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Research Article

Free Hemoglobin - Marker of General Condition of the Patient with Sepsis

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Received: March 29, 2018; **Published:** January 31, 2019

Abstract

Purpose of Research: to determine whether we can use free hemoglobin concentration as early prognostic marker and a predictor of mortality in sepsis.

Materials and Methods: In a retrospective study in 60 patients aged 47,6 ± 7,2 years with sepsis (30,4 ± 2,1 points on the Mannheim's scale for evaluation of the severity of peritonitis) modern methods of statistics (ROC-analysis) hypothesis was tested, whether level of free hemoglobin in the first 24 hours from the moment of admission can be used as a biomarker for diagnosis and prognosis for severe sepsis. Informative criterion was compared with the information of the procalcitonin test.

Results: The present study had shown that the above average free hemoglobin concentration, measured on the first day of the heavy flow of sepsis, is directly connected with increased 30-days mortality, and the level of free hemoglobin in a first day of the disease has high sensitivity, specificity, and can determine the outcome of sepsis with accuracy up to 96,7%.

Conclusion: Free hemoglobin concentration above medium size identified on the first day of the currents of severe sepsis, is directly related to increased 30-day mortality, and researched level of free hemoglobin in day 1 of the disease has a high proportion of sensitivity and specificity. Level of free hemoglobin is Predictor outcome of sepsis in the first 24 hours after the start of therapy, but the results did not rule out the need to use the necessary test from septic patients, but rather the feasibility of combining the two dictates the criteria to assess the outcome of severe septic process that requires further research.

Keywords: *Free Hemoglobin; Procalcitonin; Sepsis; ROC-Analysis*

Introduction

The septic process is characterized by persistent disturbances of microcirculation, which is regarded as a complex set of adaptive and pathological processes, the result of which can be both the restoration of the organ's function and metabolism, and its death [1]. This is especially true for patients who have undergone septic shock, since the duration of the shock (centralization of the blood circulation and subsequent persistent vasodilation) will determine not only the severity of organ disorders, but also the outcome of the disease.

The importance of hematological, biochemical and microbiological testing for the diagnosis of sepsis cannot be overemphasized. However, in recent years, serious efforts have been made to find other biomarkers of sepsis that would allow early diagnosis of this disease. In general, reliable, universally recognized markers exist today, such as C-reactive protein and procalcitonin [2]. They allow you to diagnose sepsis, to predict the outcome, to monitor its course [3] and positively proven, even in patients with a disorder of the immune status [4]. But, according to some authors, these markers cannot be used alone, but should be supplemented by a thorough clinical examination with the addition of other laboratory data [5].

To identify an early marker that determines the likelihood of developing septic shock and its outcome, a chronological analysis of events and the role of "participants" at the level of the microvasculature occurring in a particular period of time are necessary

In the condition of dehydration, hypovolemia, ischemia and hypoxia, only the erythrocyte (carrying a combination of two chemical elements - iron and oxygen, having the highest oxidation-reduction potential) can be the "main participant of events", as the most numerous cellular element of the systemic and organ blood flow. Against the background of increased plasma viscosity and hypoperfusion due to hypovolemia, intensive aggregation of erythrocytes occurs. This leads to a decrease in their ability to deformation [6]. It should be noted that hypoxia is accompanied by an intense formation of a superoxide radical (O-2), which actively attacks the red blood cell membrane, promotes changes in the erythrocyte configuration (schizocytes, poikilocytes, etc.), which results in an increase in cell volume, intravascular hemolysis and free hemoglobin release in systemic blood flow [7].

There are several possible mechanisms leading to hemolysis in sepsis. First, some pathogens themselves are capable of inducing hemolysis, releasing hemolyzing toxins [8-10]. Secondly, the filaments of fibrin can destroy erythrocytes [11,12]. Thirdly, the complement system, which is activated during sepsis, can also worsen the viability of red blood cells [13]. Fourth, lipopolysaccharides affect the mechanical properties of the membrane, contributing to the death of red blood cells [14]. Fifth, in the case of sepsis, erythrocyte death occurs by eryptosis, a process described in the literature [15]. Eryptosis, or premature aging of erythrocytes, can be caused by damage to its membrane by numerous factors, which compromises their integrity and, therefore, causes erythrocyte suicide [16]. Eryptosis is similar to apoptosis but develops without the participation of nuclei and mitochondria (since they are not present in erythrocytes a priori). Eryptosis occurs due to complex mechanisms of activation of ion channels of the erythrocyte membrane, under the influence of kinases, phosphatidylserine and phospholipases [16].

Finally, an increase in the concentration of free hemoglobin may be due to transfusion of erythrocyte mass [17].

Intravascular hemolysis is directly associated with pro-oxidant and pro-inflammatory stress. This primarily affects the endothelium with the development of endothelial dysfunction, which is characterized by a reduced contribution of nitric oxide (NO) to the regulation of vascular tone with a predominance of vasoconstriction [18], since the short-lived nitric oxide molecule is rapidly inactivated by free hemoglobin and does not allow the vasodilatation effect to be realized [19].

Thus, in terms of hypoperfusion in the splanchnic blood flow, conditions are created in which a significant number of erythrocytes is damaged, causing subsequent hemolysis and the appearance in the bloodstream of a large amount of free hemoglobin, which, in our opinion, may be one of the early markers of severe abdominal sepsis.

The aim of the study was to determine the possibility of using the concentration of free hemoglobin as an early marker and a predictor of mortality in abdominal sepsis.

Materials and Methods

A study was conducted in 60 patients (53 men and 7 women) at the age of 47.6 ± 7.2 years with diagnosed sepsis (surgical, obstetrical) treated in the intensive care unit of the Municipal Clinical Emergency Hospital No. 1 in the period 2012 - 2016. The study was officially approved by the ethics committee of the Municipal Clinical Emergency Hospital No. 1. All survived patients gave informed consent for the use of laboratory data. The diagnosis of sepsis was established in the presence of a septic focus (widespread purulent-fibrinous peritonitis, established during laparoscopy), criteria of a systemic inflammatory reaction (leukocytosis, fever, respiratory and hemodynamic disorders), organ dysfunctions (hepato- and nephropathy, encephalopathy), procalcitonin level in blood plasma.

Patients were divided into 2 groups - survivors of up to 30 (n = 22) days after surgery and deceased (n = 38) during the same period after surgery. Septic shock developed in 56 (93.3%) patients in the early postoperative period, corresponding to the severity of peritonitis and its evaluation by the Mannheim Peritonitis Index [20], which amounted to 30.4 ± 2.1 points. Diagnosis of septic shock was carried out taking into account the presence of a focus of infection (purulent fibrinous effusion in the abdominal cavity) in combination with hypoten-

sion, requiring vasopressors to maintain an average blood pressure ≥ 65 mm Hg, despite the sufficient amount of infusion therapy and adequate ventilation, which corresponds to modern recommendations of the international consensus for the determination of sepsis and septic shock [21], as well as taking into account > 5 points on the SOFA scale (Sepsis-related Organ Failure) used to assess the degree of dysfunction in sepsis [21]. Additional criteria on the recommendation of J.L. Vincent, C. Ince, J. Bakker (2012) was the presence of clinical symptoms in patients: a symptom of a "white spot" with a duration of > 3 seconds, a diuresis rate of < 0.5 ml/kg/h, a disorder of consciousness < 15 points by the Glasgow Coma Scale [22].

Patients after hospitalization underwent complex therapy up to 133,6 ± 22 minutes in order to prepare for surgical treatment, which included infusion therapy (1850 ± 550 ml), antibacterial, vasopressor therapy (dopamine 10,5 ± 4,5 mg/kg/min) and artificial ventilation according to the indications. The program of the study at hospitalization (before surgery), as well as 24 and 72 hours later, included complete blood count and blood chemistry, where, in addition to other biochemical parameters, the concentration of free hemoglobin in the blood plasma and the level of procalcitonin were studied. The concentration of free hemoglobin was determined by the hemoglobin-cyanide method in the Saveliev modification [23]. To quantify the concentration of procalcitonin in blood plasma, an immunoluminometric reagent kit (LUMI test® PCT, B·R·A·H·M·S Diagnostical GmbH, Berlin, Germany) was used, which makes procalcitonin a highly sensitive and specific marker of the systemic inflammatory reaction [24].

The criteria for inclusion in the study were: the written consent of the surviving patient to use the data of his laboratory study, the age of more than 18 years and less than 60 years, the presence of a septic focus (common purulent-fibrinous peritonitis, established during laparoscopy), the severity of peritonitis by the Mannheim Peritonitis Index [20] more than 20 points, the severity of the General state on the SOFA scale (Sepsis-related Organ Failure) more than 5 points, clinical and laboratory criteria of septic shock [21].

The criteria for exclusion from the study were: written refusal of the surviving patient to use the data of his laboratory study, death during the first 24 hours after operation, the lack of clinical and laboratory criteria for septic shock, the severity of peritonitis on the scale by the Mannheim Peritonitis Index less than 20 points, the fact of transfusion of erythrocyte mass in the period up to 3 days from the beginning of the study, age less than 18 years and more than 60 years.

The results of the biochemical parameters of patients we were interested in at different times are presented in the table 1 with the exception of data indicating the presence of organ disorders within the structure of the SOFA scale, which we assessed to determine the severity of the general state but was not the purpose of our study.

Indicators	**Survivors (n = 22)**			**Deceased (n = 38)**		
	On admission	24 hours later	72 hours later	On admission	24 hours later	72 hours later
Free plasma hemoglobin, g/l	0.72$^{\alpha}$ 0.44;0.92	0.92$^{\alpha}$ 0.87;1.21	0.51$^{\alpha}$ 0.34;0.55	1.29 0.98;1.37	1.35 0.89;1.41	1.02 0.77;1.31
Procalcitonin, ng/ml	7.82$^{\alpha}$ 6.68;8.12	8.1$^{\alpha}$ 7.88;8.92	7.7$^{\alpha}$ 8.12;8.91	9.65 9.12;11.22	12.1 11.41:12.65	10.5 9.12;11.23

Table 1: *Dynamics of laboratory parameters of blood, ME [25%; 75%]*
Note: α – *p < 0.05 compared to a group of deceased in a specified period of time.*

Statistical processing of the results was performed using Statistical 6.0 software. Nonparametric methods of statistical analysis were used due to incorrect distribution of the sample. The threshold value of the significance level is taken to be 0.05, the trend was considered to be 0.05 < p < 0.1. The accuracy of the differences between the groups was evaluated using the Mann-Whitney test. Medians and percentiles with an interval of 25 - 75% are calculated in order to exclude rare and fallen out of the total mass values of biochemical parameters. The Pearson correlation coefficient (r) was used to estimate the linear relationship between the quantitative characteristics. Evaluation of diagnostic sensitivity, specificity and diagnostic effectiveness of reliable laboratory parameters was carried out by ROK-analysis.

Results

After a correlation analysis with the calculation of Gamma correlation coefficient (G), we found a statistically significant relationship between the outcome of abdominal sepsis and the level of free hemoglobin at admission (G= 0.40, p = 0.0013), on the 1st day (G = 0.94, p = 0.0001), on the 3rd day (G = 0.43, p = 0.0007), the level of procalcitonin on admission (G = 0.58, p = 0.0003). On admission, there was also a strong correlation between the level of free hemoglobin and procalcitonin (r = 0.76, p = 0.0001 n = 60) (Figure 1).

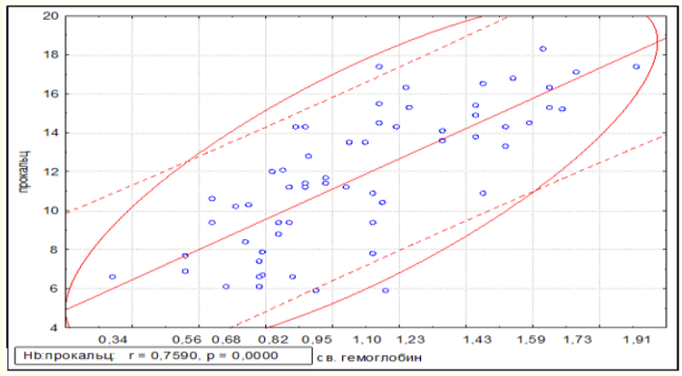

Figure 1: *Correlation between the concentration of free hemoglobin and procalcitonin in patients with sepsis on admission to the hospital.*

The results of the statistical indicator revealed that the lower the level of free hemoglobin and procalcitonin in the blood serum, the greater the chance of a positive outcome of sepsis (Figure 2).

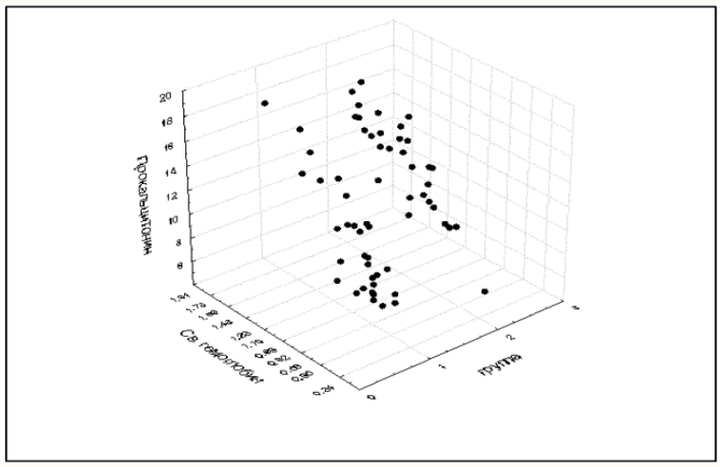

Figure 2: *The relationship between the outcome of sepsis and the level of free hemoglobin and procalcitonin on admission to the hospital.*

Conspicuous is the results of the construction of the ROC-curve for the analysis of sensitivity and specificity of the level of free hemoglobin and procalcitonin for the sepsis prognosis for the 1st day (Figure 3), which are likely to determine the outcome of the disease, and a large area under the curve (left), relatively free hemoglobin, allows to present it as a more sensitive criterion in the period of 1 day from the date of admission.

During 3 days after the development of sepsis there was a decrease in the level of free hemoglobin in the group of survivors. However, in the group with adverse outcome, the concentration of free hemoglobin remained significantly higher, especially by the end of 1 day after admission, which is more related to the period of reperfusion, which determines the severity of the general state of patients and, apparently, the outcome of the disease (Figure 4).

Based on the results obtained, we suggested that the data on the level of free hemoglobin can more accurately predict the outcome in patients with sepsis. ROC-analysis with the construction of ROC-curves and analysis of the area under the curves (AUC) confirmed the existence of the possibility to predict the outcome of sepsis on the level of free hemoglobin for 3 days after the development of the septic process, as well as on the level of procalcitonin on admission. However, it was found that the most valuable predictors are the level of free hemoglobin on the 1st day and the level of procalcitonin upon admission (Table 2).

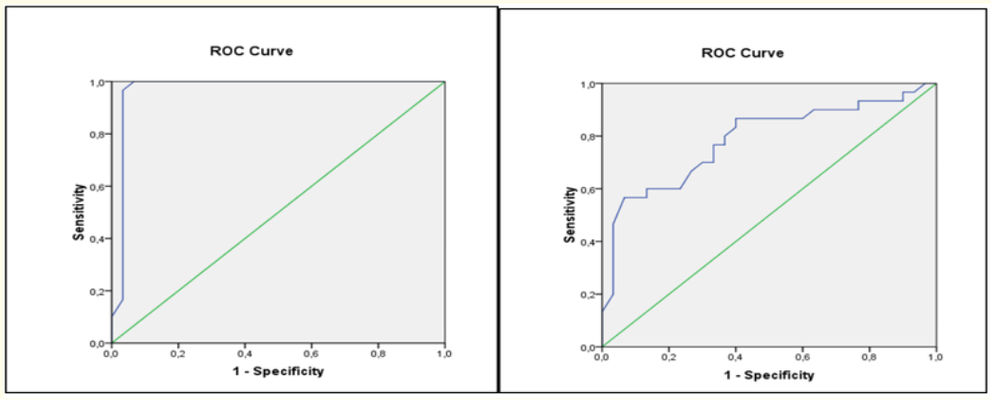

Figure 3: *ROC-curves of sensitivity and specificity analysis based on the concentration of free hemoglobin (left) and the concentration of procalcitonin (right) to predict the outcome of sepsis on the 1st day.*

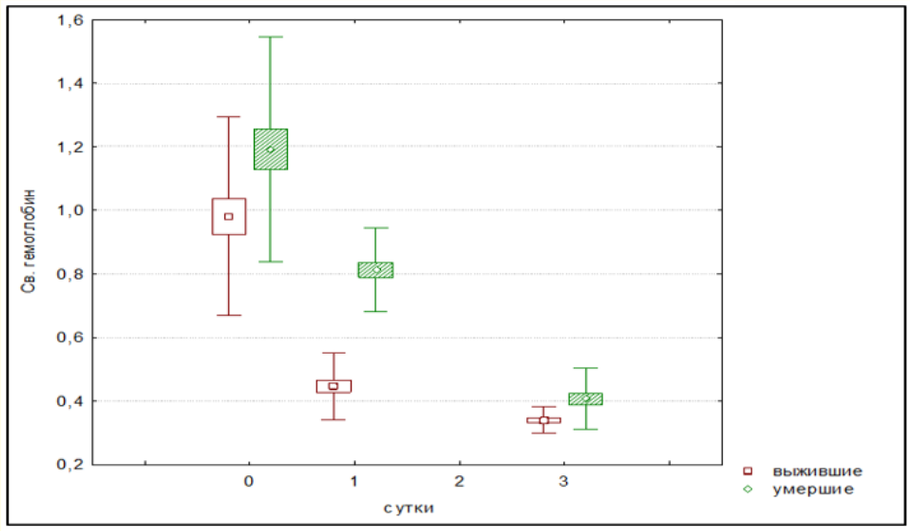

Figure 4: *The level of free hemoglobin for 3 days in survivors (n = 22) and deceased (n = 38) patients with sepsis (accuracy of differences between groups p < 0.05 on admission and 24 hours later).*

Indicators	AUC (95% CI)	p-level	Cuttoff threshold	Sensitivity (CI) %	Specificity (CI) %
Free hemoglobin on admission	0.698 (0.563 - 0.834)	0.008	1.08	58.8 (48.9 - 68.6)	60.2 (50.1 - 70.2)
Free hemoglobin 1 day later	0.971 (0.915 - 1.027)	0.000	0.63	75 (64.2 - 85.7)	72.7 (61.6 - 83.8)
Free hemoglobin 3 days later	0.687 (0.549 - 0.824)	0.013	0.34	61.7 (27.3 - 96.4)	56.3 (27.1 - 85.4)
Procalcitonin	0.786 (0.668 - 0.904)	0.000	11.55	65.2 (56.4 - 74.1)	62.7 (52.5 - 73.1)

Table 2: *The results of ROC-analysis for the identified predictors of outcome of sepsis.*

On the basis of data obtained by ROC-analysis and shown in table 2, the following may be affirmed. There is an extremely high risk of an unfavorable outcome of abdominal septic process in patients with peritonitis with a concentration of free hemoglobin in the blood plasma on admission of more than 1.08 g/l, while maintaining by the end of the first day a concentration of more than 0.635 g/l, and maintaining for the third day more than 0.345 g/l, in combination with the level of procalcitonin on admission of more than 11.55 ng/ml.

Given the two possible outcomes of sepsis (survivors and deceased), we used binary logistic regression to make a prediction. Based on the results of logistic regression of continuous signs, prognostic models of sepsis outcome were constructed on the basis of laboratory research data given in the table 3. Taking into account that the maximum level of free hemoglobin was determined by the end of the first day after hospitalization and operation, a model of prognosis for the outcome of sepsis by one indicator was calculated.

Some of the variance explained by logistic regression in this model (Table 3), is 83.7%. Thus, using even one indicator - the level of free hemoglobin on the 1st day-it is possible to determine the outcome of sepsis with an accuracy of 96.7%. Thus, using even one indicator - the level of free hemoglobin on the 1st day - it is possible to determine the outcome of sepsis with an accuracy of 96.7%.

Signs	Analysis indicators				
	B (regression coefficient)	S.E. (standard error)	df	Significance	Exp (B)
Level of free hemoglobin on the first day	19.1	5.1	1	0.0001	1.98
Constant	–12.1	3.31	1	0.0001	0.0001
Chi-sq = 59.3 df = 1 p < 0,0001					
The correctness of the predictions: 96.7%					

Table 3: *Model of prediction of sepsis outcome based on the level of free hemoglobin on the first day after admission.*

Adding to the model an indicator of the level of hemoglobin, which has been identified in patients with abdominal sepsis on admission, we obtained the following results (Table 4).

Signs	Analysis Indicators				
	B (regression coefficient)	S.E. (standard error)	df	Significance	Exp (B)
Level of free hemoglobin on admission	–8.88	3.6	1	0.057	0.001
Level of free hemoglobin on the first day	29.2	9.5	1	0.002	4.9
Constant	–10.2	3.3	1	0.002	0.001
Chi-sq = 66.3 df = 2 p < 0.0001					
The correctness of the predictions: 96.7%					

Table 4: *Model of prognosis of sepsis outcome based on free hemoglobin level on admission and in 24 hours.*

Note: the regression equation for this model $p=1/(1+e^{-10,2-8,88 \text{ free hem. on adm.}+28,2 \text{ free hem. on the first day}})$.

This model already explains 89.2% of the variance. Thus, the addition of another parameter slightly increases the significance of the model, but does not change the correctness of the prediction, which, as in the previous model, is 96.7%.

Using as prognostic factors a combination of the levels of free hemoglobin, determined on admission and 24 hours later, and procalcitonin, determined on admission to the hospital, did not improve the informativeness of the model, but on the contrary, somewhat reduced the significance of the model (Table 5).

Signs	analysis indicators				
	B (regression coefficient)	S.E. (standard error)	df	Significance	Exp (B)
Level of free hemoglobin on admission	–1.7	1.4	1	0.24	0.182
Procalcitonin	0.496	0.165	1	0.003	1.6
Constant	–4	1.3	1	0.001	0.018
Chi-sq = 18,5 df = 2 p < 0,0001					
The correctness of the predictions: 76,7%, explains 35,4% of the variance.					

Table 5: *Model of prediction of sepsis outcome on the basis of free hemoglobin and procalcitonin on admission to the hospital.*

Thus, the models, and the figures presented in tables 2 - 4, can predict the outcome of sepsis with a probability of up to 96.7%. However, the use of three indicators of the level of free hemoglobin in different periods of the study is not necessary, because it does not significantly affect the value of the model. It is enough to focus on the levels of free hemoglobin on admission or on the 1st day from the moment of hospitalization (model presented in the table 3 and 4).

Discussion

The findings of the study are not the result of statistical analysis alone. They have a certain logical pattern that explains the outcome of sepsis, depending on the level of free hemoglobin. There are various lines of evidence showing the potential pathophysiological value of hemolysis [18,19]. First of all, they relate to the direct connection of free hemoglobin excess with the development of endothelial dysfunction, in the development of which free hemoglobin plays a key "starting" role. Endothelium under hemolysis exposed to active forms of oxygen, catalyzed by heme, and the walls of the vessel are the primary tissue when exposed. In recent years, it has been shown that the oxidative stress and inflammation induced by it directly contributes to vaso-occlusive events and the formation of a thrombus in conditions of hemolysis. JD Belcher., *et al*. (2005, 2006) showed that hemoglobin, heme and iron obtained from hemolyzed red blood cells contributed to excessive production of superoxide and hydroxyl radicals. This leads to the expression of the endothelial activation of adhesion molecules on the endothelium, which in turn favors the adhesion of erythrocytes and leukocytes to the endothelium and leads to instability of blood vessels and, ultimately, to microthrombosis [25,26].

Another aspect of the pathophysiological value of free hemoglobin for its evaluation in sepsis is the effect of rapid binding of the nitric oxide molecule, causing disorders of microvascular perfusion [27].

An additional mechanism, on the basis of which free hemoglobin can be involved in the harmful effects of hemolysis in sepsis, is the direct influence of iron on the growth of microbes [4-6]. All bacterial agents without exception, and especially pathogenic (Gram- and Gram+), have a direct interest in free iron, determining its virulence just by the activity of the dependence on iron [28]. The pathogenic fungi also have a similar dependence on the free iron [29]. Today, the essence of all iron-binding proteins and a number of enzymes is considered as an immunological defense against bacteria that have different mechanisms of its consumption, without neglecting red blood cells containing 70% of the iron of organism, which is expressed in the direct aggression of bacteria to the erythrocyte membrane, which promotes haemolysis [30]. Moreover, recently S Brauckmann., *et al*. (2016) demonstrated for the first time that lipopolysaccharides induce hemolysis of erythrocytes also by direct action on their cell membrane, which leads to a decrease in their osmotic resistance, a decrease in membrane stiffness, shizocytogenesis and, subsequently, hemolysis [31].

The multiple threats from sepsis to global health protection are directly dependent on iron metabolism and, most importantly, on our growing understanding of the links between infection, its direct interest in iron, and the metabolism of iron in the body. As indicated above, there is now sufficient evidence of a causal relationship between free hemoglobin and the outcome of sepsis in conditions of simulating sepsis in animals. Free hemoglobin affects the signal transduction Toll-receptors, TNFα synthesis and directly affects the mortality of animals [32-34]. In addition, it was noted in experimental sepsis that the concentration of free hemoglobin can be reduced by administering hemopexin, thus reducing the degree of septic manifestation, which reflects the pathogenetic equivalent of iron in sepsis [35]. In recent studies, other mechanisms have been shown that lead to adverse effects of free hemoglobin, including through its oxidation and production of ferrile-hemoglobin and heme [36-38]. Together, as well as the results of our own previous studies, this shows the importance of free hemoglobin and its derivatives in the development of the septic process.

Conclusion

The concentration of free hemoglobin above the mean value found on the first day of severe sepsis is directly related to an increase in 30-day mortality, and the level of free hemoglobin on the first day of the course of the disease has a high degree of sensitivity and specificity.

The level of free hemoglobin is a sensitive predictor of the outcome of sepsis on the first day after the initiation of therapy, but the results do not exclude the need for the use of a procalcitonin test in septic patients, but on the contrary dictates the applicability of combination of the two criteria for assessing the outcome of a severe septic process, which requires continued research.

Conflict of Interest

The authors declare no conflict of interest.

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Volume 5 Issue 2 February 2019

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