HRS. Low blood pressure blood pressure is the key factor even where it does not reach the values in shock, being a simultaneous cause of vasoconstriction and renal hypoperfusion (29).

Thus, the circulating volume, a result of systolic volume is a relevant factor, and its level can be low, normal or increased, but is relatively insufficient to prevent severe decreases in the actual volume of circulating blood caused by splanchnic arterial vasodilatation in patients with hepatorenal syndrome.

**Table VII Hepatorenal syndrome type I and type II**

<table>
<thead>
<tr>
<th>creatinine</th>
<th>Evolution period</th>
<th>ascites</th>
<th>Liver damage</th>
<th>norepinephrine and renin</th>
<th>Presence of infectious process</th>
<th>prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HRS 1</strong></td>
<td>2 X N or &gt;2.5mg/dl</td>
<td>2 weeks</td>
<td>Possibly spontaneous bacterial peritonitis</td>
<td>Rapid profound damage</td>
<td>Intense activity</td>
<td>possible</td>
</tr>
<tr>
<td><strong>HRS 2</strong></td>
<td>&lt;2.5mg/dl</td>
<td>Slow progression</td>
<td>Diuretic-resistant ascites</td>
<td>Low/minimum damage</td>
<td>Minimum activity</td>
<td>may precipitate conversion from HRS1 To HRS2</td>
</tr>
</tbody>
</table>

HRS type I occurs as a consequence of severe reduction of circulating volume caused partly by a major splanchnic vasodilatation and, partly by decreased cardiac output, this type of disorder being characteristic to sepsis. HRS type II is characterized by a slow progressive loss of renal function, without being,
from the clinical point of view, the consequence of an acute deterioration of renal function, but the result of refractory ascites, and its impact on prognosis is less negative (30).

**HRS diagnosis**
Currently, there is no specific diagnostic test for a clear determination of HRS, as it is a diagnosis of exclusion on the basis of the revised criteria of the International Ascites Club (15). Primary investigations consist in identifying the glomerular filtration rate, with creatinine clearance < 40 ml/min or increased serum creatinine > 135 micromoles/L in the context of the exclusion of other causes of renal dysfunction. The most relevant indicator of functional character includes natriuresis < 10 mmol/L, urinary osmolarity > plasma osmolarity, natremia < 130 mmol/L and 500 ml diuresis <24 hrs.

**The new diagnostic criteria in HRS**
- Cirrhosis with ascites
- Serum creatinine > 133 micromol/L (1.5 mg/dL)
- Decreased serum creatinine to 133 micromol/L or less after 2 days of diuretics and administration of albumin solutions to expand volemia; the recommended dose of albumin is 1 g/kg bw/day, up to a maximum of 100 g/day
- Absence of shock
- Absence of recent treatment with nephrotoxic substances
- Absence of parenchymal diseases as an indicator of proteinuria > 500 mg/day, microhematuria (> 50 RBCs/HPF) and/or abnormal renal ultrasound.

Concerning the diagnostic criteria, it would be useful to distinguish between the concept of hepatorenal syndrome and hepatorenal insufficiency/dysfunction. In both cases, the parameters of renal function clinics are the same. However, the major difference lies in the circumstances of the occurrence and prognosis. Hepatorenal syndrome should be considered when assigning a specific status of renal function deterioration when other causes have been eliminated and precipitating factors excluded. Thus, hepatorenal syndrome is a more general term that can be applied to any deterioration of renal function in the context of liver disease or sepsis, with the involvement of portal hypertension (31).
XV. **Therapeutic principles in HRS**

- therapy in sepsis involving multiple organ dysfunction
- eliminate use of nephrotoxic substances
- eliminate gastrointestinal pain and loss compensation therapy
- eliminate use of nonsteroidal antirheumatics
- recover lost blood volume through intravascular volume supplement, preferably by correcting hypoalbuminemia (albumin is the best volume expander with the longest impact)
- remove diuretics (they increase central hypervolemia, the sympathetic activity and that of the renin-angiotensin-aldosterone system)
- avoid substitution measures to combat hyponatremia because of the risk for cerebral edema; the restriction on fluids is preferred (31).
XX. Subject importance
The term of hepatorenal syndrome (HRS), after it was described in 1863, was first used in 1932 in an effort to materialize the links between renal insufficiency which occurs after biliary tract surgery and is later used more broadly for any association between severe liver damage and kidney damage. The definition of HRS was described as renal insufficiency which arises in the context of severe liver disease, acute or chronic, in the absence of pre-existing renal pathology (32).

The first attempt to organize the diagnostic criteria was made by the International Ascites Club in 1996, when they were grouped into major and minor criteria (32, 33, 34). The use of these criteria showed, in time, the need for accuracy and additions due to the ambiguity of HRS definition. In 2006, in San Francisco, the International Ascites Club redefined HRS in terms of the diagnostic methods and taking into account the possible presence of infection, which had not been a criterion for exclusion (32, 33, 34).

The exact incidence of HRS is not known largely due to difficulties in diagnosis. In over 70% of cases of HRS, precipitating factors are identified such as infection (57%), gastrointestinal hemorrhage (36%), paracentesis (7%) or acute alcoholic hepatitis (35, 36).

It is now known that the acceptance of the infection and sepsis in defining HRS determined a decrease in mortality in patients with HRS, from 80% to 65% (32).

XXI. Material and General Methods
We have carried out the three studies in the Infectious Diseases Clinical Hospital of Iasi, here being hospitalised patients with an extremely complex pathology from the whole geographical area of Moldova and beyond, and considering the advanced technical possibilities, especially in the Intensive Care Department, specific to infectious diseases.

Both the adult age patients, or persons authorized in circumstances where it was not possible otherwise, and in the case of children, signed upon admission an informed consent formulated in accordance with the Helsinki Declaration and approved by the Ethics Committee of the University of Medicine and Pharmacy of Iasi, regarding consent to use personal data for the purpose of research.

The study included patients with the diagnosis of sepsis according to the definition of suspected or proven infection accompanied by the presence of SIRS (at least 2 of the following criteria: fever/hyphothermia, tachycardia, tachypnea, leucocytosis-leukopenia).
**Data statistics**

We set up a database in EXCEL which has included both the descriptive and qualitative and quantitative parameters, while data processing was performed using the SPSS version 16.0.

The types of statistical processing included:
1. student’s t-test
2. chi-square test
3. *Box Plot* chart type (with the median to the right of the middle, the rectangle contains 50% of the data and the extremes marked by vertical lines included data between 10 and 90%)
4. *Pearson’s* correlation for normally distributed variables
5. *Spearman* correlation - independent of the type of data distribution and based on the rank order of values
6. where data did not have a normal distribution we used the *Mann-Whitney Test* for comparing two sets of variables
7. *Pairwise Comparisons Test* for data comparison with small samples
8. for comparison of several data sets we used the *Kruskal-Wallis Test*
9. the *ROC curve* for determining sensitivity/specificity of the diagnostic tests
10. *Kaplan Meier* survival curve.

**XXII. Comparative study regarding the clinical, epidemiological, paraclinical evolution and therapeutic aspects in a group of patients who associated organ dysfunction in sepsis**

2. **STUDY OBJECTIVES**

Through this study we attempted an evaluation of epidemiological, etiological, paraclinical, laboratory and therapeutic aspects in patients diagnosed with sepsis, for which the clinical evolution context was studied, determining correlations between etiologic agents and severity of infection, establishing infection site, correlations between paraclinical parameters and APACHE II and Carmeli prognostic scores, correlations between clinical evolution model and antibiotherapy, establishing the role of associated comorbidities, and assessing the survival of these patients in the context of the different organ dysfunctions associated.

3. **MATERIAL AND METHODS**

The study included patients diagnosed with sepsis in the Infectious Diseases Clinical Hospital of Iasi, during November 2012 – April 2014, with a total of 247 patients grouped as follows:

1. a lot of 100 patients with sepsis associating multiple organ dysfunctions (called "MODS");
2. a lot of 77 patients with sepsis and hepatorenal dysfunction (also called “HRD”);  
3. a lot of 70 patients with sepsis and multiple organ dysfunction, with clinical evolution towards death (called “DECEASED”).

We have also taken into consideration the cases with discharge diagnosis of sepsis with determined etiology (positive hemocultures) or sepsis with undetermined or clinically suspected etiology (where samples from different biological products were positive). These patients had the results of the samples entered in the registry of the Microbiology Laboratory of the Infectious Diseases Clinical Hospital of Iasi. 

Patient inclusion criteria were in accordance with the standard definitions of sepsis.

4. RESULTS

We tried to determine if there is a statistical correlation between inflammatory syndrome elements throughout the lot of patients with sepsis, using two variants for the analysis of correlation: Pearson and Spearman. 

*The Pearson correlation* is used for normally distributed variables and measures a combination of linear type, while extreme values can influence strongly the value of the coefficient. 

*The Spearman correlation* is independent of the type of data distribution and is based on the rank order of values. Extreme values do not have a major influence on the calculation. 

This coefficient varies between -1 and 1.

We also verify the level of statistical significance (Table XXII. 6., Table XXII. 7.).

**Table XXII. 6. Establishing statistically significant differences between the elements of the inflammatory syndrome**

<table>
<thead>
<tr>
<th>Descriptive Statistics</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC upon admission</td>
<td>13307.98</td>
<td>8152.348</td>
<td>247</td>
</tr>
<tr>
<td>Fibrinogen upon admission</td>
<td>5.8867</td>
<td>1.53384</td>
<td>241</td>
</tr>
<tr>
<td>ESR upon admission</td>
<td>77.87</td>
<td>37.029</td>
<td>241</td>
</tr>
<tr>
<td>CRP upon admission</td>
<td>49.10</td>
<td>25.037</td>
<td>226</td>
</tr>
</tbody>
</table>

The table is symmetric, the correlation between white blood cells (WBC) and fibrinogen being equal to the correlation fibrinogen WBC, as the correlation coefficient is commutative.
**Table XXII. 7. Correlations in inflammatory syndrome in all patients with sepsis**

<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>WBC upon admission</th>
<th>Fibrinogen upon admission</th>
<th>ESR upon admission</th>
<th>CRP upon admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman’s rho</td>
<td>Correlation Coefficient</td>
<td>.210**</td>
<td>.001</td>
<td>.191**</td>
</tr>
<tr>
<td>N</td>
<td>247</td>
<td>241</td>
<td>241</td>
<td>226</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

Therefore, we obtained statistical significance for all combinations, which means that there are statistical correlations between elements of the inflammatory syndrome: an increase in WBC will determine a proportional increase in ESR, Fibrinogen, CRP, but reciprocal claims are also true (Table XXII. 8.).

**Table XXII. 8. Statistical correlations between the elements of the inflammatory syndrome in patients with sepsis**

<table>
<thead>
<tr>
<th>Coefficient of determination</th>
<th>WBC at admission</th>
<th>Fibrinogen - admission</th>
<th>ESR-admission</th>
<th>CRP-admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC at admission</td>
<td>1</td>
<td>0.0441</td>
<td>0.081225</td>
<td>0.036481</td>
</tr>
<tr>
<td>Fibrinogen at admission</td>
<td>1</td>
<td>0.191**</td>
<td>0.071289</td>
<td>0.112225</td>
</tr>
<tr>
<td>ESR at admission</td>
<td>1</td>
<td>0.071289</td>
<td>0.174724</td>
<td></td>
</tr>
<tr>
<td>CRP at admission</td>
<td>1</td>
<td>0.071289</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
The coefficient of determination multiplied by 100 represents, in percentages, the degree to which the variation in a variable is expressed by the other variable.

**Evaluating glycaemia modifications in patients with sepsis**

Of the metabolic changes that occur during sepsis, hyperglycaemia is the most important. Hyperglycaemia in severe sepsis is not merely a marker of the severity of the disease but also a predictor of adverse developments and has multiple ways of exercising the adverse effects at the level of vital organs. One of these side effects is on the innate defence system of the host against infection, resulting in a reduction of neutrophils activity such as chemotaxis and bacterial phagocytosis in spite of accelerated diapedesis of leukocytes in peripheral tissues, as well as alteration of cytokines and growth of early proinflammatory cytokines, TNF alpha and IL-6, and reducing the formation of endothelial nitric oxide (37).

<table>
<thead>
<tr>
<th>Spearman’s rho</th>
<th>WBC upon admission</th>
<th>Fibrinogen upon admission</th>
<th>ESR upon admission</th>
<th>CRP upon admission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycaemia upon admission</td>
<td>Correlation Coefficient</td>
<td>.170</td>
<td>.079</td>
<td>.057</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.007</td>
<td>.220</td>
<td>.382</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>247</td>
<td>241</td>
<td>241</td>
</tr>
</tbody>
</table>
Figure XXII. 12. Statistical correlations between glycaemia upon hospitalization (g/L) and leucocytosis in patients with sepsis (WBC/mm³)

Patients showed a variable number of organ dysfunction, and those which required monitoring in intensive care department had predominantly more than two affected organs (Figure XXII. 31.). According to studies, multiple organ failure in sepsis remains the most common cause of death in intensive therapy units (38).

Figure XXII. 31. Organ dysfunctions in patients who required monitorisation and therapy in the intensive care department
Analysis of patients who have associated multiple organ dysfunctions showed correlations between death and the presence of more than three affected organs (Figure XXII. 32).

**Figure XXII. 32. Type of evolution in patients with sepsis according to the number of organ dysfunctions**

Graphical representation of the *Kaplan Meier survival curve* on the entire group of patients showed a median estimate of 28, which suggested that 50% of cases died within the first 28 days of hospitalization, and within the first 14 days 25% of patients passed away (Figure XXII. 33.).

**Figure XXII. 33 Kaplan Meier survival curve for all patients included in the study**
Survival analysis in patients hospitalized in intensive care unit showed a median of 11 days, which means that 50% of patients die within the first 11 days, and according to the quartiles of the table, 25% of patients die within the first 4 days (Table XXII. 14, Figure XXII. 34.).

Figure XXII. 34. Kaplan Meier survival curve for patients in ICU

5. DISCUSSIONS
In patients with sepsis considered in this study, we tried a comparative evaluation of parameters related to systemic inflammatory response, so that statistical analysis of the variables of the inflammatory syndrome showed correlations between ESR, fibrinogen, CRP, leucocytosis and glycaemia, whether patients have associated or not hepatorenal dysfunction in development.

We can admit that there were biases concerning data on inflammatory syndrome, a number of factors being recognised as enhancers in relation to ESR values in the absence of inflammation, among them being age, female gender, obesity, hypercholesterolemia, nephrotic syndrome, renal insufficiency, cardiac insufficiency.

Considering that currently there is no specific diagnostic test for univocal determination of HRS, as it is a diagnosis of exclusion on the basis of the revised criteria of the International Ascites Club, we considered defining these patients according to the glomerular filtration rate, with a clearance of
creatinine < 40 ml/min or increased serum creatinine > 135 micromoles/L in the context of the exclusion of other causes of renal dysfunction (39). Although an adequate indicator of functional character of hepatorenal dysfunction includes natriuresis < 10 mmol/L, a urinary osmolarity higher than the plasma osmolarity, natremia < 130 mmol/L and diuresis < 500 ml/24 hours in normal practice, it was not possible for a full consideration of these criteria in all cases.

According to some authors, the hepatic dysfunction in sepsis represents the moment when total bilirubinemia is higher than 2 mg/dL (>34µmol/L), and alkaline phosphatase or serum aminotransferase values are greater than twice their normal value. In a study carried out by Angus and collaborators, from 192,980 cases of severe sepsis in the U.S., hepatic dysfunction was present in only 1.3% (40). However, this low incidence is explained through a highly restrictive definition of hepatic dysfunction, with the inclusion only of cases with acute and sub-acute liver necrosis. In 2001, the International Sepsis Definitions Conference has recommended the use of scoring systems such as SOFA (Sepsis-related Organ Failure Assessment), MODS or LODS (Logistic Organ Dysfunction System).

All of these scores aim to identify the degree of organ disorders during sepsis and use bilirubinemia to define disorders along with prothrombin time for LODS score (41, 42). At this conference, hepatic dysfunction was defined as total bilirubinemia greater than 4 mg/dL (µmol/L > 70), but interestingly, this cutoff value has not been proposed by any scoring system known. An exception is the acute phase of severe sepsis/septic shock where a considerable increase in the level of aminotransferase (20 times the normal limit) allows diagnosis of hypoxic hepatitis, incidentally, total bilirubinemia being used and proposed as a biomarker for the diagnosis of hepatic dysfunction in sepsis.

The nature of "systemic" evolution in sepsis, the high number of cell lines involved, organs and tissues expanding the area of candidate biomarkers, biochemical modifications determining varied responses over time, making it very difficult to define a "golden standard" in diagnosing and predicting the evolution of these patients (43).

Our analysis showed that each of the parameters studied was an independent variable in patients with sepsis, this being restrictive in terms of understanding as predicting factors, even when data integration is based on groups of patients.
XXIII. Comparative study regarding the clinical, epidemiological, paraclinical evolution and therapeutic aspects between a group of patients who associated hepatorenal dysfunction and another group with varied organ dysfunction in sepsis

2. STUDY OBJECTIVES
The trend of increase in the number of patients with sepsis hospitalised in the Infectious Diseases Clinic of Iasi in the past years was amplified by a higher degree of complexity, in many of these patients their development being accompanied by hepatorenal syndrome.
In the present study, we aimed to address the septic patient with hepatic-renal dysfunction and/or hepatorenal syndrome, from a detailed perspective, in comparative terms with the changes occurred in sepsis development involving other organ dysfunction.
Knowledge of these patients from clinical, etiological, paraclinical, prognostic score as well as therapeutic perspective represents a challenge in understanding the mechanisms underlying the hepatic/renal decompensation in sepsis. Since the redefining of the hepatorenal syndrome in 2007 and its association to decompressions in sepsis, studies have tried, in most cases, to identify similarities between the pathophysiological mechanisms of hepatorenal decompensation in liver cirrhosis versus sepsis, relatively few data being available concerning developments in patients with sepsis and hepatorenal syndrome.

3. MATERIAL AND METHODS
Our study included patients diagnosed with sepsis in the Infectious Diseases Clinical Hospital of Iasi, during November 2012 - April 2014, with a total of 117 patients who associated liver/kidney dysfunction, hepatorenal syndrome, but other organ dysfunction also.
Severe sepsis comprised patients with the acute organic dysfunction (cardiac, renal, hepatic, respiratory, circulatory insufficiency, disseminated intravascular coagulation or shock). Biological products were analysed in the Laboratory of Bacteriology by direct examination, cultures, complement-fixation test, and latex agglutination.
In patients included in the study, we have taken into consideration the following:
- Demographic data (age, gender, environment of origin)
- Period of hospitalization
- Etiology
- Paraclinical data at onset and in development
- APACHE II and CARMELI prognostic scores
- The presence of previous liver/kidney disorders
- Liver/kidney disorder in sepsis
- The presence of other organ dysfunction
- Evolution towards improvement or death
- The existence of statistical correlation of evolution parameters

4. RESULTS

![Boxplot of patients' age according to APACHE II score](image)

Figure XXIII. 5. Patients’ age according to the APACHE II score calculated

Patients’ age represented by APACHE II score calculated showed a median equal to or greater than 55 in patients with hepatorenal syndrome (Figure XXIII. 5.).
Table XXIII. 5. Correlations between uremia and creatininemia upon hospitalization

<table>
<thead>
<tr>
<th>creatinine i</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
<th>urea i</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>creatinine i</td>
<td>1</td>
<td>.496**</td>
<td></td>
<td>urea i</td>
<td>.496**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>47</td>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

Fig. XXIII. 21. Correlations between uremia (mg/dL) and creatininemia (mg/dL) upon hospitalization in patients with hepatorenal syndrome in sepsis

Correlations were also found between urea and creatinine values at discharge (Table XXIII. 6., Figure XXIII. 22.).
Table XXIII. 6. Correlations between urea and creatinine levels upon discharge

<table>
<thead>
<tr>
<th></th>
<th>creatinine (mg/dL)</th>
<th>urea (mg/dL)</th>
</tr>
</thead>
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<td>.604**</td>
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<td>Sig. (2-tailed)</td>
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<tr>
<td>Pearson Correlation</td>
<td>.604**</td>
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<td>Sig. (2-tailed)</td>
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<td>N</td>
<td>46</td>
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**. Correlation is significant at the 0.01 level (2-tailed).

Figure XXIII. 22. Correlations between urea (mg/dL) and creatinine (mg/dL) levels upon discharge, in patients with hepatorenal syndrome in sepsis

Comparison of the data sets was made using the Kruskal Wallis nonparametric test for independent samples (ANOVA equivalent), the test yielding a $p$ value
of 0.44. By comparing groups of pairs two by two, we found significance only between creatinine upon admission for APACHE I in comparison with APACHE III. As seen in Figure XXIII. 23., creatininemia is higher in the APACHE III group.

![Figure XXIII. 23. Correlations between creatininemia (mg/dL) and APACHE II calculated score](image)

For ALT values, we obtained the *ROC curve* according to the graph in Figure XXIII. 24.

![Figure XXIII. 24. ROC curve for TGP values](image)
Therefore, the optimal value of diagnostic decision, or the moment when the sensitivity/specificity ratio was at its peak for ALT is 78 IU/l.

For AST, we obtained the data from the *ROC curve* graphical plot in Figure XXIII. 25.

![ROC Curve for AST](image)

**Figure XXIII. 25. ROC curve for AST values**

According to the data obtained from the statistical software, the optimal value of diagnostic decision, or the moment when the sensitivity/specificity ratio was at its maximum for AST, is 75.5 IU/l.

The optimal value of diagnostic decision, or when the sensitivity/specificity ratio has been fullest for TB is 25.5 mg % (Figure XXIII. 26.).

![ROC Curve for TB](image)

**Figure XXIII. 26. ROC curve for total bilirubinemia values**

**(TB: mg‰)**
In those patients with hepatorenal dysfunction in sepsis, we tried to determine if there is a statistical correlation between values of bilirubinemia and altered neurological status, using the chi-square test (Figure XXIII. 31.).

**Figure XXIII. 31. Statistical correlations between bilirubinemia and neurological status alteration**

Representation of the correlation between glycaemia levels upon admission in hospital and the number of organ disorders showed higher values of blood glucose for patients who associated more than three organ dysfunctions (Figure XXIII. 34.).

**Figure XXIII. 34. Correlations between glycaemia (g/L) and organ dysfunctions in patients with sepsis**
According to the statistical data presented in Figure XXIII.44, correlations have been identified between antibiotherapy modification and death in patients with hepatorenal dysfunction in sepsis, which, on the one hand, could highlight the increasing risk of inducing resistance to antibiotics by frequently changing the therapeutic scheme, and on the other hand the difficulty of choosing an appropriate treatment and the complexity of severe cases.

![Figure XXIII. 44. Statistical correlations between antibiotherapy modification and death in patients with sepsis](image)

5. DISCUSSIONS
Although the standard definition of hepatorenal syndrome refers to the precise values of biochemical parameters in the development of patients with impaired renal/liver function as well as consequences related to diselectrolitemia, urinary osmolarity and diuresis, enclosing the critical patient with sepsis in the diagnosis of hepatorenal syndrome remains a challenge for the clinician, as the elements of the clinical development aspect are often not correlated mathematically with the modified biochemical parameters.

In this second study were included by selection patients with sepsis who, in addition to multiple organ dysfunctions, associated hepatic/renal dysfunction, while in those who developed both types of disorder, we tried to make an evaluation of the hepatorenal syndrome under various aspects: clinical, paraclinical, etiological, as well as from the point of view of patient evolution and therapy. The absence of shock prior to the diagnosis of hepatorenal syndrome was considered according to the latest consensus of International Ascites Club which allows the association of hepatorenal syndrome in sepsis (44, 45).
In our approach, we considered useful to try to determine the significance level through tests of sensitivity/specificity for serum urea, serum creatinine, serum transaminases, bilirubinemia and even proteinemia, cholesterol in the hepatorenal syndrome in sepsis, the threshold from which these parameters become suggestive or not along with elements from the patient’s clinical septic evolution.

For accuracy in the use of laboratory tests values modified in the hepatorenal syndrome, we took into considered the data from patients in which the algorithm for differential diagnosis ruled out other entities that could be causes *per se* for renal damage, according to currently accepted definitions for hepatorenal syndrome in sepsis (44).

At present, there are not any known differences between the pathophysiological mechanisms of HRS type I and type II. However, a number of precipitating factors type I have been signalled, such as bacterial infection, especially spontaneous bacterial peritonitis, paracentesis with large volumes and without compensating plasma substitute, gastrointestinal bleeding and alcoholic hepatitis, but there are also unknown precipitating factors. There have been reported a series of controversies related to dividing HRS type I into 2 types according to the existence or not of the precipitating factors in the evolution of the syndrome, and the result was that infection should be considered as an element of HRS, such that outside spontaneous bacterial peritonitis, any kind of infection may be considered a precipitating factor of HRS. Renal dysfunction which occurring after the infection presents the following risk factors: severity of infection, MELD score (Model for end-stage liver of disease) and the lack of response to antibiotherapy (45).
**XXIV. Research on the sensitivity/resistance profile of the etiologic Gram-positive and Gram-negative agents isolated from patients with sepsis hospitalized in the Infectious Diseases Clinical Hospital of Iasi from November 2012 to August 2014**

1. **INTRODUCTION**

We are currently confronted with the emergence of bacterial resistance to numerous antibiotics, as the number of systemic infections with multiresistant germs resister is increasing significantly in Europe and in other parts of the world (4).

In the 60 years since their introduction, millions of tons of antibiotics have been produced and used in a huge variety of situations. There is now a "glut" in the use of these "toxic" agents, who contributed significantly to the development of resistance and the selection pressure, which it is not a natural process, but a process conducted by the man and imposed on nature (4). The successful use of therapies can be compromised by the development of tolerance or resistance, involving a whole range of biochemical and physiological mechanisms.

2. **STUDY OBJECTIVES**

For patients with positive hemocultures, hospitalised in the Infectious Diseases Clinic of Iasi from November 2012 to August 2014, we made estimates concerning microbiological data, both for Gram-positive and Gram-negative germs. During this interval, we considered a number of 256 in-patients with positive hemocultures.

3. **MATERIAL AND METHODS**

We drew up an evaluation of the microbiological data in terms of sensitivity/resistance rates for the most common strains identified in the hemocultures of in-patients, admitted from November 2012 until August 2014. Isolation of microorganisms was performed in the Microbiology Laboratory of the Infectious Diseases Clinical Hospital of Iasi, by using classical methods. Hemoculture follow-up was carried out on brain-heart infusion broth and trypticase soy broth, the first 3 days (twice a day), then daily until positive culture was obtained, extending the examination period to a maximum of 14 days. We excluded from the database the stems from the same bacterial species isolated during the same infective episode in the same patient.

The identification of cultures was based on tracking microorganisms’ culture characteristics: morpho-tinctorial, metabolism, and on special tests for identifying coagulase positive staphylococci (*Staphylococcus aureus*),
coagulase negative (*Staphylococcus epidermidis* and other species), other Gram-positive bacteria (*Enterococcus spp, Streptococcus spp.*) and Gram-negative bacteria (*Pseudomonas spp., Klebsiella spp., Escherichia coli, Proteus spp., Acinetobacter spp., Enterobacter spp.*).

4. RESULTS
We studied if there were statistical correlations between patients who had received antibiotherapy prior to hospitalization in our clinic and the type of development classified according to APACHE II score.

![Figure XXIV. 4. Correlations between previous antibiotherapy in patients with positive hemocultures and APACHE II score calculated](image)

Figure XXIV. 4 with *chi-square test* showed that the proportions of patients with previous antibiotic differ significantly in the two APACHE groups II and III.

Etiologic agents identified in hemocultures were in close proportions, both Gram positive and Gram negative, and their distribution was represented in Figures XXIV. 5.
Fig. XXIV. 5. Etiologic agents identified in hemocultures

38% of the Staphylococcus species were Methicillin-resistant strains, while ESBL-producing Enterobacteriaceae were in variable proportions, as follows:

- Escherichia coli: 27.6%
- Proteus spp.: 93%
- Klebsiella spp.: 13.4%
- Serratia spp.: 32%

4. 2. Evaluation of sensitivity/resistance profile of isolated Gram-positive bacteria in patients with positive hemocultures during November 2012 - August 2014

**Gram-positive bacteria**

The considerably increasing resistance to antibiotics of Gram-positive bacteria, and in particular *Staphylococcus aureus* in the past 20 years, with the development of resistance to methicillin is accompanied recently by an emergent resistance to vancomycin.

Staphylococci registered a record value with regard to rapidly developed resistance to antibiotics as a consequence of the acquisition and transfer of antibiotic resistance-carrying plamids, as well as possessing intrinsic mechanisms of resistance (47).

In the patients included in our study, we identified 14 strains (36%) of methicillin-resistant *Staphylococcus aureus*, with a resistance rate of 32.3% in to oxacillin in the Gram-positive strains isolated.

Strains of *Staphylococcus coagulase negative* were isolated especially from diabetic patients and 8 oxacillin-resistant strains were identified.
*Group B streptococcus* was present in 5 patients also in diabetic context, all strains tested being sensitive to penicillin, ampicillin, oxacillin, 3\textsuperscript{rd} generation cephalosporins (Figure XXIV. 7).

![Figure XXIV. 7. The spectrum of sensibility/resistance of Gram-positive germs (both MRSA and MSSA) to Betalactamines](image)

**Figure XXIV. 7** Sensitivity/resistance of Gram-positive bacteria to Rifampicin, Trimethoprim-Sulfamethoxazole (STX), Tetracycline
The patients under study showed a resistance rate of 15.4% to Gram-positive bacteria with ciprofloxacin and 16% with norfloxacin, while for aminoglycosides there were statistically significant differences in terms of resistance, sensitivity, and “intermediate” sensitivity between gentamicin and amikacin (Figure XXIV. 9).

![Figure XXIV. 9. Sensitivity/resistance of Gram-positive germs to Quinolones/Aminoglycosides](image)

![Figure XXIV. 11. Sensitivity/resistance of Gram-positive bacteria to Carbapenems and Oxazolidinones](image)
The patients in our study produced a very good sensitivity/resistance ratio of Gram-positive bacteria to carbapenems and oxazolidinones, and so, we can say that these remain the first choice antibiotics in cases of resistance difficulties encountered in other classes of antibiotics (Figure XXIV. 11.).

4. 3. Evaluation of sensitivity/resistance profile of Gram-negative bacteria in isolated in hemocultures of patients hospitalized during November 2012-August 2014 in the Infectious Diseases Clinic of Iasi
Gram-negative bacteria

Infections with Gram-negative germs often have severe developments, with high mortality, as the difficulty of choosing antibiotherapy is increased by the emergence of numerous resistant strains of *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Acinetobacter spp.*, *Proteus spp.*, and not only. *Pseudomonas aeruginosa* is an invasive Gram-negative germ responsible for a wide variety of systemic infections including pneumonia, urinary tract infections, and infections of the soft parts (48). This pathogen is inherently susceptible to a limited number of antibacterial agents due to low permeability of its cell wall (49).

Of the total number of patients in our study for which etiology was identified, 22% have presented systemic infections with *Escherichia coli*, 9% with *Pseudomonas spp.*, 7% with *Klebsiella spp.*, *Proteus spp.*, 5%, but we also identified in smaller proportions *Acinetobacter spp.*, *Morganella spp.*, *Serratia spp.*

The profile of susceptibility/resistance to cephalosporins, aminopenicillins (Figure XXIV. 12.) showed a response rate of 62-64% to 3rd generation cephalosporins and a low rate of response to aminopenicillins, aminopenicillins, and inhibitors of beta-lactamases.
Our patients have responded with a rate of 64.5% to ciprofloxacin, almost 68% for norfloxacin, and with a response rate of over 60% to aminoglycosides (Figure XXIV. 13).

Carbapenems maintained a high level of efficiency even in the case of ESBL-producing strains, remaining the “rescue” solution in first intention therapy in sepsis with Gram-negative germs. In our study, the sensitivity of the Gram-negative bacteria to carbapenems had values above 80% for all three tested antibiotics (ertapenem, imipenem, meropenem), which enabled the appreciation of this therapeutic class as a very good option of first intention in severe infections with Gram-negative germs.
Figure XXIV. 14. Sensibility/resistance of Gram-negative bacteria to Carbapenems and Piperacillin/Tazobactam

At present, there is limited information regarding resistance mechanisms to colistin. A number of strains of *Pseudomonas aeruginosa* showed an increase in sensitivity to other antibiotics such as chloramphenicol or tetracycline (to which they commonly show resistance) while developing resistance to colistin (50).

Figure XXIV. 15. Sensibility/resistance of Gram-negative bacteria to Colistin and Biseptol
5. DISCUSSIONS
If classical antibiograms offer information on the sensitivity of germs, they don’t automatically give the solution regarding the optimal association of antibiotics for targeted treatment of an infection (51). Sepsis with multiple organ dysfunction often affected patients who were assigned prognostic score APACHE II and III, with values higher than 15-20, requiring associations of antibiotics from the moment of hospitalization, and these associations do not prevent the emergence of anti-microbial resistance.

“Combined” antibiogram could provide important information on the degree sensitivity of an etiological agent to an antibiotic, in the context of resistance to another.

Laboratory data of the patients studied were integrated into the dynamics, using the results from the study of antibiograms when they were available, and it is difficult to appreciate why a particular choice of antibiotics, although in conformity with microbiological data, did not represent a solution, being necessary to reshuffle schemes.

Over a third of patients considered in the study of antibiograms of etiologic agents isolated from hemocultures have received antibiotic treatment before hospitalization and in our clinic. These antibiotics do not induce directly mechanisms of resistance but exert a bacterial selection pressure, and in some cases can facilitate the emergence of some resistant mutants or of pre-existing subpopulations.

While determining the rates of sensitivity/resistance in our patients through antibiograms, we did not consider separately those who had received antibiotics prior to hospitalization in our clinic, so the results include a case selection bias. Special consideration of patients with previously administered antibiotics would have led to the formation of a lot too small to obtain relevant medical and statistical information.
CONCLUSIONS

- As it is presented in literature, the evolving trend of sepsis, in recent years, affecting predominantly the advanced ages, according to studies presented, more than 50% of inpatients in the Infectious Diseases Clinical Hospital of Iasi were over 65 years old, the median age of patients falling into the APACHE II score prognostic values of over 30 being 68 years.

- Research of data sheet concerning inflammatory response showed the existence of statistical correlations between parameters of the inflammatory syndrome in all patients with sepsis included in the study, which reflected an activation of the inflammatory markers on all lines, and also statistical correlations were underlined between blood glucose and inflammatory syndrome, and between blood sugar and the number of organ dysfunctions.

- Values referring to the inflammatory syndrome and creatinine clearance were statistically correlated with death in patients with sepsis, such data highlighting the role of individual risk factors for renal insufficiency and inflammatory response in sepsis.

- In patients with sepsis studied, hepatorenal syndrome was present in 20% of cases which deceased, representing an important percentage if we the result is related to the great variety of organ dysfunctions in sepsis, however, we consider this value indicative on the reason that data were collected only in a single center and targeted a selection of patients with liver/kidney dysfunction in sepsis.

- The ROC curve (sensitivity/specificity) in hepatorenal dysfunction in the sepsis revealed a cutoff value of 78 IU/L for TGP: 75.5 IU/L for TGO, 25.5 mg% for total bilirubinemia and 66% for QI, and we consider these relative values as threshold values for the diagnosis of hepatorenal syndrome, which is largely a diagnosis of exclusion in accordance with the definitions.

- Total bilirubinemia was statistically correlated with alteration of neurological status in patients with hepatorenal dysfunction in sepsis, which suggests that the cholestasis syndrome has an influence per se on clinical status changes in these patients.
• Statistical correlations were found between the antibiotherapy modification and death in patients with hepatorenal dysfunction in sepsis, which, on the one hand it could highlight the increasing risk of resistance to antibiotics by frequent changes in the therapeutic scheme, and on the other hand the difficulty of choosing an appropriate treatment and the complexity of severe cases.

• A very good therapeutic response has been noted in patients where Gram-positive etiologic agents had been identified, for which there were used glycopeptides and lincosamides, with 86.7% of tested strains sensitive to vancomycin and 72.5% to clindamycin.

• For Gram-positive bacteria, we have obtained a very good report on sensitivity/resistance to carbapenems and oxazolidinones, with rates of over 90% sensitivity, these antibiotics being used successfully in the most complex clinical situations with Gram-positive germs involved.

• Fluoroquinolones were a good therapeutic option, with a sensitivity of Gram-negative germs of up to 68% to norfloxacin, aminoglycosides showed a response rate of over 70%, and carbapenemes were also effective, with a sensitivity of up to 87% to imipenem.

• Colistin remains the therapeutic option of choice when in sepsis with Gram-negative germs resistant to other therapeutic classes, tested sensitivity in our patients being 87%.

• The profile study in patients with hepatorenal dysfunction in sepsis is one of the few in the country, and, in the context in which the data in the literature relating to sepsis bring comparative information in the area of pathophysiological mechanisms and therapeutic effectiveness in HRS in sepsis vs. HRS in liver cirrhosis, it is a difficult to frame our results into data relating to other groups of patients.
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