ORIGINAL ARTICLE

CONTENTS

Dynamics of changes in proteins of the acute phase of inflammation in the postoperative period in patients with disseminated peritonitis

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ABSTRACT

Aim: To determine the effect of the developed complex treatment of patients with peritonitis on the dynamics of humoral factors of nonspecific reactivity in the course of the disease.

Materials and Methods: The study included 124 patients with toxic and terminal stages of peritonitis, who were divided into 3 groups. Group I (main) included 39 patients whose complex treatment included cytochrome C. Group II (main) included 41 patients whose complex treatment included cytochrome C and a solution containing levocarnitine and arginine hydrochloride. The comparison group comprised 44 patients who did not receive the specified drugs. The patients underwent determination of the levels of fibronectin, ceruloplasmin, and procalcitonin in the serum during the course of the disease.

Results: In patients of the I and II main groups, the use of the proposed treatment contributed to the optimization of the production of acute phase proteins: a decrease in procalcitonin production during the study, optimization of ceruloplasmin and fibronectin production, especially in the II main group. In patients of the comparison group, decompensation in the production of humoral inflammatory factors was determined, associated with a significant increase in fibronectin production, a decrease in ceruloplasmin content, and an increase in procalcitonin throughout the entire period.

Conclusions: The use of cytochrome C and a solution containing levocarnitine and arginine hydrochloride in the complex treatment of patients with disseminated peritonitis helps to optimize the production of acute phase proteins, which leads to a decrease in inflammation and the preservation of factors of nonspecific humoral activity at a subcompensated level.

KEY WORDS: peritonitis, acute phase proteins, multiple organ failure, endogenous intoxication, abdominal sepsis

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INTRODUCTION

Peritonitis is a vivid example of inflammation, which has evolved in the course of evolution and is an adaptive and protective reaction of the body that occurs in response to various damaging factors, consists of alternative, vascular and proliferative components and is a good model for studying the general mechanisms of inflammation. As you know, inflammation is one of the oldest forms of the body's defense response to damage factors (mechanical, chemical, thermal, bacterial, ischemic).

Widespread peritonitis and abdominal sepsis is a serious medical and, especially, surgical problem characterized by significant morbidity and mortality. The consequences of peritonitis depend on the local peritoneal and systemic response, as well as on the type, amount of pathogen and duration of its persistence in the abdominal cavity. In a systemic bacterial infection, an intense body response causes excessive production of inflammatory mediators and, as a result, the development of systemic inflammatory response syndrome (SIRS) [1].

Secondary peritonitis is a systemic manifestation of severe peritoneal inflammation secondary to rupture of a hollow organ caused by ischemia or necrosis, previous surgery, or trauma and is associated with an in-hospital mortality rate of approximately 30%, long-term morbidity, and reduced health-related quality of life after 6 months, which leads to increased healthcare costs [2].

Secondary peritonitis is a severe infectious disease of the body characterized by a rapid reaction of the innate immune system and leading to a severe inflammatory process. The initial response is usually accompanied by a depression of innate immunity in various types of sepsis. However, there is a lack of data on the systemic and local innate immune response during peritonitis in humans and its relationship to disease prognosis.

The outcome of secondary peritonitis is influenced by several clinical and bacteriologic features of the disease. For example, two or more microorganisms in the culture of peritoneal fluid, anaerobes, yeasts, or Enterococcus species are associated with a worse prognosis. Also, postoperative peritonitis is associated with a worse outcome and prognosis [3].

Peritonitis is a disease that requires immediate surgical treatment, and abdominal sepsis is a serious problem in the postoperative period. Repeated surgical intervention for abdominal septic foci can be planned or on demand, and improves survival, but relaparotomies are an additional risk factor for patients. Therefore, it is very important to find optimal diagnostic markers for early, noninvasive and reliable diagnosis of intra-abdominal infection and sepsis [4].

Secondary peritonitis is an intra-abdominal infection that requires urgent intervention and rapid antimicrobial therapy to achieve acceptable outcomes. However, a longer duration of antimicrobial therapy is not always associated with improved outcomes and may increase the incidence of resistant strains [5].

Therefore, the determination of humoral factors of nonspecific body reactivity in the treatment of peritonitis can help in the development of a comprehensive treatment of this disease and determine the correct treatment tactics.

AIM

The aim of the study is to determine the effect of the developed complex treatment of patients with peritonitis on the dynamics of humoral factors of nonspecific reactivity in the course of the disease.

MATERIALS AND METHODS

A single-center, single-arm, open-label observational study was conducted at the Brovary Multidisciplinary Clinical Hospital. In 2022, 222 patients with peritonitis were operated on in the surgical department, aged 18 to 93 years. Among them, there were 119 men (53.6%) and 103 women (46.4%).

Patients were examined in accordance with the Declaration of Helsinki of the World Medical Association (Seoul, 2008), orders of the Ministry of Health of Ukraine (№ 281 of November 01, 2000, № 355 of September 25, 2002, No. 356 of 05.22.2009 as amended by the Order of the Ministry of Health of Ukraine No. 574 of 08.05.2009, No. 1118 of 12.21.2012) and with the permission of the Ethics Committee of the Shupyk National University of Health of Ukraine (Protocol No. 8 of 11.7.2022). All patients gave informed consent to participate in the study.

All patients underwent clinical and laboratory examination. The material for this article was based on the medical records of inpatients treated by us, the number of which corresponded to the number of patients in the study (124 records) and the results of laboratory tests. Study inclusion criteria: peritonitis in patients over 18 years of age caused by the following diseases: acute appendicitis, acute cholecystitis, perforated gastric ulcer or duodenal ulcer, tumor perforation, acute destructive pancreatitis, abdominal injuries, small intestinal perforation, abdominal trauma, colonic obstruction of non-tumor origin, small intestinal obstruction. Criteria for non-inclusion in the study: patients with peritonitis of gynecologic genesis.

The study included patients with toxic and terminal stages of peritonitis (124 patients) who were treated in 2022. They were divided into 3 groups. Group I (main) consisted of 39 (31.4%) patients whose complex treatment included cytochrome C, which was administered at a dose of 4 ml (10 mg) twice daily intravenously for 7-10 days. Group II (main) included 41 (33.1%) patients whose complex treatment was supplemented with cytochrome C and a solution containing levocarnitine and arginine hydrochloride, which was administered to patients 100 ml intravenously once daily for 7-10 days. The comparison group consisted of 44 (35.5%) patients in whom the above drugs were not used in the complex treatment of peritonitis. The groups were identical in terms of age and gender.

We studied the activity of humoral inflammatory factors in patients with peritonitis during the course of the disease. Patients underwent the determination of fibronectin, ceruloplasmin and procalcitonin in the blood serum during the course of the disease. The level of fibronectin was determined by enzyme-linked immunosorbent assay, ceruloplasmin - by immunoturbidimetric method, procalcitonin - by immunochemiluminescent assay.

Statistical processing of the study results was performed using the Statistical software EZR v. 1.64 (graphical user interface for R statistical software version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria). Provided that the sample conforms to the law of normal data distribution, statistical hypotheses for equality of means in two dependent or independent samples were evaluated using Student's t or Fisher's F tests at a significance level of 95% ($\alpha = 0.05$). When the indicators did not conform to the law of normal distribution of data, nonparametric statistics methods were used with the use of criteria for testing Wilcoxon-Mann-Whitney (U) and Wilcoxon rank sums (T) statistical hypotheses. In all cases of statistical evaluation, the value of $p<0.05$ was considered significant.

Table 1. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the first main group, M±m, u.s., (n=19)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	105,9±14,7 *p<0,01	120,9±19,2 *p<0,01 **p<0,05	206,1±14,5 *p<0,01 **p<0,01	313,0±22,7
Ceruloplasmin content	mg/dl	66,2±2,7 *p<0,01	57,3±1,9 *p<0,05 **p<0,05	52,4±2,02 *p<0,05 **p<0,05	37,8±4,7
Procalcitonin content	ng/ml	1,41±0,32 *p<0,001	1,12±0,29 *p<0,001 **p<0,05	0,66±0,24 *p<0,001 **p<0,05	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

Table 2. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the second main group, M±m, u.s., (n=21)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	109,5±10,7 *p<0,01	139,2±17,4 *p<0,01 **p<0,05	257,2±12,7 *p<0,05 **p<0,01	313,0±22,7
Ceruloplasmin content	mg/dl	64,7±1,7 *p<0,01	55,7±1,2 *p<0,05 **p<0,05	46,5±1,9 *p<0,05 **p<0,05	37,8±4,7
Procalcitonin content	ng/ml	1,34±0,1 *p<0,001	0,71±0,14 *p<0,001 **p<0,05	0,31±0,37 *p<0,05 **p<0,001	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

RESULTS

When determining the content of fibronectin in the blood serum of patients with peritonitis of the first main group (Table 1), we found a decrease in these indicators relative to the reference values by 2.96 times ($p<0.01$) on the first day of the study.

An increase in the content of ceruloplasmin in the blood plasma was determined. The determined indicators were 1.75 times ($p<0.01$) higher than the reference values. At the same time, an increase in the content of procalcitonin was found in relation to the reference values by 14.1 times ($p<0.001$).

On the 3rd day of the study, we found an increase in fibronectin content by 1.14 times ($p<0.05$) compared to the baseline values, while these values were reduced by 2.59 times ($p<0.01$). A 1.16-fold decrease in ceruloplasmin activity was found relative to the baseline values ($p<0.05$). At the same time, these values exceeded the reference values by 1.52 times ($p<0.05$). There was a tendency to decrease the content of procalcitonin relative to the baseline values by 1.26 times ($p<0.05$), while increasing relative to the reference values by 11.2 times ($p<0.001$).

On day 7 of the study, a tendency to increase the content of fibronectin relative to the baseline values by 1.95 times ($p<0.01$) was determined, but it was reduced relative to the reference values by 1.52 times ($p<0.01$). A decrease in the content of ceruloplasmin and procalcitonin relative to the baseline values was found by 1.26 times ($p<0.05$) and 2.14 times ($p<0.05$), respectively, but they exceeded the reference values by 1.39 times ($p<0.05$) and 6.6 times ($p<0.001$), respectively.

In the study of the content of proteins of the acute phase of inflammation in patients of the second main group (Table 2) on the first day of the study, we found a decrease in the content of fibronectin relative to the reference values by 2.86 times ($p<0.01$). An increase in the content of ceruloplasmin relative to the reference values by 1.71 times ($p<0.05$) and procalcitonin by 13.40 times ($p<0.001$) was determined.

On day 3 of the study, we found a 1.27-fold increase in fibronectin content compared to baseline values ($p<0.05$), while these values were 2.25-fold lower than the reference values ($p<0.01$). A decrease in the concentration of ceruloplasmin relative to the baseline values was determined by 1.16 times ($p<0.05$), while an increase relative to the reference values was 1.47 times ($p<0.05$). A decrease in the content of procalcitonin in the blood plasma relative to the baseline values was

Table 3. Indicators of activity of humoral inflammatory factors in patients with peritonitis of the comparison group, M±m, u.s., (n=17)

Analyzed indicators	Units of measurement	Duration of the study, day			Reference values
		1 day	3 days	7 days	
Fibronectin content	μg/ml	108,5±7,7 *p<0,01	257,7±11,2 *p<0,05 **p<0,01	423,1±16,9 *p<0,05 **p<0,001	313,0±22,7
Ceruloplasmin content	mg/dl	61,4±1,5 *p<0,05	40,1±2,1 *p<0,05 **p<0,05	22,1±1,02 *p<0,05 **p<0,01	37,8±4,7
Procalcitonin content	ng/ml	1,26±0,21 *p<0,001	1,16±0,24 *p<0,001 **p<0,05	1,04±0,31 *p<0,001 **p<0,05	0,1±0,8

Notes: * - probability of differences relative to the reference values; ** - probability of differences relative to the initial values.

found by 1.89 times (p<0.05), but they exceeded the reference values by 7.1 times (p<0.001).

On day 7 of the study, we determined an increase in the content of fibronectin in the blood plasma of patients of the second group compared to the baseline values by 2.35 times (p<0.01), while decreasing relative to the reference values by 1.22 times (p<0.05). An increase in the content of ceruloplasmin relative to the reference data by 1.23 times (p<0.05) was found, while they were reduced relative to the baseline by 1.39 times (p<0.05). At the same time, a decrease in the content of procalcitonin was determined relative to the baseline values by 4.32 times (p<0.001), but they exceeded the reference values by 3.1 times (p<0.05).

In the study of the content of proteins of the acute phase of inflammation in patients of the comparison group (Table 3), we found an increase in fibronectin values relative to baseline values on days 3 and 7 of the study by 2.38 times (p<0.01) and 3.90 times (p<0.001), respectively.

A tendency to decrease ceruloplasmin on days 3 and 7 of the study relative to the baseline values by 1.53 times (p<0.05) and 2.78 times (p<0.01), respectively, was determined. An increase in the content of procalcitonin relative to the reference values was found throughout the study.

Thus, as a result of our studies, we found that in patients with peritonitis, changes in the content of proteins of acute phase of inflammation are determined throughout the study.

DISCUSSION

Fibronectin is a high molecular weight glycoprotein involved in many processes, including cell adhesion, proliferation, embryonic development, and matrix remodeling [6]. Fibronectin significantly accelerates healing and reduces areas of inflammation, and is a significant component of blood clots [7]. In addition, fibronectin plays an important role in the response to infection, participating in maintaining vascular integrity and wound healing, as well as triggering blood clotting processes [8]. It mediates important interactions between phagocytes throughout the inflammatory process and, by forming a three-component bridge, promotes bacterial colonization of endothelial and epithelial cells. A decrease in plasma fibronectin levels is associated with acute inflammation, as well as recent surgical trauma and disseminated intravascular coagulation. In a study of 159 patients with systemic inflammatory response syndrome (SIRS), the authors found a lower level of fibronectin in patients with the presence of microorganisms in the blood than in patients without it (373 μg/ml and 409 μg/ml, respectively) [9]. In our study, we also noted a decrease in fibronectin levels in patients with peritonitis, which on the first day amounted to 105.9-109.5 μg/ml with reference values of 313.0 μg/ml. Under the influence of treatment in patients of the main groups on day 7, the level of this indicator increased to 206.1-257.2 μg/ml.

Ceruloplasmin is an acute-phase plasma protein produced mainly by hepatocytes and activated monocytes and macrophages [10]. In the acute phase, levels of ceruloplasmin, as an inflammatory factor, increase due to the response to infection and inflammation. The role of ceruloplasmin in the body's immunity may be associated with the elimination of free radicals, oxidation and apoptosis of neutrophils, and the inflammatory process [11]. In addition, the ferroxidase activity of ceruloplasmin inhibits the production of reactive oxygen species mediated by iron ions, and thus ceruloplasmin has a powerful antioxidant activity [12]. Despite the fact that the mechanism of ceruloplasmin in copper and iron metabolism has been thoroughly studied,

some questions remain unresolved. Many authors have asked the following questions: Does ceruloplasmin work primarily as an antioxidant or oxidant during oxidative stress? Does it play an anti-inflammatory role in the inflammatory response? Why do studies of ceruloplasmin using different research methods on similar populations give different or even opposite results? In addition, the stability of the physiological functions of ceruloplasmin has not been determined [11]. Our study revealed elevated levels of ceruloplasmin in patients with peritonitis, indicating its active participation in the inflammatory process. Thus, in patients on the first day of the study, ceruloplasmin levels were in the range of 61.4-66.2 mg/dL (with reference values of 37.8 mg/dL). However, with effective treatment and a good patient response to it, the level of ceruloplasmin already on day 7 approached the reference values and amounted to 46.5-52.4 mg/dl in the main groups.

Procalcitonin, a member of the calcitonin superfamily, can be an important tool for the diagnosis of sepsis. Procalcitonin concentrations are associated with the severity of multiple organ dysfunction syndrome, which is secondary to systemic inflammation of infectious origin. A multivariate model showed that higher procalcitonin levels were independent risk factors for septic shock (p=0.046). Thus, according to the authors, the procalcitonin level in patients with septic shock was 54.48±62.14 μg/dL, while in patients without it, it was 0.57±1.31 μg/dL [13]. Procalcitonin helps to increase the concentration of intracellular calcium ions, which facilitate the body's response, decreasing the phagocytic activity of neutrophils in a dose-dependent manner, causes an increase in pro-inflammatory cytokines, aggravates the dysfunction of neutrophils, lymphocytes and macrophages, acts as a powerful trigger of the inflammatory cascade, increases body temperature and motor activity, affects energy homeostasis, cardiovascular stability. These effects can affect the course of the disease in patients with sepsis and are associated with morbidity and mortality. According to the authors, procalcitonin levels in patients with sepsis who survived were 24.90 ± 53.61 μg/dL, while in deceased patients they were at the level of 59.22 ± 99.87 μg/dL [14]. Our study showed the connection of the procalcitonin level with the presence of peritonitis and its dependence on the effectiveness of treatment, as we found significantly elevated levels of procalcitonin in patients with peritonitis on day 1 of the disease. The values were 1.26-1.41 ng/mL (normal range: 0.1 ng/mL). At the end of the study, these values in patients of the main groups were close to the reference values.

In patients of the first and second main groups, the use of the proposed treatment helps to optimize the production of acute phase proteins. These trends were manifested in a decrease in procalcitonin production during the study, which leads to a decrease in inflammation and the preservation of factors of nonspecific humoral activity at a subcompensated level. The optimization of ceruloplasmin and fibronectin production, especially in the second main group, was also determined, which leads to a decrease in oxidative activity and preservation of the antioxidant activity of peripheral blood in the conditions of a generalized infectious process, reduces the risk of complications associated with increased blood coagulation potential, helps maintain vascular integrity and wound healing.

At the same time, patients in the comparison group showed decompensation in the production of humoral inflammatory factors associated with a significant increase in fibronectin production and a decrease in ceruloplasmin content, which may indicate a risk of thrombosis and activation of processes associated with oxidative stress. An increase in procalcitonin throughout the period indicates a significant activity of the inflammatory response and the risk of developing multiple organ failure.

CONCLUSIONS

Determination of the dynamics of the levels of proteins of the acute phase of inflammation, such as fibronectin, ceruloplasmin and procalcitonin in the blood serum, in the course of complex treatment of patients with peritonitis is of great practical and theoretical importance, is an objective indicator of the quality of treatment and allows you to choose the right treatment tactics.

The use of cytochrome C and a solution containing levocarnitine and arginine hydrochloride in the complex treatment of patients with disseminated peritonitis helps to optimize the production of acute phase proteins, which leads to a decrease in inflammation and preservation of factors of nonspecific humoral activity at a subcompensated level. Thus, in patients of the first main group on day 7 of the study, the level of fibronectin was 206.1±14.5 μg/ml, ceruloplasmin - 52.4±2.02 mg/dl, procalcitonin - 0.66±0.24 ng/ml. In patients of the main group II on day 7 of the study, the level of fibronectin was 257.2±12.7 μg/mL, ceruloplasmin - 46.5±1.9 mg/dL, procalcitonin - 0.31±0.37 ng/mL. Whereas in the patients of the comparison group on day 7 of the study, the level of fibronectin was 423.1±16.9 μg/ml, ceruloplasmin - 22.1±1.02 mg/dl, procalcitonin - 1.04±0.31 ng/ml, which indicated decompensation in the production of proteins of the acute phase of inflammation.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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