https://doi.org/10.23934/2223-9022-2020-9-3-348-355

Association of SP-A and SP-D Surfactant Proteins with the Severity of Community-Acquired Pneumonia

O.S. Kharlamova1, 2*, K.Y. Nikolayev1, 3, Y.I. Ragino1, M.I. Voyevoda1

Laboratory of emergency treatment
1Research Institute of therapy and preventive medicine – branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences
175/1 Borisa Bogatkova St., Novosibirsk 630089, Russian Federation
2Novosibirsk Region City Clinical Hospital No. 25
1a Alexandra Nevskogo St., Novosibirsk 630075, Russian Federation
3Novosibirsk State University
1 Pirogova St., Novosibirsk 630090, Russian Federation

*** Contacts:** Olga S. Kharlamova, postgraduate student of the Laboratory for Emergency Therapy, Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences; head of the Therapeutic Department, Novosibirsk Region City Clinical Hospital No. 25. Email: olga.kharlamova2016@yandex.ru

RELEVANCE In current clinical practice, there is a need for research to find new diagnostic tests for the purpose of determining the patients with the highest risk of death from pneumonia. Surfactant proteins SP-A and SP-D play a key role in the pathogenesis of the response to microbial invasion of lung tissue, which participate in a cascade of reactions of both innate and adaptive immunity, and therefore proteins SP-A and SP-D may be considered as markers of the severity of community-acquired pneumonia (CAP).

AIM OF STUDY To evaluate the associations of surfactant proteins SP-A and SP-D in blood plasma with the severity of CAP.

MATERIAL AND METHODS The study included 247 patients admitted to the therapeutic department. The group of patients with CAP (n=188) was divided into groups of severe (n=103) and non-severe (n=85) pneumonia. The comparison group (n=59) consisted of patients without acute and chronic diseases of the bronchi and lungs. The mean age (years, Me, 25th; 75th percentile) of patients was 55 (47; 68), 55 (47; 70), and 61 (37; 63) years, respectively. All patients underwent clinical, functional, diagnostic and laboratory studies (including determination of the content of SP-A and SP-D proteins by enzyme immunoassay).

RESULTS In the group of patients with severe pneumonia unlike mild pneumonia, and group of comparison higher levels of proteins SP-A and SP-D were observed. Correlation analysis described below revealed statistically significant connection: protein SP-D — direct relation with leukocyte levels (r=0.320, p<0.0001), erythrocyte sedimentation rate (r=0.331, p<0.0001), inverse relation with blood oxygen saturation (r=-0.407, p<0.0001), for SP-A protein — direct relation with body temperature (r=0.355, p<0.0001), erythrocyte sedimentation rate (r=0.369, p<0.0001) in the blood C-reactive protein (r=0.446, p<0.0001), SP-D (r=0.357, p<0.0001), and also relation with the duration of clinical symptoms (r=0.528, p<0.0001) and blood oxygen saturation (r=-0.401, p<0.0001). When conducting ROC- analysis for the surfactant protein SP-A, the area under the ROC-curve was 0.70, the optimal sensitivity for severe pneumonia was 68%, the specificity was 69% at the SP-A level in blood plasma equal to 42.9 ng/ml. When performing ROC analysis for the surfactant protein SP-D, the area under the ROC curve was 0.64 for severe pneumonia, the optimal sensitivity was 62%, and the specificity was 62% at the SP-D content in blood plasma equal to 319.2 ng/ml.

CONCLUSION According to the results of this study, the SP-A and SP-D proteins are associated with clinical and laboratory signs that reflect the severity of CAP. Thus, SP-A and SP-D are new laboratory markers of CAP severity.

Keywords: surfactant, surfactant protein A, surfactant protein D, biomarker, community-acquired pneumonia

For citation Kharlamova OS, Nikolayev KY, Ragino YI, Voyevoda MI. Association of SP-A and SP-D Surfactant Proteins with the Severity of Community-Acquired Pneumonia. Russian Sklifosovsky Journal of Emergency Medical Care. 2020;9(3):348–355. https://doi.org/10.23934/2223-9022-2020-9-3-348-355 (in Russ.)

Conflict of interest Authors declare lack of the conflicts of interests

Acknowledgments, sponsorship The material of the article is part of the budgetary topic of Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences. The work was carried out according to the State assignment within the framework of the budgetary topic № AAAA-A17-117112850280-2.

The work was carried out on the basis of Novosibirsk Region City Clinical Hospital No. 25, Novosibirsk, Russian Fedration. The work was carried out within the framework of the state assignment for the integration project (0324-2018-0040) “Development of new methods for the rapid diagnosis of human diseases based on the detection of organ-specific markers using modern physical and physicochemical approaches”.

Affiliations

Olga S. Kharlamova	postgraduate student of the Laboratory for Emergency Therapy, Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences; head of the Therapeutic Department, Novosibirsk Region City Clinical Hospital No. 25; https://orcid.org/ 0000-0001-8788-685X, olga.kharlamova2016@yandex.ru; 40%, collection and processing of material, statistical processing, analysis and interpretation of data, writing text
Konstantin Yu. Nikolayev	Doctor of Medical Sciences, Professor, Head of the Laboratory of Emergency Therapy, Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences; https://orcid.org/0000-0003-4601-6203, nikolaevky@yandex.ru; 20%, research concept and design, data analysis and interpretation, editing
Yuliya I. Ragino	Doctor of Medical Sciences, Professor, Corresponding Member of the Russian Academy of Sciences, Acting Head of the Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian

	Branch of the Russian Academy of Sciences; https://orcid.org/ 0000-0002-4936-8362, ragino@mail.ru; 20%, the concept and design of the study, obtaining data for analysis, analyzing and interpreting data, editing
Mikhail I. Voyeboda	Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Head of the Scientific Direction of Fundamental and Clinical Research, Research Institute of therapy and preventive medicine — branch of the Federal Research Center Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences; https://orcid.org/0000-0001-9425-413X, mvoevoda@ya.ru; 20%, research concept and design, data analysis and interpretation, editing

CAP – community-acquired pneumonia
ARDS – acute respiratory distress syndrome
ESR – erythrocyte sedimentation rate
RR – respiratory rate
CRP – C- reactive protein
SP-A – surfactant protein A
SP-D – surfactant protein D

INTRODUCTION

Lower respiratory tract infections, in particular community-acquired pneumonia (CAP), are the fourth leading cause of death worldwide (about 2.4 million deaths in 2016) [1, 2]. In Russia, in 2018, the incidence rate of CAP was 492.2 per 100,000 population against 413.2 in 2017, that is, an upward trend (by 19.1%) in the number of CAP cases is outlined [3].

Considering the prevalence of pneumonia and the incidence of life-threatening complications, numerous works have been devoted to its diagnosis and treatment: in 2019, at the request of "Community-acquired pneumonia", according to the Medline database, more than 2000 articles were published. Considerable information has been accumulated for understanding the comprehensive pathogenesis of pneumonia. However, the authors of the 2019 American Thoracic Society (ATS) EP guidelines emphasize that few key clinical issues have been studied extensively enough to provide rigorous guidelines for standard of care. [4]. ATS recommends the use of the Pneumonia Severity Index (PSI), which includes a number of biochemical indicators: blood acidity (pH), the content of urea nitrogen, sodium, glucose in it, as well as hematocrit and partial pressure of oxygen in arterial blood; and the British Thoracic Society's CURB-65 scale, assessing confusion, blood urea, respiratory rate, blood pressure, and patient age (over 65) [5, 6]. However, the use of the PSI index in clinical practice is quite difficult due to the need to use a number of biochemical parameters that are routinely determined not in all medical institutions in Russia, the PSI index also does not always accurately determine the indications for referring a patient to the intensive care unit. [1, 7]. All of the above confirms that in modern clinical practice there is a need for further research in order to find new diagnostic tests to determine the intensity of treatment and identify patients with the highest risk of death from pneumonia.

In the pathogenesis of the response to microbial invasion of lung tissue, one of the key roles is played by the surfactant proteins SP-A and SP-D, which are involved in the cascade of reactions of both innate and acquired immunity. Surfatant proteins SP-A and SP-D are considered in modern literature as a diagnostic and prognostic markers of such severe lung diseases as chronic obstructive pulmonary disease [8-11], bronchial asthma [12-14], lung cancer [15, 16], interstitial lung diseases [17-19], idiopathic pulmonary fibrosis [20], pulmonary aspergillosis [21], sarcoidosis [22], acute respiratory distress syndrome (ARDS) [23–26]. It has been established, for example, that the content of the surfactant protein SP-D in blood plasma is a sensitive and specific biomarker of ARDS [27], and the content of SP-A is a sensitive and specific prognostic biomarker of the risk of death in ARDS [28].

Currently, more and more data are being published on the role of the SP-A and SP-D proteins at the earliest stages of the response to foreign invasion of the lung tissue. Proteins SP-A and SP-D are involved in the activation of the complement pathway, opsonization of foreign microorganisms, positively regulate the expression of cell surface receptors responsible for the recognition of pathogens and phagocytosis, and are also inducers of systemic inflammation, leading to a cascade of cytokine reactions [29]. The complex interactions of the SP-A and SP-D proteins are among the key ones in the regulation of pneumonia [26]. Only a few studies of the blood levels of SP-A and SP-D proteins in CAP compared with healthy individuals showed not only the presence of higher indicators, but also the association of proteins with the development of non-threatening complications and mortality in CAP. [30, 31].

In view of the above, the surfactant proteins SP-A and SP-D can be considered as diagnostic and prognostic markers for CAP.

OBJECTIVE: To evaluate the associations of surfactant proteins SP-A and SP-D in blood plasma with the severity of community-acquired pneumonia.

MATERIAL AND METHODS

The study included 247 patients admitted to the therapeutic department of the City clinical hospital No. 25 in Novosibirsk. Inclusion criteria for the study: patients of both sexes aged from 18 to 75, consent to participate in this study and filling out the corresponding informed consent form, absence of acute and chronic diseases of the bronchi and lungs, as well as the presence of a verified CAP diagnosis. General criteria for excluding patients from this study: the presence of an acute infectious process and oncological diseases at the time of enrollment in the study, previous courses of chemotherapy or radiation therapy, immunodeficiency disease, previous / active pulmonary tuberculosis, clinically significant (according to the researcher) unstable cardiac disease, for example , uncontrolled symptomatic arrhythmia, atrial fibrillation, heart failure with congestive symptoms of the 3rd or 4th degree according to the classification NYHA (New York Heart Association Functional Classification – functional classification of chronic heart failure), severe renal failure, determined by the value of the glomerular filtration rate less than 15 ml / min / 1.73 m2, calculated by the

formula CKD-EPI taking into account the serum creatinine concentration, type I diabetes mellitus (DM), pregnancy or lactation, a known concomitant life-threatening illness in which life expectancy may be less than 18 months from the date of enrollment.

The group of patients with CAP, n = 188 (the diagnosis was verified according to the diagnostic criteria of the Russian Respiratory Society in 2010), was divided into groups of severe (n = 103) and mild (n = 85) pneumonia [7]. In severe CAP 78.8% of patients had more than 3 criteria of severity, 2 criteria – 18.3%, 1 criterion – 2.9% of patients. Upon admission to the emergency department, the decision on the place of hospitalization was made based on the calculation of points on the CURB / CRB-65 scale (according to the criteria of the Russian Respiratory Society, 2014) [32], patients with severe CAP belonged to group III and needed urgent emergency hospitalization. The comparison group (n = 59) consisted of patients without acute and chronic diseases of the bronchi and lungs, hospitalized with a worsening of the course of essential hypertension in the therapeutic department and the day hospital. All patients and persons included in the comparison group underwent clinical, functional-diagnostic and laboratory studies within 24 hours from the moment of admission to the hospital. Laboratory diagnostics was performed using a biochemical analyzer Beckman Coulter AU 480 and hematology analyzer Siemens Healthineers ADIVA2120i, vs 5300. Concentration of surfactant proteins SP-A и SP-D in serum determined by enzyme immunoassay on an analyzer Multiscan EX using a test system ELISA BioVendor within a day from the moment of hospitalization with an assessment of vital functions at the time of collection of laboratory data. Blood oxygen saturation was also determined before blood sampling for the study of the content of surfactant proteins by a pulse oximeter Armed YX200. X-ray of the chest organs was carried out using the "TeleKord-MT" apparatus (complex X-ray diagnostic telecontrolled).

Statistical processing of the data obtained was carried out using the software package SPSS 10.05. The nature of the distribution of quantitative traits was determined using the Kolmogorov – Smirnov test. In the case of a normal distribution, the mean value (M) and standard deviation (SD) were calculated. When comparing two normally distributed samples, we used Student's t-test. In the absence of a normal distribution, the interquartile range was calculated – the median (Me) and the 25% and 75% percentiles. The relationships between the traits were assessed by calculating the Spearman correlation coefficient (r). When evaluating qualitative features, the criterion χ2 was used. The dependences of the sensitivity and specificity of biomarkers on prognostic estimates were built using the area under the ROC curve (AUC) during the ROC analysis. Во всех процедурах статистического анализа критический уровень значимости нулевой статистической гипотезы (p) принимался равным 0,05.

Протокол исследования одобрен локальным этическим комитетом по месту его проведения.

RESULTS

The clinical characteristics of patients in groups are presented in Table 1.

Table 1

Clinical characteristics of patients

	Comparison group, n = 59	The group of patients with non-severe community-acquired pneumonia, n = 85	The group of patients with severe community-acquired pneumonia, n = 103
Gender, male / female, n (%)	32 (54.2) / 27 (45.8)	42 (49.4) / 43 (50.6)	60 (58.3) / 43 (41.7)
Age, years, Me (25%; 75%)	55 (47; 68)	55 (47; 70)	61 (37; 63) #
Height, (M±SD), cm	169.2 ± 9.0	168.1 ± 9.5	168.6 ± 8.8
Body weight, Me (25%; 75%), kg	75 (69; 85)	75 (68; 85)	70 (61; 78) *#
Body temperature upon admission, Me (25%; 75%), ° ºC	36.7 (36.5; 37.0)	37.6 (36.9; 37, 9) **	37.9 (37.5; 38, 2) **##
Respiratory rate (M±SD), respirations/minute	17.6 ± 4.8	19.6 ± 2.0 *	22.4 ± 2.6 **##
Heart rate (M±SD), beats/minute	84.2 ± 10.2	90.0 ± 15.1 *	94.6 ± 16.3 **#
Patients secreting sputum, n (%)	8 (13.5)	42 (49.4) **	86 (83.5) **##
Patients with hemoptysis, n (%)	0 (0)	0 (0)	9 (8.7) **##
Patients with chest pain, n (%)	0 (0)	9 (10.6) **	37 (35.9) **##

Notes: comparison of groups of community-acquired pneumonia, severe and non-severe degree with a comparison group: * - p <0.05, ** - p <0.001; comparison of groups of community-acquired pneumonia of severe and non-severe degree: #- p <0.05, ## - p <0.001

As can be seen from Table 1, in patients with mild and severe CAP, when compared with the data of the comparison group at admission, there was a statistically significantly higher body temperature and respiratory rate (RR). Also, statistically significant differences were obtained in the

proportion of patients with sputum production, hemoptysis and chest pain. In patients from the group of severe CAP, the body weight was lower than in the groups of comparison and non-severe CAP. When comparing groups with severe and non-severe CAP, statistically significant differences were obtained in the following parameters: temperature on admission, heart rate, respiratory rate, proportion of patients with sputum production, hemoptysis and chest pain. Also, the groups of non-severe and severe CAP statistically significantly differ in the level of blood oxygen saturation: 98.3% (98.0; 99.0) and 96.7% (96.0; 98.0), respectively (p<0,001).

Data on concomitant diseases in patients are presented in Table 2.

Table 2
Comorbidities in patients

	Comparison group, n = 59	The group of patients with non-severe community-acquired pneumonia, n = 85	The group of patients with severe community-acquired pneumonia, n = 103
Gender, male / female, n (%)	32 (54.2) / 27 (45.8)	42 (49.4) / 43 (50.6)	60 (58.3) / 43 (41.7)
Age, years, Me (25%; 75%)	55 (47; 68)	55 (47; 70)	61 (37; 63) #
Height, (M±SD), cm	169.2 ± 9.0	168.1 ± 9.5	168.6 ± 8.8
Body weight, Me (25%; 75%), kg	75 (69; 85)	75 (68; 85)	70 (61; 78) *#
Body temperature upon admission, Me (25%; 75%), ° C	36.7 (36.5; 37.0)	37.6 (36.9; 37, 9) **	37.9 (37.5; 38, 2) **##
Respiratory rate (M±SD), respirations/minute	17.6 ± 4.8	19.6 ± 2.0 *	22.4 ± 2.6 **##
Heart rate (M±SD), beats/minute	84.2 ± 10.2	90.0 ± 15.1 *	94.6 ± 16.3 **#
Patients secreting sputum, n (%)	8 (13.5)	42 (49.4) **	86 (83.5) **##
Patients with hemoptysis, n (%)	0 (0)	0 (0)	9 (8.7) **##
Patients with chest pain, n (%)	0 (0)	9 (10.6) **	37 (35.9) **##

Notes: comparison of groups of community-acquired pneumonia, severe and non-severe degree with a comparison group: * - p <0.05, ** - p <0.001; comparison of groups of community-acquired pneumonia of severe and non-severe degree: #- p <0.05, ## - p <0.001

When comparing the groups for concomitant diseases, statistically significant differences were revealed in the proportion of patients with a history of tobacco smoking in the comparison and severe CAP groups compared with the non-severe CAP group. The proportion of patients with essential hypertension was statistically significantly higher in the comparison group than in the group of non-severe CAP.

In addition, for all patients with CAP, the duration of the clinical symptoms of CAP was assessed at the outpatient stage (until admission to the hospital). In the group of severe CAPs, 32 people (31.1%) had symptoms of more than 10 days, in the group of non-severe CAPs, 12 (14.1%) respectively, which is statistically significantly less ($\chi 2=132,6$, p<0,001).

The characteristics of laboratory parameters of patients are presented in Table. 3.

Table 3
Indicators of general and biochemical blood test in patients

	Comparison group, n = 59	The group of patients with non-severe community-acquired pneumonia, n = 85	The group of patients with severe community-acquired pneumonia, n = 103
White blood cells, Me (25%; 75%), * 10 9 / L	8.1 (6.6; 10.1)	10.4 (8.1; 13.3)	13.3 (9.6; 17.0) **##
Red blood cells, (M ± SD), * 10 9 / L	4.5 ± 0.7	4.4 ± 0.9	4.3 ± 0.7
Hemoglobin, (M ± SD), g / L	135.3 ± 22.7	130.2 ± 18.5	127.6 ± 19.8
Platelets, Me (25%; 75%), * 10 9 / L	225 (176; 267)	231 (188; 299)	206 (170; 275)
Stab neutrophils, Me (25%; 75%), %	2 (2; 3)	3 (2; 4)	5 (3; 6) **##
Segmented neutrophils, Me (25%; 75%), %	62 (57; 69)	66 (60; 72) *	70 (60; 78) *#
Lymphocytes, Me (25%; 75%), %	26 (19; 31)	23 (18; 28)	16 (11; 25) **##
Monocytes, Me (25%; 75%), %	6 (4; 8)	6 (4; 8)	6 (4; 8)
Erythrocyte sedimentation rate (ESR) , Me (25%; 75%) mm/hour	8 (5; 13)	20 (12; 30)	29 (19; 40) **##
Alanine aminotransferase (ALT), Me (25%; 75%), IU/L	18.5 (12.0; 29.2)	22.4 (15.2; 39.5) * *	22.5 (16.3; 35.0) *
Aspartate aminotransferase (AST), Me (25%; 75%), IU/L.	21.3 (17.1; 35.0)	24.4 (18.2; 38.0) *	28.4 (20.4; 41.8) *
Total protein, (M ± SD), g / L	71.0 ± 26.5	71.3 ± 6.2	68.6 ± 7.6 *
Creatinine, Me (25%; 75%), mmol / L	99.0 (80.9; 21.6)	93.6 (80.3; 106.0)	90.4 (80.5; 120.7)
C- reactive protein (CRB), Me (25%; 75%), mg / L	10.7 (0; 18.0)	12.0 (0; 24.0) *	24 (12.0; 48.0) **
Fibrinogen, Me (25%; 75%), g / L	3.9 (3.4; 5.9)	5.0 (4.1; 6.8)	5.8 (4.5; 7.2)
SP-A, Me (25%; 75%), ng / ml	34.2 (27.0; 46.0)	36.9 (28.8; 46.8)	57.6 (39.7; 92.9) **##
SP-D, Me (25%; 75%), ng / ml	274.1 (174.0; 484.2)	273.9 (189.4; 425.7)	411.8 (249.3; 649.9) *##

Notes: comparison of groups of community-acquired pneumonia, severe and non-severe degree with the comparison group: * - $p < 0.05$, ** - $p < 0.001$; comparison of groups of community-acquired pneumonia of severe and non-severe degree: #- $p < 0.05$, ## - $p < 0.001$

Table 3 shows that in the group of severe pneumonia, when compared with the data of the group of non-severe CAP and the comparison group, higher levels of the following hemogram parameters were obtained: the number of leukocytes, stab neutrophils, lymphocytes, as well as the erythrocyte sedimentation rate (ESR). All patients with CAP in the blood had higher levels of segmented neutrophils, alanine aminotransferase, aspartate aminotransferase, and C-reactive protein (CRP) than in the comparison group. At the same time, the levels of surfactant proteins SP-A and SP-D in the group of severe CAPs exceeded those in the groups of comparison and non-severe CAPs.

Correlation analysis of surfactant proteins with clinical and laboratory characteristics revealed the most significant statistically significant relationships for the following indicators. For surfactant protein SP-D – a direct relationship with the level of leukocytes ($r = 0.320$, $p < 0.0001$), ESR ($r = 0.331$, $p < 0.0001$), feedback with blood oxygen saturation ($r = -0,407$, $p<0,0001$). Для сурфактантного белка SP-A – прямая связь с температурой тела ($r=0,355$, $p<0,0001$), ESR ($r=0,369$, $p<0,0001$), the level of CRP in the blood ($r=0,446$, $p<0,0001$), surfactant protein SP-D ($r=0,357$, $p<0,0001$), and, the strongest, statistically significant association with the duration of clinical symptoms of CAP ($r=0,528$, $p<0,0001$), inverse statistically significant relationship between SP-A and blood oxygen saturation ($r=-0,401$, $p<0,0001$).

In the ROC analysis for the surfactant protein SP-A for severe pneumonia, the area under the ROC curve was 0.70, the optimal sensitivity was 68%, and the specificity was 69% at a blood SP-A level of 42.9 ng / ml. In the ROC analysis for the surfactant protein

SP-D for severe pneumonia, the area under the ROC curve was 0.64, the optimal sensitivity was 62%, and the specificity was 62% at a blood SP-D content of 319.2 ng / ml.

THE DISCUSSION OF THE RESULTS

Our results with respect to the surfactant protein SP-D are consistent with modern studies, which show not only the presence of a higher blood level of SP-D in patients with CAP compared with healthy patients, but also the relationship of this protein with respect to the development of life-threatening complications and mortality in CAP [30, 31]. Thus, in a study by the Ovidius group (2016), it was shown that patients with severe CAP had statistically significantly higher levels of SP-D compared to patients with non-severe CAP, and it was found that SP-D is a more informative predictor of long-term mortality in follow-up period compared with CRP and procalcitonin [33]. There are few studies on the surfactant protein SP-A in CAP. S. Spadaro and M. Park (2019) did not find significant differences in the total SP-A level in patients with CAP and the control group without bronchopulmonary pathology, however, the same study showed a difference in serum SP-A levels in patients with CAP in combination with type II diabetes from their values in patients with CAP only [34].

In our study, in patients with severe CAP, compared with non-severe CAP, patients were found to have higher body temperature upon admission to the hospital, increased respiratory rate, decreased blood oxygen saturation, leukocytosis with a shift in the formula to the left, and increased markers of acute phase reactions (ESR, CRP). These results are consistent with numerous studies due to the fact that these parameters are widely used to stratify risk and severity in patients with CAP. [35, 36]. No studies were found in the available literature on the association of clinical and laboratory characteristics with the surfactant proteins SP-D and SP-A in CAP. We have determined higher serum levels of SP-A and SP-D proteins in patients with severe CAP compared with their values in patients without broncho-pulmonary diseases and with non-severe pneumonia. This direct relationship between the SP-A blood level and the duration of the clinical symptoms of CAP is due to the important role of this protein in the immune response in the lungs, the presence of its direct bactericidal activity, which confirms the possibility of using SP-A as a marker of epithelial damage in the lungs.

The highest area under the ROC curve for severe pneumonia was associated with the surfactant protein SP-A (0.70), indicating moderate marker prediction accuracy. Our data slightly differ in area under the ROC curve for most of the known biomarkers in CAP. [37–40].

Evaluating the SP-A and SP-D correlations obtained in our study, we can conclude that surfactant proteins are associated with the main clinical manifestations of acute infectious lung injury: respiratory failure, fever, and duration of clinical symptoms. A connection was also obtained with key laboratory parameters (leukocytosis, accelerated ESR, increased blood levels of CRP), which reflect the activity of the inflammatory process, its severity, as well as the reactivity of the body in response to a foreign invasion of the respiratory system. The direct relationship between the blood content of surfactant proteins SP-A and SP-D is probably due to the similar protective functions of these proteins.

CONCLUSION

According to the results of this study, surfactant proteins SP-A and SP-D are associated with clinical and laboratory signs that reflect the severity of the course of community-acquired pneumonia. The obtained results, in our opinion, indicate the involvement of the surfactant proteins SP-A and SP-D in the pathogenesis of clinical manifestations of community-acquired pneumonia, which reflects an active inflammatory process and damage to the lung parenchyma. Thus, surfactant proteins SP-A and SP-D are new laboratory markers of the severity of community-acquired pneumonia.

FINDINGS

1. Blood levels of SP-A and SP-D proteins in severe community-acquired pneumonia ((ng / ml, Me, 25th; 75th percentiles) 57.6 (39.7; 92.9) and 411.8 (249.3; 649.9), respectively) higher than with mild community-acquired pneumonia (36.9 (28.8; 46.8) and 273.9 (189.4; 425.7), respectively) and the absence of bronchopulmonary diseases (34.2 (27.0; 46.0) and 274.1 (174.0; 484.2), respectively).

2. For the surfactant protein SP-A, a statistically significant direct relationship between its blood level and body temperature ($r = 0.355$, $p < 0.0001$), erythrocyte sedimentation rate ($r = 0.369$, $p < 0.0001$), blood C-reactive protein ($r = 0.446$, $p < 0.0001$), SP-D ($r = 0.357$, $p < 0.0001$), as well as the duration of clinical symptoms ($r = 0.528$, $p < 0.0001$) and inverse relationship with saturation blood oxygen ($r = -0.401$, $p < 0.0001$).

3. For the surfactant protein SP-D, statistically significant direct associations of its blood level with leukocytosis ($r = 0.320$, $p < 0.0001$), erythrocyte sedimentation rate ($r = 0.331$, $p < 0.0001$) and feedback with blood oxygen saturation were obtained ($r = -0.407$, $p < 0.0001$).

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Received on 25.11.2019
Accepted on 23.04.2020