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"CAROL DAVILA", BUCHAREST
THE DOCTORAL SCHOOL
IN THE FIELD OF MEDICINE

Prognosis Assessment in Acute Peritonitis Based on the Dynamics of Sepsis Biomarkers

ABSTRACT OF THE DOCTORAL THESIS

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Introduction

This work aimed initially, as a simple scientific intention, to highlight some biological markers that are relevant to the clinician and easy to use in the current practice, which are released by a human body undergoing septic aggression, more specifically in abdominal sepsis installed in patients with secondary peritonitis. With this approach combining clinical experience with the findings of studies in the literature on the intimate pathogenetic mechanisms of the septic syndrome and on their continuous interdependence with the body's immune system, we tried to formulate certain working hypotheses for the detection of specific and significant biological "markers" in the early and stratified diagnosis of this pathology, in order to assess the risk of severe complications and thus the opportunity of certain targeted therapeutic interventions.

Currently defined as a "life-threatening organ dysfunction caused by an uncontrolled host response to an infection" [1], sepsis remains a highly complex condition with many unknowns and major implications for healthcare systems around the world; 2017 estimates show an incidence of 48.9 cases per year worldwide, with a mortality of approximately 19.7% [2]. Through the application of evidence-based medicine, the treatment of sepsis and septic shock has been greatly improved, in successive stages, over the last two decades, according to international guidelines ("Surviving Sepsis Campaign"), of which the latest was developed in 2021 [3], while international medical statistics have recorded a 53% decrease in mortality between 1990 and 2017. However, a recent meta-analysis [8] surprisingly shows stagnation in mortality due to septic shock from 2011 to the time of the study (2019). The application of initiatives promoted by international sepsis guidelines has led to the early recognition and diagnosis of sepsis and associated risk factors and to the implementation of protocolised management, actions that have resulted in consistent improvements in survival rates over the last 20 years; on the other hand, the long-term outcomes of caring for patients who were treated in the intensive care unit and survived an episode of sepsis remain very modest, with 1 in 3 adults dying within a year and 1 in 6 patients having long-term co-morbidities: prolonged immunosuppression and frequent reinfections (the so-called "persistent inflammation, immunosuppression and catabolism syndrome" [11]), significant acquired cognitive deficits, physical limitations, all requiring frequent hospital readmissions [10]. From this perspective, in the ICU, the unveiling of the sepsis paradigm still faces high barriers.

For the last decade, the "precision medicine" [4], sometimes referred to as "personalised medicine", has been attempting to redefine the approach to sepsis. After decades of efforts in refining the methods of sepsis detection and in its personalised management, in accordance with the individual response of each body, the topic of sepsis still remains a major burden, and the shift from a deterministic approach to a complex, stochastic approach to the sepsis phenomenon is slow. With the understanding of the need to integrate each body's particular response to septic aggression into a complex, nonlinear, predictable mathematical model and into the longitudinal dynamics, the approach to the pathophysiology and therapy of sepsis has entered a new phase. Integrating conclusions drawn from hundreds of clinical trials on the pathogenesis of sepsis undertaken over the last three decades into new types of clinical trials, with very large databases, distributions and non-Gaussian statistics, data to be analysed using algorithms that learn from the data (machine-learning and artificial intelligence techniques) [5], will pave the way for personalised medicine.

This goal requires the appropriate stratification of patients into cohorts based on clinical, biological and biomarker criteria, including immunological tests, phenotypic markers, specific "omic" (transcriptomic, metabolomic and proteomic) techniques [21], as well as routine laboratory, microbiological and imaging tests. This stage that consists in defining groups and subgroups of sepsis patients and tracking the evolution of the condition in its dynamics for each individual is the most challenging one, and depends exclusively on the researcher's judgment. The final step is the phase in which each patient is assigned a specific therapeutic intervention, according to his/her individual "pattern" of response to the sepsis aggression, an intervention that is likely to be often based on specific immunotherapies.

I. Working hypothesis and general objectives

In view of the sepsis characterisation as an "uncontrolled immune response of a host to infection", it appears necessary to profile some immune typologies and to classify patients into those endotypes, with a predictive role in the evolution of sepsis. At the same time, it appears necessary to highlight the roles of immunological biomarkers in the diagnosis and predictability of the sepsis evolution, as well as their subsequent use in targeted immunotherapy. Of course, these approaches are in their early stages, but important initial steps have already been taken, namely the recalibration of the approach to sepsis and septic shock, not as an aberrant immune response requiring immunosuppression to be brought to normal homeostasis, but as an imbalance that needs to be addressed by immune-targeted therapies, applied according to the stratification of patients into endotypes, and based on precise biomarkers and molecular mechanisms [4].

This research is based on a prospective, single-centre study that comprised a group of 32 patients with sepsis or septic shock, who were admitted to the ICU clinic of the Bucharest Emergency University Hospital with the diagnosis of secondary peritonitis and underwent emergency surgery between June 2020 and June 2022. The study attempted to substantiate the architecture of future large studies, establishing correlations that remain to be further verified to address this pathology, therefore it is essentially a pilot study.

In shaping our research, to this approach angle we added the known elements about the specificities of abdominal sepsis. Abdominal sepsis accounts for approximately 20% of sepsis cases [9], the second most common cause of sepsis syndrome after pulmonary sepsis. Approximately 74% of the abdominal sepsis cases develop single-organ failure and 20% develop multiple-organ failure, according to a recent observational study undertaken in the United States that included 11200 patients (similar statistics to the European ones), and mortality amounts to approximately 35% for patients with severe sepsis; mortality in secondary peritonitis appears to correlate with older age, pre-existing disease, non-appendiceal source, extension of peritonitis, and delay of surgery beyond 24 hours [12].

During the last 7 years, a mortality of 43.09% was reached in our hospital in cases of secondary peritonitis with sepsis admitted to the intensive care unit; the patients required 3896 days of hospitalisation in the ICU and 6233 total hospitalisation days.

One aspect that stands out in this pathology is the heterogeneity of patients with acute peritonitis, due to pre-existing associated conditions and to the different immunogenetic background of the patients, as well as to the type of the infecting pathogen, the size of the inoculum, the perforated digestive organ, and the delay between the time of infection and the time of presentation at the hospital. The concept of heterogeneity in precision medicine refers primarily to the incredible variability of reactions triggered in individual patients, not only to inter-individual differences [13]. Therefore, we collected various patient clinical data for stratification, data on associated conditions, we calculated the most relevant prognosis scores (APACHE II, SOFA and qSOFA scores), and added microbiological and laboratory data, data on the evolution of organ dysfunctions (especially renal dysfunction, which severely alters prognosis); in order to characterise the immune response in the evolution of patients, we linked the dynamic values of some biomarkers that we considered significant to the pro- and anti-inflammatory cascade in the septic syndrome, based on the study of the pathogenic mechanisms known to date and the findings of previous studies in this field.

Another concept that we considered is the phenomenon of compartmentalisation of the immune response, a hypothesis according to which immune processes differ between compartments of the human body, which explains the failures of immunomodulatory therapies in sepsis to date. By using, most commonly, the blood compartment as a "window" to quantify the immune response, we do not take into consideration the possible existence of a completely different immune status, perhaps even at the extreme, at the tissue level; moreover, pathogenic mechanisms predominant in one compartment may be irrelevant for another [14] [16]. In order to overcome such inadequacies, in this study we associated blood samples collected from patients to samples collected intraoperatively from peritoneal fluid.

The most important step in this study consisted in selecting the biomarkers, after reviewing recent literature, taking into account the availability of tests and testing methodology, and finally trying not to base our choices on data from other studies, but rather on the pathogenic mechanisms in which the biomarkers are involved, pro- and/or anti-inflammatory mechanisms that we will detail throughout the paper (the activation of molecular pattern recognition receptors-PRRs, associated to cell septic aggression-DAMPs or pathogen-associated-PAMPs, the neuro-endocrine reaction with release of precursor hormones/peptides, the activation of

membrane receptors with formation of differentiation clusters (DC) expressed on cells with a role in immunity, the formation of protein molecules with chemoattractant role, and others).

In 2019, a total of 5367 studies on biomarkers for sepsis identified 258 biomarkers of which 27% with a diagnosis role, 39% with a prognosis role and 34% with both roles; only 12 studies used validation cohorts [20]. In this study, we therefore chose a panel consisting of 5 known biomarkers, selected from among the cytokines and chemokines synthesised by the body in the immune response – interleukin-6, interleukin-8, interleukin-10, high-mobility-group box-1 and monocyte chemoattractant protein-1 - whose plasma levels we correlated with the baseline levels of known biomarkers proven to reflect the inflammatory and/or septic syndrome: serum lactate, procalcitonin [193] and presepsin-CD14 [16][17][18][19][22].

Lastly, we analysed the sensitivity and specificity of the biomarkers with various statistical methods, according to the patients belonging to a certain typology established based on clinical criteria manifested upon the patients' admission and in the dynamics, and according to the occurrence and evolution of severe organ dysfunction.

Following this hypothesis, we searched the literature for relevant data on the involvement of cytokines and other biomarkers in the development of the pro- and anti-inflammatory septic cascade. We thus chose a set of biomarkers to analyse in the group of patients with secondary peritonitis based on the following characteristics:

- *Serum lactate* – is an intensively studied inflammatory marker, but does not represent the septic phenomenon itself, however it is associated with tissue hypoperfusion, and in septic shock is correlates with increased mortality at values greater than or equal to 2mmol/L at 90 days [179];

- *Procalcitonin* – its normal plasma concentrations are very low, under 0.1 ng/ml, while values above 0.5 ng/ml suggest infection, reaching values above 1000 ng/ml in severe sepsis. It is known to be a valuable early marker of bacterial infection [192], before cultures are obtained (it has a peak in plasma levels on day 1 and decreases by half on day 2-3 postoperatively, with an in vivo half-life of 25-30 hours), but is of no value in localised infections that do not have a systemic inflammatory reaction. High levels are found in infections with gram-negative bacilli, and very high levels are found in infections with gram-positive cocci or gram-negative bacilli with multiple drug resistance (MDR) to antibiotics.

As a singular biomarker, procalcitonin cannot be a predictor of sepsis prognosis, since differences in plasma values are insignificant for survivors, perhaps requiring association, correlation with the values of another specific biomarker of the septic syndrome. On the other hand, PCT is very well used as an indicator of a sepsis source that is not adequately controlled, and is also a good guide in antibiotic therapy [177]; it is superior to other biomarkers as a predictor of severe septic complications and mortality in patients with secondary peritonitis [192];

- *Presepsin* – has very rapid kinetics, showing elevated values at 2 hours, with a half-life of 4-5 hours. It has been intensively studied because a good prognosis accuracy was observed for this biomarker (the ALBIOS study). It also revealed a statistically significant differential cut-off value between sepsis and septic shock (500 pg/ml). Non-specifically, presepsin develops higher values in older age and in renal dysfunction, requiring interpretation precautions in such cases;

- *Interleukin-6* – is a proinflammatory cytokine with very fast plasma kinetics, a half-life of 1-3 hours, increasing more than 10-fold, preceding C-reactive protein and, together with it, representing the primary phase of the septic process. Therefore, IL-6 has been intensively studied for more than 20 years, in more than 55 studies until 2015, which investigated it in isolation or in combination with other cytokines, revealing its rapid increase in dynamics, peaking at 24 hours and normalising at about 3 days. The highest plasma concentrations of IL-6 occur in sepsis but its role in diagnosis or prognosis remains unclear, with cut-off values varying widely (12-2760 pg/ml). Thus, at least 15 studies conclude that IL-6 appears to be a good predictor of sepsis mortality, while 9 others claim that it is a poor predictor of disease severity [164];

- *Interleukin-8* (half-life of 10 hours) – a chemokine that appears very early in sepsis, triggered by IL-1 and TNF, induces fever and neutropenia, and decreases with antibiotic administration. Plasma levels of IL-6 and IL-8 have been closely correlated with severity and the final prognosis in septic patients [166], while some studies support the correlation of IL-8 values with progression to septic shock [115];

- *Interleukin-10* – has a very short half-life (60 minutes), and was originally described as the main anti-inflammatory cytokine; it is now considered to be a pleiotropic cytokine with

numerous immunomodulatory roles [180]; its elevated levels appear to correlate with septic shock and 28-day mortality [166].

A combined score consisting of serum IL-6, IL-8 and IL-10 concentrations proved predictive in an observational study, showing progression to worsening and death, and was better in predicting mortality than the CRP-PCT combination [167];

- *HMGB-1* (half-life between 17 minutes and 3 hours) – shows more delayed kinetics and reaches a plateau of blood levels at 18-32 hours, which seems to correlate with the severity of sepsis by the degree of organ dysfunction. To this end, HMGB-1 was investigated for its prognosis role in a multi-centre randomised trial, which showed that serum levels were elevated in septic patients but could not be correlated with in-hospital mortality [166];

- *MCP-1* – a proinflammatory chemokine that has been independently and repeatedly associated with an early prognosis of sepsis, a property that makes it attractive in terms of possible therapy with neutralising antibodies. At the same time, its elevated values may reveal tumour progression and/or metastasis.

Based on this hypothesis, we chose to investigate, in the group of patients with secondary peritonitis, 3 biomarkers established in clinical practice for their prognosis qualities in cases of sepsis of any kind (lactate, procalcitonin and presepsin), to which we added the investigation of 5 biomarkers involved in the septic cascade (IL-6, IL-8, IL-10, HMGB-1, MCP-1), measured simultaneously in blood and peritoneal fluid at three different times: at time T0, representing the time of surgery, then at time T1, 24 hours and T2, 48 hours after surgery.

Consistent with the above principles and trying to give more clarity to the studied pathology, we formulated the following questions as objectives of the study:

- Is there a significant correlation between serum and/or intraoperatively collected peritoneal fluid concentrations of the biomarkers studied in dynamics in patients with secondary peritonitis and sepsis, and what would be the relevant cut-off point for clinical practice?
- Is there a significant correlation between the concentrations of the biomarkers studied in dynamics in blood and/or peritoneal fluid collected intraoperatively in patients with secondary peritonitis and severe organ dysfunction, and what would be the relevant cut-off point for clinical practice?

- Does combining the results obtained for the studied biomarkers with the results of those associated to sepsis and proven in previous literature studies (lactate, procalcitonin and presepsin) to be statistically relevant lead to earlier and more specific stratification of the risk of severe organ dysfunction in patients with sepsis due to secondary peritonitis?

The limitations of the study consist mainly in the small number of cases investigated, in the associated pathology of the patients and finally in the impossibility to homogenise the types of response to the septic aggression according to a stratification of the patients into subphenotypes and endotypes predictive of the evolution in dynamics. Moreover, we believe that monitoring the levels of the studied biomarkers over a longer period of time, possibly until their values become normal, would have been useful in defining their "behaviour" and in outlining correlations with the severity of the sepsis evolution.

Essentially, the aim of the studies on biomarkers in sepsis would be to provide pragmatic solutions and realistic combinations of biomarkers in order to refine early diagnosis, to assess the risk of each patient and, in the case of a high risk, to establish the targeted immunotherapy that would benefit the patient. The rigour in selecting and validating such prognosing but also "predicting" biomarkers in the sepsis syndrome would add value to the current protocolised management of sepsis, and their applicability in any hospital, at the bedside of the patient, would be the turning point towards fundamentally changing the approach to sepsis for the future generations.

II. General research methodology

This research is a cross-sectional prospective observational clinical trial, approved by the Ethics Committee of the Bucharest Emergency University Hospital (32485/2018), which comprised patients diagnosed with secondary acute peritonitis, selected over a period of 3 years from among the patients of the general surgery clinics of the hospital.

The selected studied group included 32 adult patients diagnosed with secondary peritonitis on which emergency surgical intervention was performed. Patients with recent chemotherapy or biological immunosuppressive treatment, pre-existing ascites or peritoneal dialysis, and those with a body mass index, BMI ≥ 40 kg/m² were excluded from the study.

At the time of the surgery, standard biological samples were collected from venous blood (blood count, coagulogram with fibrinogen, extended biochemistry panel), blood culture, serum markers of inflammation (procalcitonin, presepsin and samples for determinations of the studied inflammatory cytokines), serum lactate from arterial blood (blood acid-base balance); intraoperative peritoneal fluid was also collected for microbiological culture as well as samples for determinations of the studied inflammatory cytokines from the intraperitoneal fluid. The venous blood samples for the determination of inflammatory cytokines were collected at the initial time T0 (intraoperatively) and then after 24 hours (T1) and 48 hours (T2). The peritoneal fluid samples, 4 ml, were collected in sterile conditions intraoperatively (T0) in two 1.8 ml cryotubes and stored at -20 degrees Celsius initially, and then cryopreserved at -80 degrees Celsius.

The cytokine concentration was measured using the enzyme-linked immunosorbent assay (ELISA) method, the non-competitive variant.

The presepsin measurement (sCD14-ST) was performed with the PATHFAST analyser using the chemiluminescence enzyme immunoassay (CLEIA) and the Magtration technology, with values expressed in pg/ml. The procalcitonin (PCT) measurement was performed with the same device, as the sensitivity of PATHFAST B.R.A.H.M.S PCT is very good, allowing the measurement of concentrations below 0.1 ng/ml.

Admitted to the intensive care unit after surgery, patients received treatment according to current guidelines and protocols [3], individualised to each patient according to the severity of the sepsis and the pre-existing conditions. The treatment was the responsibility of an attending ICU physician who was not the same with the investigator in this research.

The following were taken into account for the assessment of evolution and prognosis:

- the clinical status on admission according to the qSOFA score - state of consciousness (Glasgow Coma Score < 15), systolic blood pressure ≤ 100 mmHg, respiratory rate ≥ 22 breaths/min.
- the SOFA, APACHE II severity scores calculated in the first 24 hours after admission to the ICU, retaining the highest severity value
- the presence of renal dysfunction on admission to ICU (including serum urea and creatinine values).

The group of patients was divided into 3 subgroups according to the Sepsis-3 Consensus Guideline criteria [1]:

- patients with sepsis - subgroup 1
- presence of sepsis with severe multiple-organ dysfunction with SOFA ≥ 7 and APACHE ≥ 24 - subgroup 2
- lack of sepsis criteria - subgroup 3.

The main objectives of the research conducted were outlined as follows:

1. To identify a demographic profile of patients with abdominal sepsis due to secondary peritonitis.
2. To determine the mean values of the selected biomarkers, in peritoneal fluid and blood, for the group of studied patients; to determine the predictability of their evolution using the levels of the biomarkers studied in dynamics in patients with sepsis, and separately for those with severe multiple-organ dysfunctions (SOFA≥ 7).
3. To determine the predictive role of presepsin, procalcitonin and lactate in the studied group and to identify possible correlations with the studied markers.

Currently, there are methods and systems to detect the biomarkers present in inflammatory processes, both in blood and in the peritoneal fluid, but the critical problem remains the lack of a specific combination of known biomarkers, which could be easily detected, for an accurate prediction of the onset and severity of abdominal sepsis, a combination of tests that could greatly reduce the enormous costs of the consequences of this pathology and improve the final prognosis of patients in such cases.

III.1. Descriptive analysis of the patient group

The group of patients selected for this pilot study included various pathogeneses of secondary peritonitis, spread across all the subgroups of patients, with perforation of a cavitary organ being by far the most common pathology, 19 cases of which 7 patients with gastric perforations. Of the cases with intestinal perforations, 4 cases of peritonitis were due to appendicular pathology and only 1 case was classified in the subgroup of patients without sepsis; also classified in this subgroup was the only traumatic case in the group of patients, a trauma of the left hypochondrium in a car accident that produced the rupture of the sigmoid colon.

Table 6.1

	Non-septic patients	**Septic patients**	**Patients with sepsis and severe organ dysfunctions**
Total number	10/32	7/32	15/32
Age, median (years)	40	67	70
Sex M , No./total (%)	6/32 (18%)	5/32 (15%)	8/32 (25%)
Cause of secondary peritonitis, No./total			
Perforation	7/32	5/32	7/32
Occlusion	0/32	2/32	6/32
Anastomotic fistula	0/32	0/32	1/32
Fissured abscess	3/32	0/32	1/32
Renal dysfunction, No./total patient category	1/10	2/7	13/15

It is worth noting that an oncological condition was present in 12 cases, of which 4 without digestive localisation.

As regards the number of patients in the subgroups created, it is important to mention that the largest subgroup is the one with patients that developed multiple and severe organ failures, namely 15 patients, representing 46.87% of the total group.

An examination of the subgroups of patients shows that there is a predominance of older ages in the subgroup of patients with sepsis and severe organ dysfunctions, the median age being 67 and 70 years, respectively.

As regards the positive cultures in the peritoneal fluid collected intraoperatively, the data obtained in the studied group shows the following:

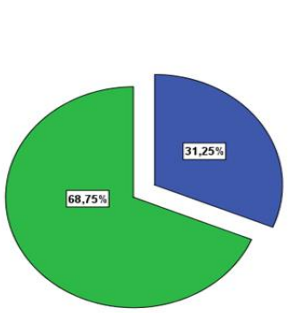


Figure 6.5 shows that 68.75% of the patients included in the study had positive cultures in the peritoneal fluid.

The distribution of negative cultures mirrored the selection of patients according to septic manifestations, i.e. 6 of the non-septic patients had negative cultures and only 3 cultures were negative in the group of patients with sepsis, of which 1 patient also had severe organ dysfunction.

Table 6.2

	Non-septic patients	**Septic patients**
No. of positive cultures in the peritoneal fluid	4/10	18/21

For all patients, the qSOFA, SOFA and APACHE II scores were calculated in order to characterise as accurately as possible the organ dysfunction and the severity of sepsis in the group of studied patients; the minimum and maximum values in each subgroup were as follows:

Table 6.3.

	Non-septic patients (N=10)	**Septic patients (N=7)**	**Patients with sepsis and severe organ dysfunction (N=15)**
qSOFA	0 - 1	2 - 3	2 - 3
SOFA	0 - 4	2 - 6	7 - 10
APACHE II	5 - 11	17- 23	24 - 29

Although the APACHE II score is a standard in intensive care units, stratifying patients with the aim of predicting mortality rates, it lacks sensitivity and specificity when it comes to patients with multiple comorbidities [181].

The mortality at less than 14 days in the studied group was 40.6%, and the total mortality recorded was in most cases due to associated conditions, occurring after a long evolution (3 deaths occurred 3-7 weeks after surgery), burdened more by associated conditions and a consequent immunosuppression background.

The most common organ dysfunction developed by septic patients is **renal dysfunction**, and its evolution during hospitalisation depends on the initial stage of nitrogen retention at the time of the patient's presentation; it should be mentioned that none of the patients in the group had renal failure as a pre-existing condition, with or without the need for chronic hemodialysis.

At T0, urea and creatinine values in the studied group after data normalisation (by logarithms, square root extraction) were grouped as follows:

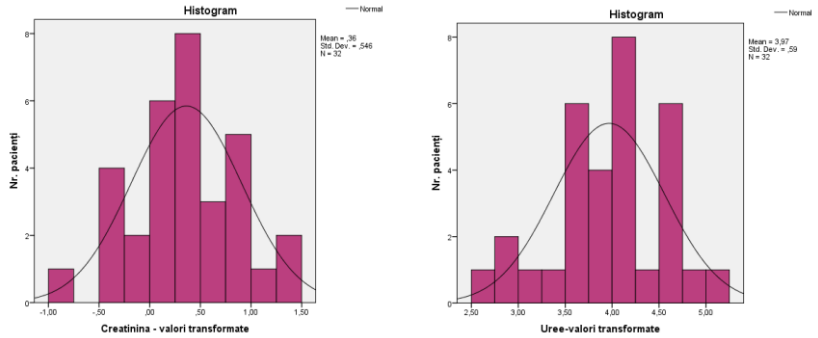


Figure 6.10 Histograms of the distribution of urea and creatinine values converted

According to the t-test the following differentiating variables were identified between the subgroups of septic and non-septic patients.

- For creatinine values: there was a statistically significant difference between the subgroups of septic and non-septic patients as regards the mean creatinine value (t=3.00, p=0.005). Thus, patients with sepsis had a higher mean creatinine level (0.53 vs. 0.02).
- For urea values: there was a statistically significant difference between the subgroups of septic and non-septic patients as regards the mean urea value (t=4.22, p=0.001). Thus, patients with sepsis had a higher mean urea level (4.20 vs. 3.44).

III.2. Distribution analysis of biomarker values in the peritoneal fluid and blood

A simple analysis of the values obtained in the total studied group of patients with secondary peritonitis suggests the following:

- The peak IL-6 values were detected in the peritoneal fluid compared to blood values, and the latter decreased progressively from the first determination to the 48-hour determination; the magnitude of the values was of the order of thousands (pg/ml) at T0, both serum and peritoneal, and then of the order of hundreds at systemic level. There were also a few cases that did not fall into this profile.
- The peak IL-8 values were also found in the peritoneal fluid, the serum values progressively decrease in dynamics but differ from IL-6 in terms of order of magnitude, being several times lower at systemic level.
- IL-10 showed similar values in dynamics but much lower overall, even compared to IL-8 values, with very large differences between peritoneal and plasma levels.
- MCP-1 and HMGB-1 had values similar to IL-6 in terms of magnitude, especially in the peritoneal fluid, of the order of thousands of ng/ml, with some cases reaching values above 30,000 pg/ml.

Figure 7.5 Mean serum concentration in dynamics IL-6

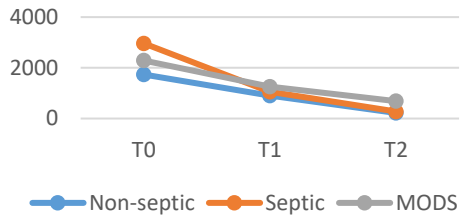
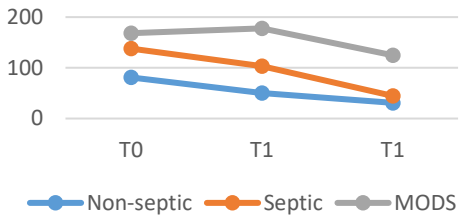
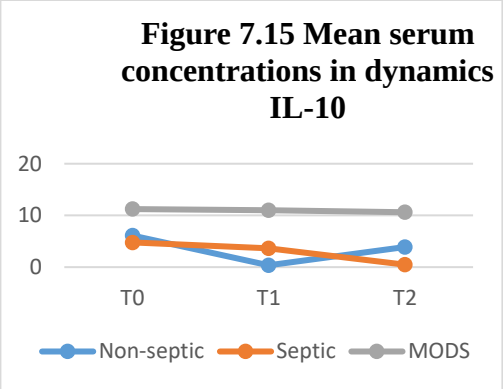
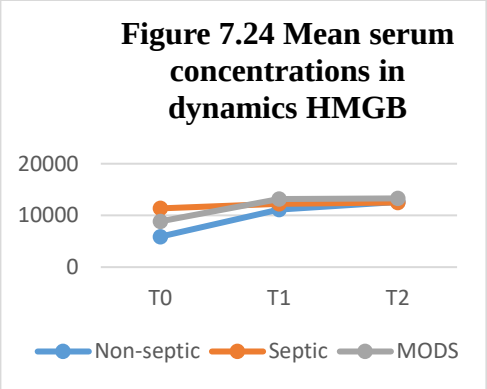
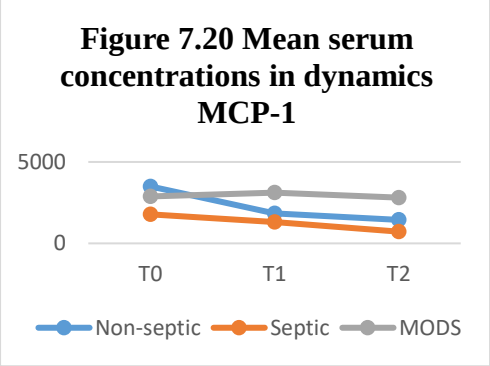


Figure 7.10 Mean serum concentrations in dynamics IL-8









[193]

Summarizing the distribution of IL-6, IL-8, IL-10, MCP-1 and HMGB-1 values **in the peritoneal fluid**, we can conclude that large amounts of cytokines are released in the peritoneal cavity of patients with secondary peritonitis, which functions as a real reservoir of inflammatory mediators, but shows significant differences across the 3 subgroups of patients. The highest levels of intraperitoneal IL-6, IL-10 and HMGB-1 were recorded in the group of septic patients and, even more importantly, there were high concentrations of those cytokines in non-septic patients as well, the lowest levels being seen in patients with severe multiple-organ dysfunctions (Figures 7.26-7.28). In this regard, we note a similarity between the IL-8 and MCP-1 values, with the highest values measured in the peritoneal fluid of patients without sepsis criteria (Figures 7.29-7.30).

In conclusion, based on the data obtained in this study, we believe that there is no correlation between the evolution of organ dysfunction and the cytokine levels in intraperitoneal fluid in patients with secondary peritonitis. In certain studies in the literature, findings have been divergent [34, 184], showing a correlation of levels of some peritoneal cytokines with the

severity of sepsis progression. More rigorous stratification of patients and the creation of much larger databases is probably needed in order to draw a more accurate conclusion.

Figure 7.26 IL-6 peritoneal fluid T0

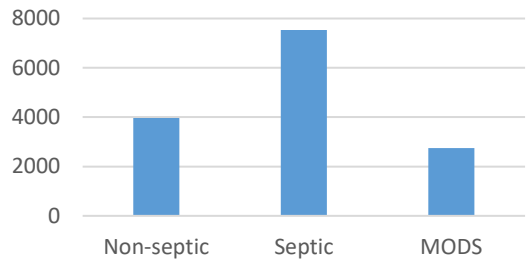


Figure 7.29 IL-8 peritoneal fluid T0

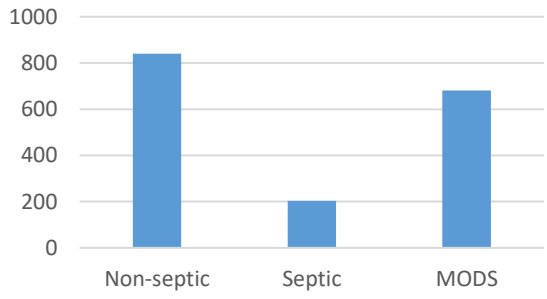


Figure 7.27 IL-10 peritoneal fluid T0

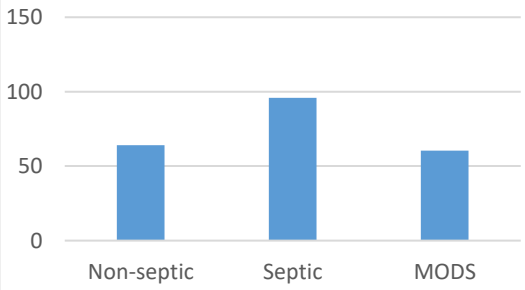


Figure 7.30 MCP1 peritoneal fluid T0

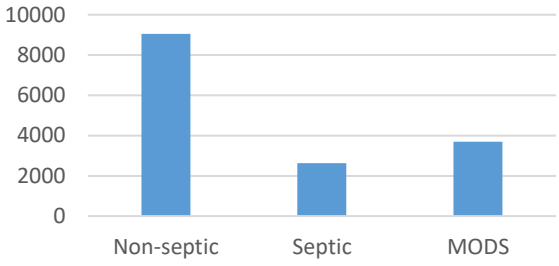
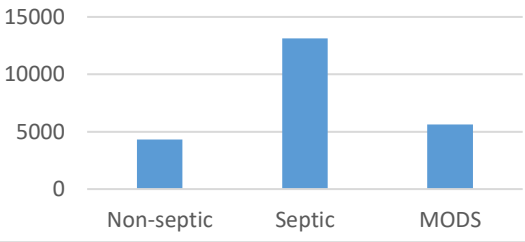


Figure 7.28 HMGB1 peritoneal fluid T0



[193]

It is important to note that the subgroup of patients with severe and persistent organ dysfunctions (SOFA score ≥ 7 and APACHE II score ≥ 24) is designated by the abbreviation MODS for easier graphical illustration of this selected group of septic patients.

III.3. Analysis of biomarkers in patients with secondary peritonitis and sepsis

In order to verify the correlations between the septic patients, defined according to the Sepsis-3 Consensus Guidelines [1], and the cytokine levels in peritoneal fluid (PF) at time T0 and at systemic level at times T0, T1, T2, ROC curves (Receiver Operating Characteristics) were used to measure the efficiency of the various models proposed.

Summarising the areas under the curves (AUC) created by the serum values of the cytokines with relevance to the presence of sepsis in the subgroup of patients with secondary acute peritonitis and sepsis, the serum cytokines collected from the patients at T0, T1 and T2, we notice significant values only for the serum samples collected 24 or 48 hours after surgery. At T0, the time of surgery, none of the selected cytokines showed useful sensitivity and specificity in the early detection of intra-abdominal sepsis in the patients of the studied group, both in terms of serum and intra-peritoneal concentrations.

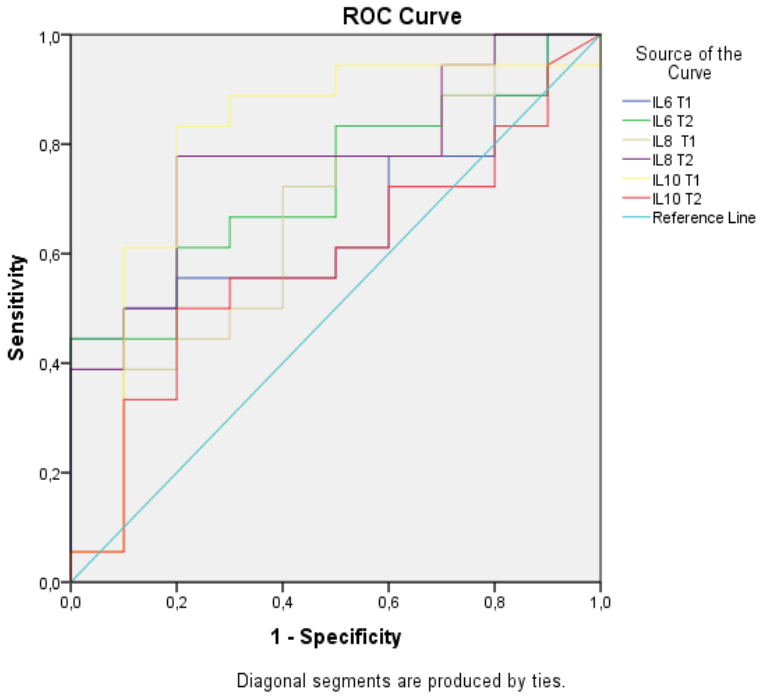


Figure 8.9 ROC curves for serum IL-6, IL-8 and IL-10 at T1 and T2

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL6 T1	.667	.102	.150	.467	.867
IL6 T2	**.728**	**.095**	**.049**	**.541**	**.914**
IL8 T1	.656	.111	.179	.437	.874
IL8 T2	**.772**	**.091**	**.019**	**.595**	**.950**
IL10 T1	**.800**	**.100**	**.010**	**.604**	**.996**
IL10 T2	.586	.112	.457	.367	.805

Table 8.9 AUC values for serum IL-6, IL-8 and IL-10 at T1 and T2

As shown by the table, for IL-6 at T2, IL-8 at T2 and IL-10 at T1, the areas under the curve have values above 0.7 (0.728, 0.772, 0.800), indicating that the models obtained are satisfactory to good, and all are statistically significant as the p-values are less than 0.05.

The following relevant threshold values can be specified for those biomarkers:

- For IL-6 T2 the threshold value of 307.628 pg/ml identifies 61.1% true positive patients with sepsis and 20% false positive patients; specificity increases to a cut-off value of 411.930 pg/ml (0% false positive cases), but sensitivity decreases to 44.4%.
- The threshold value for the IL-8 level at T2 was 42.047 pg/ml, identifying 72.2% true positive and 20% false positive patients.
- The IL-10 collected at T1 has the best model of the area under the curve, and at the threshold value of 2.652 pg/ml we will identify 83.3% true positive cases with sepsis and 20% false positive cases.

For the *MCP-1* protein, the values do not create a sufficiently high area under the curve at any of the times of the determinations, and all p-values (asymptomatic sig.) are above 0.05, therefore without statistical significance of the results.

For the *High Mobility Group Box 1* protein, both peritoneal fluid and serum values were statistically insignificant. The values obtained at T0 can be considered a satisfactory model, AUC = 0.636, but p is 0.223, therefore not statistically significant.

III.4. Analysis of biomarkers in patients with secondary peritonitis and sepsis with severe organ dysfunction

For *interleukin-6*, the ROC curve analysis for patients with severe multiple-organ dysfunction shows a satisfactory/good model for T2 values, with the area under the curve equal to 0.701, but with p greater than 0.05, therefore not statistically significant. A threshold value of 411,930 pg/ml is recorded at T2, which could be relevant, identifying 54.5% true positive and 11.8% false positive patients.

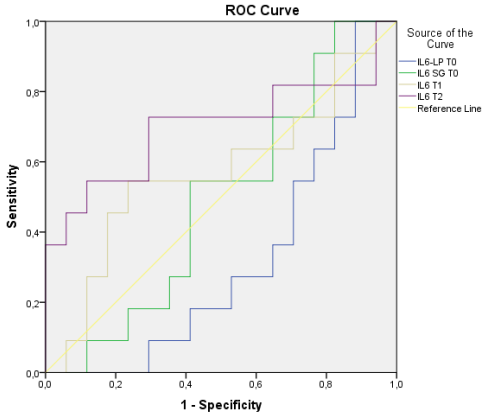


Figure 9.1 The ROC curves for IL-6

The representation of the *interleukin-8* values showed statistically significant results at T2 (p=0.04), the AUC 0.829 having a good model, with 72.7% sensitivity and a high specificity of 88.2%, for a threshold value of 59.476 pg/ml. For the IL-8 values at T1, the model is satisfactory/good, but not statistically significant (p=0.63); a threshold value in this model would be 155,842 and it identifies 54.5% true positive and 11.8% false positive patients.

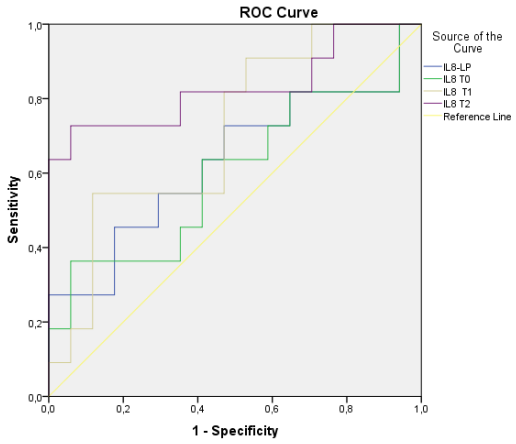


Figure 9.2 The ROC curves for IL-8

The modelling performed for *interleukin-10* on the same subgroup of patients shows a good model (AUC = 0.797) for the values at T1, statistically significant (p=0.009), with a threshold value of 4.81 pg/ml, for which the sensitivity of the test is 81.8% (true positive cases) and the specificity is 82.4%.

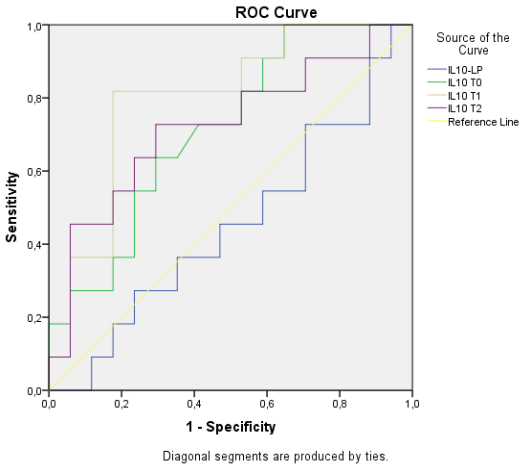


Figure 9.3 The ROC curves for IL-10

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL10-LP	.449	.113	.655	.227	.671
IL10 T0	.714	.097	.060	.523	.905
IL10 T1	**.797**	**.088**	**.009**	**.625**	**.969**
IL10 T2	.722	.104	.051	.519	.925

Table 9.3 The AUC values for IL-10

The *monocyte chemoattractant protein-1* appears to present the closest correlation with the severe outcome in patients with abdominal sepsis and multiple-organ failure, achieving statistically significant ROC curves both at T1 and T2.

At T1, the area under the curve is 0.786, with p=0.012, and there is a threshold value of 2602.03 for which the calculated sensitivity is 81.8% and the specificity is 77.5%.

At T2, the area under the curve is 0.727, with p=0.046 statistically significant, and the threshold value of 2270,730 identifies 72.7% true positive and 23.5% false positive patients (specificity 77.5%).

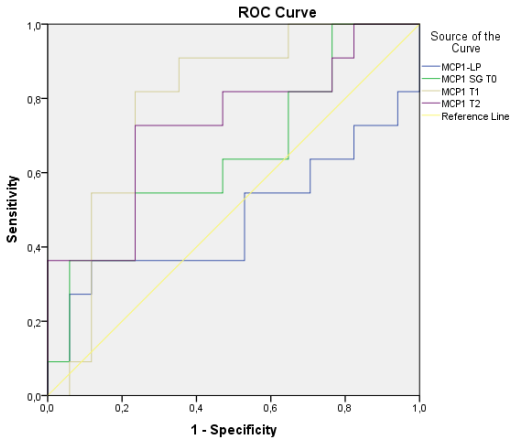


Figure 9.4 The ROC curves for MCP-1

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
MCP1-LP	.476	.126	.832	.230	.722
MCP1 SG T0	.642	.110	.213	.426	.858
MCP1 T1	**.786**	**.089**	**.012**	**.611**	**.961**
MCP1 T2	**.727**	**.103**	**.046**	**.526**	**.929**

Table 9.4 The AUC values for MCP-1

As in the septic patients with lower severity scores, the *HMGB-1 protein* does not validate the prognosis of abdominal sepsis at any time.

III.5. The predictive role of presepsin, procalcitonin and lactate in the studied group

As established mediators of sepsis, intensively studied in the recent decades, in our study of the group of patients with sepsis by secondary peritonitis we chose **presepsin, procalcitonin and lactate**, measured on admission to the ICU, their profile being relevant to the critically ill septic patient requiring intensive care measures.

According to the t-test the following differentiating variables were identified between the subgroups of septic and non-septic patients:

- *Presepsin*: There is a statistically significant difference between the subgroups of non-septic and septic patients in terms of the mean presepsin level (t=2.90, p=0.007). Thus, septic patients had a higher mean presepsin level (754 vs. 672).
- *Procalcitonin*: There is a statistically significant difference between the subgroups of non-septic and septic patients in terms of the mean procalcitonin level (t=4.32, p=0.001). Thus, septic patients had a higher mean procalcitonin level (1.99 vs. 0.62).
- *Lactate*: There is a statistically significant difference between the subgroups of non-septic and septic patients in terms of the mean lactate (t=3.47, p=0.002). Thus, septic patients had a higher mean lactate level (1.18 vs. 0.52).

As a final step to test certain predictors, *the ROC curves for presepsin, procalcitonin and lactate, collected at T0 in patients with sepsis*, show the following:

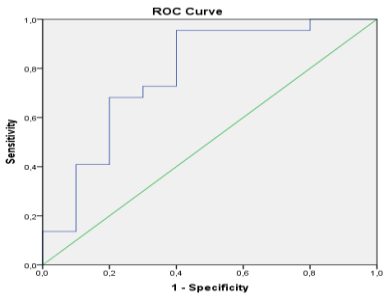


Figure 9.8 The ROC curve for presepsin at T0 – septic patients

The presepsin values at T0 in the subgroup of septic patients show an area under the curve of 0.77, the model being satisfactory/good, with a statistically significant threshold value of 0.013 [193]; the cut-off value is 1438.00 pg/ml and will identify 68.2% true positives and 20% false positive patients in the studied group.

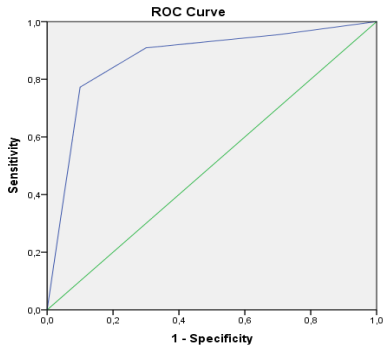


Figure 9.9 The ROC curve for procalcitonin at T0 – septic patients

The procalcitonin values at T0 in the subgroup of septic patients have an area under the curve of 0.873, the model is good/very good; the threshold value is 0.001 (statistically significant).

A procalcitonin cut-off value of 7.5 ng/ml will identify 77.3% true positive and 10% false positive patients.

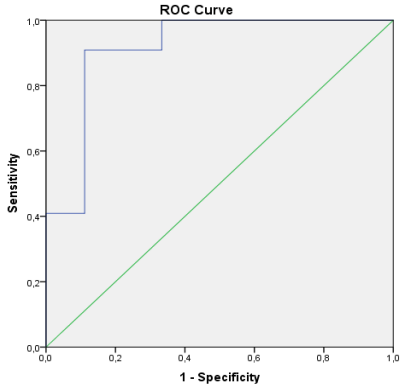


Figure 9.10 The ROC curve for lactate at T0 - septic patients

The serum lactate values at T0 in the septic patients selected according to the "Sepsis-3" Consensus Guideline criteria create an area under the curve of 0.914, the model being very good, with a statistically significant threshold value of 0.000; the cut-off value of 1.8750 mmol/L identifies 90.9% true positive and 11.1% false positive patients.

For the patients with severe organ dysfunctions, the ROC curve for serum lactate at T0 looks like this:

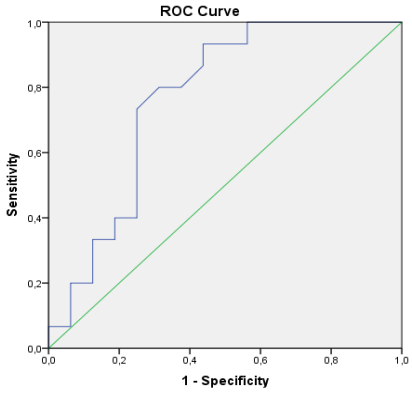


Figure 9.11 The ROC curve for lactate at T0 – patients with severe organ dysfunctions

Serum lactate at T0 for patients with secondary peritonitis who developed severe organ dysfunctions in the course of sepsis shows an AUC of 0.767, the model is good, with a statistically significant threshold value of 0.011; at a cut-off value of 2.1650 mmol/L the model identifies 73.3% true positive and 25% false positive patients.

The checks for possible correlations of presepsin, procalcitonin or lactate with the values of the other biomarkers studied in the dynamics had no statistical significance except in the following cases:

Table 9.15

Correlation	Pearson test value	p	Interpretation
Procalcitonin -> Lactate	0.46	0.02	There is a moderate and statistically significant correlation (Figure 9.12)
Lactate -> IL6-T0	0.65	0.001	There is a moderate and statistically significant correlation (Figure 9.13)
Lactate -> IL8-T1	0.42	0.04	There is a moderate and statistically significant correlation (Figure 9.14)

IV. Conclusions

Sepsis remains to date a complex phenomenon, the vital prognosis of the patient depending on the quick diagnosis and application of therapeutic measures specific to a given case prototype. Although the strategy of an early and correct application of the direct treatment for the infection, the outbreak cleaning and the targeted antibiotic therapy have led to an improved prognosis of sepsis, results are still unsatisfactory for the patients' final verdict. The refinement of microbiological methods for pathogen detection, the rapid PCR techniques, and the refinement of antibiotic formulas, with pK/pD monitoring of the therapeutic agent, have not produced the effects initially expected as statistical results worldwide. It is obvious that the approach to dealing with sepsis needs to change, starting from the fundamental analysis principles of this condition.

By moving away from defining sepsis based on its manifestations, within the systemic inflammatory response syndrome, to defining the concept of sepsis as an inflammatory response to an infection that is not properly controlled, which will lead to the onset of organ dysfunction, the medical world has perhaps more accurately and sharply perceived the seriousness of the evolution of uncontrolled sepsis, because of the invariable presence in sepsis of organic dysfunction, sometimes concealed but undeniably present, dysfunction that reveals an alteration of the mitochondrial metabolism, the energetic essence of the body. On the other hand, the problems arising from this new conceptualisation have been linked to the diagnosis and quantification of the seriousness of certain cases.

At this stage we consider that stratifying the patients and the type of septic response becomes the primary tool that needs to be analysed and conceived as a work plan for routine, easy bedside use.

For this purpose, in this paper we chose only one type of septic condition - secondary peritonitis – and tried to supplement the usual means of diagnosis and prognosis by adding concentration profiles of certain known mediators of the septic cascade, hoping to benefit from their use as serum and/or peritoneal biomarkers. We chose the time of emergency surgery as the easiest and most defining time to use for patients with this condition, which in fact, in the vast majority of cases, is within a few hours of admission to hospital and in many cases represents the peak of clinical, paraclinical and septic cascade signs.

In this study, the sample collections of inflammatory biomarkers up to 48 hours postoperatively followed the evolution of the septic process, but now, after interpreting the results, we believe that measurements should be repeated more frequently or according to the kinetics of each mediator, until the time of their normalisation; only in this way it will be possible to identify relevant profiles for a particular subtype of patient. At the same time, we believe that systemic biomarker concentrations appear to be more relevant to the clinical case than local peritoneal ones, and this is based both on the results of this pilot study and on the argument of the more important systemic effects of such mediators. Not without importance in this context are also be the problems encountered in the laboratory, in the measurements of peritoneal fluid samples contaminated with spilled material from the intestine, especially colonic material. Finally, we must point out that the number of tests used in the study was limited, according to the possibilities that were available.

Another limitation of the study was the presence of an associated pathology for many patients with peritonitis, a pathology that alters the inflammatory immune response but is frequently present in the clinical practice and therefore unavoidable. We believe that this would be a relevant point for a large study in the future, not to exclude certain comorbidities, but to stratify the types of patients as adequately as possible by using machine learning software to manage large databases.

In order to differentiate the negative evolution of the septic process we valued the experience of previous studies and used as a control group patients with the same underlying condition - secondary acute peritonitis - but without sepsis criteria according to the Sepsis-3 Consensus Guideline. Another possible error factor, as mentioned earlier in the paper, can be the clinical and paraclinical criteria of sepsis severity adding to pre-existing comorbidities, which are difficult to differentiate and therefore patient stratification becomes error prone. Exclusion criteria for patients in this study were immunosuppressive treatment of any type administered in the last 6 months and body mass index $\geq$ 40 kg/mpc, but it would probably be useful to exclude other old conditions as well, especially pulmonary ones. We mention that patients with chronic kidney disease in the hemodialysis stage were not included in the study.

A single-centre observational study conducted during 2017-2020 on a cohort of 1313 patients shows that organ failure, acute or chronic, is associated with increased 30-day mortality in patients with suspected sepsis at the time of hospital admission; the patients with infections had lower mortality, and their SOFA score predicted mortality more accurately than for patients who did not have a proven subsequent infection. Chronic organ failures, which naturally do not improve during sepsis-specific therapies, contribute to higher mortality rates, and therefore total SOFA score values should be interpreted with caution when assessing the therapeutic effects in interventional studies [187].

Finally, also in the context of the limitations of the study, it should be noted that the number of patients included was small and that the lack of patient phenotyping is a decisive factor in the immune response to the septic aggression.

The pilot role of this study has led to several key conclusions:

1. The biomarker reservoir role of peritoneal fluid, with highly elevated IL-6, IL-8, IL-10, HMGB-1 and MCP-1 levels, is obvious in all cases of secondary acute peritonitis, but the possibility of using those mediator concentrations as stratifying factors or as part of a prognosis index is likely to be difficult to clarify, due to the low specificity for severity of sepsis and organ dysfunction. A particular line to explore could be the role of the concentration dynamics of certain peritoneal biomarkers in monitoring patients developing postoperative digestive fistula.

At the same time, based on the increased levels of cytokines in the peritoneal fluid and the biologically plausible possibility of them aggravating sepsis, the therapeutic value of peritoneal fluid drainage in patients with very high intraperitoneal concentrations of sepsis mediators is worth exploring.

2. The systemic values of pro-/anti-inflammatory biomarkers, of chemokines and tissue injury-associated biomarkers decrease progressively after surgery in secondary peritonitis, and it would probably be useful to monitor them as prognosis factors at least during the first week after surgery, or even for a longer period for patients with persistent infections or immunosuppressed status.

3. The very high systemic values of interleukin-6 recorded in the study confirm the known proinflammatory role, at least in the early stages of sepsis with a peritoneal starting point; peak IL-6 values were detected in peritoneal fluid compared to blood values, values that progressively decreased from the first determination to the 48-hour determination.

For patients with sepsis, the area under the curve obtained for IL-6 values collected at T2, the model obtained is satisfactory/good (AUC=0.728) with a statistically significant p=0.049. At a cut-off value of 307.628 pg/ml, the model identifies 61.1% true patients with sepsis and 20% false positive patients. For patients with severe organic dysfunctions (SOFA ≥ 7 and APACHE II ≥ 24), the ROC curve analysis shows a satisfactory/good model for T2 values, with an area under the curve equal to 0.701 but with p greater than 0.05, therefore statistically insignificant.

4. The interleukin-8 determinations showed peak values also in peritoneal fluid, while the serum values progressively decrease in dynamics, but different orders of magnitude were noticed compared to IL-6, several times lower at systemic level. At T2, septic patients show, for IL-8 values, an area under the curve equal to 0.772, with a satisfactory/good model, and a statistically significant p value (0.019); the relevant cut-off value is 42.047 pg/ml, identifying 72.2% true positive and 20% false positive patients. For patients with high severity of organ dysfunctions, IL-8 values are statistically significant for T2 (p=0.04), the area under the curve is 0.829 and has a good model, with a sensitivity of 72.7% and a high specificity of 88.2%, for a threshold value of 59.476 pg/ml.

5. The interleukin-10 values obtained show similar values in dynamics but much lower overall, even compared to the IL-8 values, with very large differences between peritoneal and plasma levels. In septic patients, the IL-10 values at T1 create a good model, with a high area under the curve (0.800) and p equal to 0.010, therefore statistically significant. By selecting a cut-off value of 2.652 pg/ml we will identify 83.3% true positive cases with sepsis and 20% false positive cases (specificity of 80%). In patients with severe organ dysfunctions, the IL-10 values show a good model (AUC=0.797) for values at T1, statistically significant (p=0.009), with a threshold value of 4.81 pg/ml, for which the sensitivity of the test is 81.8% (true positive cases) and the specificity is 82.4%.

6. The MCP-1 and HMGB-1 concentrations had similar values to IL-6 in terms of magnitude, especially at peritoneal level, of the order of thousands pg/ml, with some cases reaching values of over 30,000 pg/ml.

The MCP-1 concentrations appear to be most consistent with the severe outcome of patients with abdominal sepsis and severe multiple-organ dysfunctions, achieving statistically significant ROC curves both at T1 and T2. At T1, the area under the curve is 0.786, with

p=0.012, and the threshold value is 2602.03 pg/ml, for which the calculated sensitivity is 81.8% and the specificity is 77.5%. At T2, the area under the curve is 0.727, with a statistically significant p=0.046, and the threshold value of 2270.730 pg/ml identifies 72.7% true positive and 23.5% false positive patients (specificity of 77.5%).

7. The HMGB-1 concentrations recorded in patients with secondary peritonitis were considerably elevated, correlating according to some studies with the degree of tissue injury and shock; at the same time, it is the only cytokine that had an upward dynamic of the mean value. In this study however both peritoneal fluid and serum values were statistically insignificant.

In conclusion, based on the data obtained in this study, we believe that there is no correlation between the evolution of organ dysfunction and the intraperitoneal fluid cytokine levels in patients with secondary peritonitis. In certain studies in the literature, findings have been divergent [34, 184], showing a correlation between the levels of some peritoneal cytokines and the severity of sepsis progression. Most probably a more rigorous stratification of patients and much larger databases, processed by machine learning analysis methods, are needed in order to draw a more accurate conclusion.

8. The role of the established biomarkers of sepsis - presepsin and procalcitonin - is confirmed by this study as follows:

- The presepsin values collected at T0 in the subgroup of septic patients show an area under the curve of 0.77, the model being satisfactory/good, with a statistically significant threshold value of 0.013; the cut-off value is 1438.00 pg/ml and will identify 68.2% true positive and 20% false positive patients in the studied group.
- The procalcitonin values collected at T0 in the subgroup of septic patients show an area under the curve of 0.873, the model is good/very good; the threshold value is 0.001 (statistically significant). At a procalcitonin cut-off value of 7.5 ng/ml, it will identify 77.3% true positive and 10% false positive cases.

9. As regards the serum lactate values, the results obtained are in agreement with the literature, as follows:

- The serum lactate values collected at T0 from septic patients selected according to "Sepsis-3" Consensus Guideline criteria create an area under the curve of 0.914, the model being very

good, statistically significant; the cut-off value of 1.8750 mmol/L identifies 90.9% true positive and 11.1% false positive cases.
- The serum lactate values at T0 for patients with secondary peritonitis who developed severe organ dysfunctions in the course of sepsis show an area under the curve of 0.767, the model being good, with a statistically significant threshold value of 0.011; at a cut-off value of 2.1650 mmol/L the model identifies 73.3% true positive and 25% false positive patients.

With regard to multiple-organ dysfunction, important steps have been taken in the last decade in detecting and understanding the intimate pathophysiological mechanisms of this syndrome, regardless of its triggering cause, septic or otherwise; however basic and systematically effective treatments and a prognosis system that could provide certainty in the clinician's choice of the appropriate therapy have not yet been developed [190]. The complex evolution of the multiple-organ dysfunction syndrome, the onset manner and the self-perpetuation in various pathophysiological circumstances are still under the attention of researchers and require clarification; the common pathway of the dysfunction progression, for multiple conditions and types of patients, will probably be revealed with new genetic and molecular discoveries that will identify the biological basis as well as the common biomarkers that trigger, potentiate and shape the outcome of the multiple-organ dysfunction syndrome. In the context of sepsis, the lack of coordination between the immune, endocrine and central nervous systems inexorably evolves towards septic shock, an entity with increased mortality but whose distinction from the septic state itself is still being researched; what is certain is the metabolic, cellular, bioenergetic alterations that ultimately occur and lead to multiple ways of cell death, however the intimacy of the pathophysiological processes taking place successively in all tissues and organs remains to be clarified.

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