EVALUATION OF PROGNOSTIC SIGNIFICANCE OF BLOOD ENDOTOXIN ACTIVITY INDICATOR IN PATIENTS WITH SEPSIS

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Introduction. *Laboratory indicators of endotoxinemia in conjunction with the clinical picture have been used as the criteria to diagnosticate sepsis for quite a long time. The objective of the study is to analyze the prognostic significance of endotoxin activity indicator in patients with sepsis that undergo treatment in conditions of surgical resuscitation and intensive care at multidisciplinary hospital.*

Materials and methods. *One-center prospective study on the basis of ICU department of the Republic Clinical Hospital (Ufa, Russia) during the period of 2015- 2016. We have performed screening of 60 patients with sepsis and 15 healthy volunteers.*

Results. *The median of the value of endotoxin activity in group of patients with sepsis amounted to 0.42 vs. 0.1 of group of healthy volunteers. It was found that the level of endotoxin activity correlates with the severity of the disease: low scores on the scale of the APACHE II severity are consistent with low endotoxin activity (r=0.985, r2=0.971, p=0.0001). Findings show that the median of sensitivity of the medical test corresponds to 87.5% and that of specificity - to 32.6%. The prognostic value of a positive result amounted to 27.5%, and the prognostic value of a negative result - 97.8%. The area under the ROC-curve for the level of endotoxin activity is equal to 0.749±0.084, 95% confidence interval (CI) 0.620-0.852. Sensitivity-61.5% (54.8-86.0), specificity is 93.6% (82.4-98.6). The optimum point of separation was 0.88. Positive predictive level amounted to 72.7%, negative one - 89.8%.*

Conclusions. *The test to determine the level of endotoxin activity of blood has high sensitivity and negative prognostic value, allowing you to use it as an effective screening method to assess the probable invasion of gram-negative flora, days before the results of bacteriological research are available.*

Keywords: *lipopolysaccharide A, endotoxinemia, sepsis, septic shock, predictor*

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ОЦЕНКА ПРОГНОСТИЧЕСКОЙ ЗНАЧИМОСТИ ПОКАЗАТЕЛЯ АКТИВНОСТИ ЭНДОТОКСИНА КРОВИ У ПАЦИЕНТОВ С СЕПСИСОМ

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Введение. *Лабораторная оценка эндотоксинемии, совместно с клинической картиной, уже достаточно давно используется в качестве критериев диагностики сепсиса. Однако, на сегодняшний день недостаточно данных, позволяющих оценить прогностическую эффективность теста активности эндотоксина. Цель исследования - анализ прогностической значимости показателя активности эндотоксина у пациентов с сепсисом, находящихся на лечении в условиях отделения хирургической реанимации и интенсивной терапии многопрофильного стационара.*

Материалы и методы. *В рамках одноцентрового проспективного исследования на базе анастезиолого-реанимационного отделения №1 ГБУЗ РКБ им. Г.Г. Куватова (г. Уфа) в период октябрь 2015 - май 2016 года проведен скрининг 60 пациентов с сепсисом и 15 здоровых добровольцев.*

Результаты. *Установлено, что медиана значения активности эндотоксина в группе больных сепсисом составила 0,42 против 0,1 группы здоровых добровольцев. Уровень активности эндотоксина коррелирует с тяжестью заболевания: низкие баллы по шкале тяжести состояния APACHE II соответствовали низким показателям активности эндотоксина (r=0.985, r2=0.971, p=0.0001). Медиана чувствительности диагностического теста соответствует 87,5%, а специфичности - 32,6%. Прогностическая ценность положительного результата составила 27,5% (вероятность наличия инвазии грамотрицательной флоры у пациента с положительным результатом теста), а прогностическая ценность отрицательного результата − 97,8% (вероятность отсутствия инвазии грамотрицательной флоры у пациента с отрицательным результатом теста). Оценка прогностической роли в развитии септического шока демострирует: площадь под ROC − кривой для уровня активности эндотоксина оказалась равной 0,749±0,084, 95% доверительный интервал (ДИ) 0,620 - 0,852. Чувствительность − 61,5% (54,8-86,0), специфичность − 93,6% (82,4-98,6). Оптимальная точка разделения составила 0,88. Положительный предсказывающий уровень составил 72,7%, отрицательный 89,8%.*

Заключение. *Таким образом, установлено, что высокий уровень эндотоксинемии является предиктором развития тяжелого сепсиса/септического шока, органных дисфункций, высокой летальности. Тест определения уровня активности эндотоксина крови обладает высокой чувствительностью и негативной прогностической ценностью, что позволяет использовать его в качестве эффективного скринингового метода оценки вероятной инвазии грамотрицательной флоры до получения результатов бактериологических исследований.*

Ключевые слова: *липополисахарид А, эндотоксинемия, сепсис, септический шок, предиктор*

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INTRODUCTION

Pyoinflammatory diseases of the abdominal cavity and, as a consequence, the resulting abdominal sepsis, remain challenging medical problem and today continues to be one of the leading causes of mortality, duration of treatment and disability [1]. The number of deaths from abdominal sepsis varies every year, from 30% to 90% [2,3]. According to [4,5], frequency of gram-negative sepsis is 40% of the cases. Endotoxin or lipopolysaccharide A is an obligate component of the outer cell membrane of gram-negative bacteria and the primary product of gram-negative bacteria responsible for septic shock. High level of endotoxin in blood causes changes in the expression of more than 300 genes, activated macrophages, neutrophils, endothelial cells,

causes activation of the blood clotting system, that leads as the result to the development of septic process [6, 7]. Laboratory indicators of endotoxinemia in conjunction with the clinical picture have been used as the criteria to diagnosticate sepsis for quite a long time [8]. In recent years, upon evaluation of the concentration of endotoxin researchers have been using analysis of Endotoxin Activity Assay (EAA) (Spectral Diagnostics Inc., Canada). However, to date, there are insufficient data to evaluate the predictive efficacy of EAA test [9].

The objective of the study is to analyze the prognostic significance of endotoxin activity indicator in patients with sepsis that undergo treatment in conditions of surgical resuscitation and intensive care at multidisciplinary hospital.

MATERIALS AND METHODS

Design.

One-center prospective study on the basis of ICU (intensive care unit) department of the Republic Clinical Hospital named after G.G. Kuvatov (Ufa, Russia) during the period of October 2015- May 2016. We have performed screening of 60 patients with sepsis and 15 healthy volunteers. The specialists of the University Hospital Regensburg (Regensburg, Germany) provided advice in the organization and planning of this study. The work has been approved by the local Ethics Committee of the Republic Clinical Hospital (№. 212 dated 11.09.2015).

Target of research.

Does the Result of the Endotoxin Activity Assay correlate with the severity of disease and severitiy of infection in patients getting treated in an intensive care unit in comparison with healthy volunteers?

To monitor the patient's disease severity and extent of organ dysfunctions, we measured biochemical indicators, coagulation values , levels of endotoxin and procalcitonin daily (along with combined intensive therapy.)

Combined intensive therapy of sepsis in all patients was conducted in accordance with the recommendations of the Russian Association of Surgical Infection Specialists and The Third International Consensus Definitions for Sepsis and Septic Shock [10]. Bacteriological researches were carried out on the first day of hospitalization, on the 7th day of stay at the intensive care unit and, if necessary, were repeated depending on the clinical situation. The average duration of bacteriological research with determination of sensitivity of microorganisms to the applied antibacterial medicines was 5 days.

Endotoxin activity analysis was conducted using standard sets of EAA (Spectral Diagnostics Inc., Canada) according to the manufacturer's instructions. According to the manufacturer the Endotoxin Activity Assay uses spezific Immunoglobulin M (IgM) which binds to Lipopolysaccharide A. The developing immune complex activates neutrophils to trigger an oxidative burst. Luminol responds to those released reactive oxygen species with emitting light, which is then detected with a chemiluminometer. Each examination has to be done with two controls.

The patient's blood sample, stabilized with EDTA, was placed into three test tubes from the set. Tube 1 measured the basal activity in the absence of specific antiendotoxic antibodies (IgM) to determine the activity of neutrophils in the patients whole blood at the specific time without an additional trigger. Tube 3 measured the maximum oxidative burst of neutrophils of the patient in response to an excess amount of exogenous endotoxin to display the maximal activity of neutrophils and luminescence for the patient. Test tubes 1 and 3 were used as internal control. Tube 2 measured the level of activity in the sample in the presence of endotoxin-antibody (specific IgM). The Emission of light from those three tubes was detected by a chemiluminometer. The EAA is a semi quantitative test to stratify the Endotoxin level. The device calculates the endotoxin activity level and displays the results in the form of activity of endotoxin or EAA units.

Statistical analysis.

The findings are processed using the statistical package Statistica 10.0 (StatSoft Inc, USA). The normality of the distribution of actual data was checked by using the criterion of Shapiro-Wilk. The groups were described using the median and interquartile interval. Variance analysis was performed using the criterion of Kruskal-Wallis test (for independent observations) and Friedman (for repeated observations). Critical level of p significance for statistical criteria was taken equal to 0.05. The prognostic value of the activity level of endotoxin in the development of severe sepsis/ septic shock and lethality was assessed with ROC-analysis (Receiving Operating Characteristics), taking into account the true-and false-positive results and calculating the area under the ROC-curves and consent criterion (goodness-of-fit).

RESULTS AND DISCUSSION

Based on laboratory evaluation of the endotoxic load the patients were distributed in 3 groups. (table 1). The 1-st group comprised patients with low EBA <0,4; the 2-nd group included patient with intermediate value of EAA which is equal to 0,4-0,6; the 3-rd group had patients with high level of EAA>0.6.

Table 1 – Clinical and demographic characteristics of patients

Characteristic	Group I, n = 20	Group II, n = 19	Group III, n = 21	Total, n = 60
Age ± SD, years	53,7±7,9	58,1±8,4	55,8±7,1	57,9±8,5
Male (%)	11 (55,0)	9 (47,4)	11 (52,4)	31 (51,6)
The severity at the time of hospitalization ± SD				
APACHE II	13,4±3,7	16,8±4,3	20,9±5,1	17,4±5,2
SOFA	2,3±1,8	5,5±2,7	13,8±3,1	7,3±3,1
Diagnosis upon hospitalization, n (%)				
Pancreato-necrosis	11 (55,0)	10 (52,6)	13 (61,9)	34 (56,7)
Respiratory infection	2 (10,0)	3 (15,9)	1 (4,8)	6 (10,0)
Peritonitis	7 (35,0)	6 (31,5)	7 (33,3)	20 (33,3)
Infectious agent 1, n (%)				
Gram-positive flora	6 (30,0)	4 (21,1)	5 (23,8)	15 (25,0)
Gram-negative flora	11 (55,0)	6 (31,5)	7 (33,3)	24 (40,0)
Phungi	2 (10,0)	1 (5.3)	3 (14,3)	6 (10,0)
Mixed infection	1 (5,0)	8 (42.1)	6 (28,6)	15 (25,0)
Lethality 2, n (%)	2 (10,0)	3 (15,7)	8 (38,1)	13 (21.7)
Laboratory indicators at the time of hospitalization, Me (25-75)				
Body temperature,	38.1 (37,2-38,9)	38.2 (37,7-38,6)	38.1 (37,4-38,8)	38.1 (37,2-38,5)
Leukocytes, x 109/l	16.7 (11,7-19,4)	13.7 (9,1-15,2)	17.9 (14,7-23,9)	15.4 (10,2-19,1)

Note: 1- according to the results of bacteriological researches made on the first day of hospitalization in the INTENSIVE CARE UNIT, 2 - 28-day mortality.

Among the patients included into the 3 groups there were no observed differences in terms of age, gender and body weight. The severity of the disease of the studied patients was assessed by using the APACHE II score, which reached an average of 17 points, and the degree of organ failure associated with sepsis, according to the SOFA scale reached an average of 7 points.

Figure 1 represents the level of activity of endotoxin in patients and healthy volunteers. The level of activity of endotoxin of patients is in the range 0.1-0.9, with maximum activity of endotoxin in 95% of healthy volunteers does not exceed 0.1. The median of the value of endotoxin activity in group of patients with sepsis amounted to 0.42 vs. 0.1 of group of healthy volunteers.
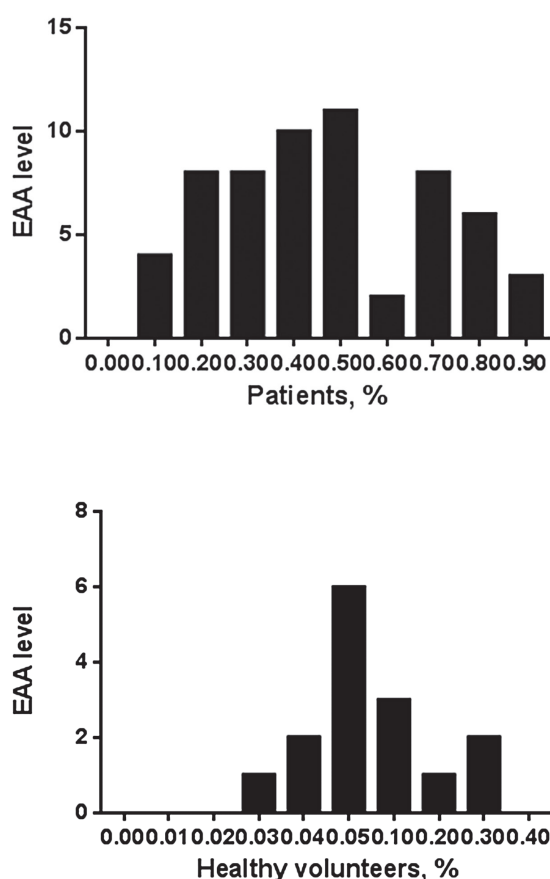

Figure 1 – Indicators of activity of endotoxin in groups of patients with sepsis (a) and healthy volunteers (b).

It was found that the level of endotoxin activity correlates with the severity of the disease: low scores on the scale of the APACHE II severity are consistent with low endotoxin activity (r = 0.985, r2 = 0.971, p = 0.0001). Patients with severe sepsis and septic shock are predominantly in the group with a high level of endotoxin activity.

The next step was to assess prognostic value of high level of blood endotoxin activity as a criterion for gram-negative invasion flora (table 2) and predictor of developing of severe sepsis/septic shock (table 3).

Table 2 – Evaluation of the prognostic value of endotoxin activity of blood in diagnosis of invasion of gram-negative flora

Indicator	Value
Diagnostic sensitivity,%	87,5 (76,5-96,7)
Diagnostic specificity,%	32,6 (21,2-44,3)
Prognostic value of a positive result,%	27,5 (24,3-35,1)
Prognostic value of a negative result,%	97,8 (95,2-98,3)

Findings show that the median of sensitivity of the medical test corresponds to 87.5% and that of specificity - to 32.6%. The prognostic value of a positive result amounted to 27.5% (probability of infestation of gram-negative flora in a patient with a positive test result), and the prognostic value of a negative result - 97.8% (probability of no infestation of gram-negative flora in a patient with a negative test result). On the first day of hospitalization at the intensive care unit among 60 surveyed patients 8 patients showed high (> 0.60) EAA level values without development of severe sepsis. However, within the next 48 hours 13 patients of Group III showed: shock (n = 4), hypoxemia (n = 8), chronic renal insufficiency (n = 4), repeated restoration operations (n = 13). It should be noted that most patients showed combination of these conditions.

Table 3 – Risk of severe sepsis/septic shock during the first 48 hours, depending on the level of endotoxin activity of blood

Group	Development of severe sepsis/ septic shock,%	RR	x^2 values; r
Group I, n = 20	5.6	-	
Group II, n = 19	8.7	2.0 [1,57; 2,61]	5.2; r < 0.01
Group III, n = 21	17.2	3.5 [1,8; 3,62]	9.7; r < 0.01

Note: RR-relative risk with the calculated confidence interval.

The study was completed with analyzing the interrelation of mortality to the level of endotoxin activity. Mortality from severe sepsis and septic shock within 28 days after being hospitalized in the ICU surveillance was 32.4% in the group with high level and 11.9% in the group with low level of endotoxinemia. The area under the ROC-curve (W) for the level of endotoxin activity is equal to 0.749 ± 0.084, 95% confidence interval (CI) 0.620-0.852. Sensitivity-61.5% (54.8 -86.0), specificity is 93.6% (82.4 -98.6). The optimum point of separation was 0.88. Positive predictive level amounted to 72.7%, negative one - 89.8%.
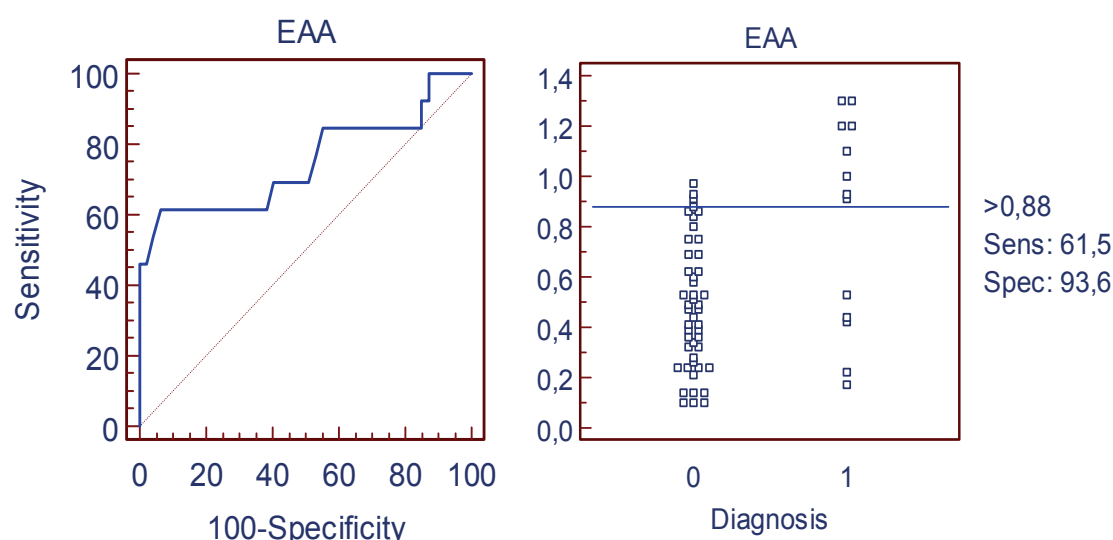
Figure 2 – The distribution of the deceased and survivors and ROC–curve of endotoxin activity.

Thus, the findings show that the high level of endotoxinemia is a predictor of severe sepsis/septic shock, organ dysfunction and high letality. The test to determine the level of endotoxin activity of blood has high sensitivity and negative prognostic value, allowing you to use it as an effective screening method to assess the probable invasion of gram-negative flora, days before the results of bacteriological research are available.

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