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THE DEGREE OF INVOLVEMENT OF THE LEVEL OF CIRCULATING IMMUNE COMPLEXES AND RED BLOOD CELLS IN THE PATHOGENESIS OF COMMUNITY-ACQUIRED PNEUMONIA

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The paper presents the findings of the study of the degree of involvement of the level of circulating immune complexes and red blood cells in the pathogenesis of community-acquired pneumonia. Marked disorders in immunological indices of the severity degree of the disease in patients with community-acquired pneumonia have been established, which must be taken into account when diagnosing the disease: elevated circulating immune complexes level in the blood serum of patients and an increase in the total circulating immune complexes level, mainly due to an increase in the most toxigenic small- (<11 S) and medium molecular weight (11 S–19 S) fractions of the immune complexes. The higher the level of circulating immune complexes, especially small molecular weight ones, the more likely a severe course of community-acquired pneumonia is. In community-acquired pneumonia, oxidative stress and intensified generation of reactive oxygen species develop. Changes in the quantity and quality of blood corpuscles necessitate the inclusion of agents that improve blood oxygenation into standard antibacterial therapy.

Key words: community-acquired pneumonia, circulating immune complexes, erythrocytes, haemoglobin.

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СТУПІНЬ УЧАСТІ РІВНЯ ЦИРКУЛЮЮЧИХ ІМУННИХ КОМПЛЕКСІВ І ЕРИТРОЦИТІВ У ПАТОГЕНЕЗІ ПОЗАЛІКАРНЯНОЇ ПНЕВМОНІЇ

У роботі представлені результати вивчення ступеня участі рівня циркулюючих імунних комплексів та еритроцитів у патогенезі позалікарняної пневмонії. Встановлено виражені порушення з боку імунологічних показників ступеня тяжкості у хворих з позалікарняною пневмонією, які необхідно враховувати при діагностиці захворювання: підвищення рівня циркулюючих імунних комплексів у сироватці крові хворих та збільшення загального рівня ЦВК, в основному, за рахунок збільшення найбільш токсигенних дрібно- (<11 S) та середньомолекулярних (11 S–19 S) фракцій імунних комплексів: чим вищий рівень циркулюючих імунних комплексів, особливо дрібномолекулярних, тим найбільш ймовірним є тяжкий перебіг позалікарняної пневмонії. При позалікарняній пневмонії розвивається окислювальний стрес та посилення генерації активних форм кисню. Зміни кількості та якості формених елементів крові зумовлюють необхідність включення до стандартної антибактеріальної терапії засобів, що покращують оксигенацію крові.

Ключові слова: позалікарняна пневмонія, циркулюючі імунні комплекси, еритроцити, гемоглобін.

The study is a fragment of the research project *"Mucoactive and herbal medicines for the treatment of cough in acute infectious and inflammatory diseases of the lower respiratory tract", state registration No. 0117U000595*

The search for new targets for targeted therapy of pathological conditions is one of the primary tasks of contemporary fundamental and applied in medicine. It is very important that not only genes and their products (receptors, signalling molecules, etc.) can act as such targets, but also biological processes that play a key role in the pathogenesis of certain diseases. One of these universal processes is oxidative stress, which to some extent occurs in most pathological conditions and plays a crucial role in the

pathogenesis of alterative and inflammatory diseases [5]. Tissues are most sensitive to oxygen deficiency, last of all they are adapted to the anaerobic means of obtaining energy [3, 4]. Blood, as a liquid connective tissue of the body, not only ensures the interconnection of all organs and systems, being an indicator of the body state, but also directly reacts to oxygen deficiency, thus, blood corpuscles (white blood cells, platelets, granulocytes, white blood cells, plasma cells and monocytes) are interesting objects for study in community-acquired pneumonia (CAP), since they differ from each other not only in the functions they perform, but also in the nature of metabolic processes, the degree of oxygen utilization, the ability to generate ROS and resistance to them. Red blood cells are unique in that they are constantly in contact with oxygen, transporting it to all tissues, though they do not use oxygen for themselves. Red blood cells with its exclusively anaerobic metabolism, do not contain the main oxygen-using systems: mitochondria and endoplasmic reticulum. The formation of energy in them occurs by substrate phosphorylation of ADP in glycolysis reactions, they are not capable of synthesizing proteins and do not have DNA. On the other hand, red blood cells are cells that constantly have oxygen, are contained in hemoglobin, and are maximally resistant to the damaging effects of its active forms. Constant interaction with oxygen causes autooxidation of red blood cells' hemoglobin with the formation of superoxide radicals, as well as other ROS, mainly hydrogen peroxide and hydroxide radicals [6]. The above processes are the key pathogenetic components in the development of community-acquired pneumonia (CAP); however, this aspect has practically not been studied to date. [5, 7].

The purpose of the study was to clarify the involvement and significance of the circulating immune complexes and red blood cells in the pathogenesis of community-acquired pneumonia.

Material and methods. 104 (63 men and 41 women) patients, aged 20 to 80 years, with community-acquired pneumonia, who received treatment in the Therapeutic Department of the Kharkiv Municipal Clinical Hospital No. 25, have been examined. CAP was diagnosed on the basis of the findings of the epidemiological, clinical, laboratory, and X-ray studies. Patients with such pathologies as tuberculosis, bronchial asthma, Hepatitis B, C and D, HIV, blood diseases and oncological diseases were excluded from the study [7]. The control group was formed of 20 apparently healthy individuals (AHI) of the same age and gender.

During hospitalization, all examined patients, according to the standards of the International Society of Pulmonologists and the Recommendations of the F.G. Yanovsky National Institute of Phthisiology and Pulmonology (Kyiv, 2019), received standard antibiotic therapy.

Patients have been examined in accordance with the Medical Standards (F.G. Yanovsky National Institute of Phthisiology and Pulmonology). CAP causative agents were verified by the conventional microscopic and bacteriological methods.

The parameters of the immunological study included: determination of the concentration of circulating immune complexes (CICs) by precipitation in a solution of polyethylene glycol (PEG) with a molecular weight of 6000 Daltons. The molecular composition of the CICs was determined by differentiated precipitation in 2 %, 3.5 % and 6 % PEG solutions [2].

To study the reaction of red blood cells in CAP, a working suspension of red blood cells was obtained by three-fold washing with a solution of low ionic strength Liss (manufactured by LLC "Hematolog") by the centrifugation mode at 2700 rpm for 8 minutes. The finished red blood cells suspension was diluted in a ratio of 1:200. The parameters of hemograms were determined in the blood using an automatic hematological analyzer MINDRAV VS-3000. The study of the hemogram parameters included determination of the red blood cells count (RBC), hemoglobin (Hb), hematocrit level (Ht), expressing the red blood cells count in the total blood volume (normally the hematocrit level is 0.36 – 0.48 g/l). The mean content of hemoglobin in each red blood cell was calculated from the ratio of blood hemoglobin to hematocrit, which reflected the saturation of red blood cells with hemoglobin. Red blood cells indices corresponded to the mean of the red blood cells volume (RBC_m). The morphometry of red blood cells in the peripheral blood was investigated to construct a histogram of the distribution of red blood cells by the content and level of hemoglobin, geometric parameters of red blood cells and their statistical characteristics [2].

Statistical processing of the digital data was carried out by the methods of parametric and nonparametric statistics on a personal computer with the "Statistica 8.0" StatSoft USA using Student's t-test. The level of reliability was taken at $p < 0.05$ [1].

Results of the study and their discussion. The findings of the immunological study of the humoral component of the immune system in patients with CAP have found disruptions of studied parameters that were similar to the AHI group. They were characterized, first of all, by an increase in the

CICs concentration, mainly due to the most pathogenic medium molecular weight (11 S–19 S) and small molecular weight (<11 S) fractions.

The findings of the analysis showed that the absolute content of the medium molecular weight fraction (11 S–19 S) in the group of patients with CAP was by 2.0 times higher ($p<0.05$). As for the concentration of small molecular weight immune complexes, this index was increased by 1.74 times ($p<0.05$). Noteworthy, the absolute concentration of high molecular weight CICs (> 19 S) in most patients with CAP was at the level of the indices of the AHI group or even slightly higher than the upper limit of the reference value ($p<0.05$). At the same time, the relative concentration of this fraction of the immune complexes decreased by 1.28 times.

Thus, elevated CICs level in the blood serum was caused by the significant increase in the concentration of the medium and small molecular weight fraction of the CICs.

The findings of the study of the reaction of red blood cells in patients with CAP have established that the level of Hb within one day after hospitalization increased by 8% and subsequently decreased by 10–15 % (on day 2, 3); on day 5 it decreased by 30 % compared to the AHI group. On day 6 and 7, the Hb concentration remained reduced by 25 % and 30 %, respectively, compared to the indices of the AHI group (table 1).

Table 1

The progression of changes in hemoglobin, red blood cells count and hematocrit level in patients with community-acquired pneumonia, ($X \pm S_X$)

Period of observation	Index		
	RBC, 10^{12}/l	Hb, g/l	Ht, l/l
AHI	6.75±0.12	116.0±3.6	36.5±1.8
Day 1	8.23±0.25*	125.0±2.9*	46.0±1.2*
Day 2	6.1±0.18*	104.0±2.2*	33.6±1.7
Day 3	6.3±0.15*	98.0±4.1*	33.0±2.0
Day 4	6.5±0.11*	100.0±3.7*	35.1±2.2
Day 5	4.1±0.46*	81.0±4.0*	23.0±3.0*
Day 6	4.0±0.51*	88.0±3.6*	21.0±2.1*
Day 10	4.8±0.44*	82.0±4.1*	28.0±1.1*

Note: * – $p<0.05$ compared to the values of the AHI group.

Notably, exogenous hemin is able to easily penetrate into the membrane, destabilizing it and causing hemolysis. Apparently, the changes in red blood cells count and Hb level are also caused by fluctuations in the hematocrit level, which indicates redistribution of blood and hemodynamic disturbances during inflammation. The hematocrit index in patients with CAP increased dramatically on day 1 (by 26% higher compared to AHI group), which indicated blood thickening. On day 2, the Ht level returned to normal. However, on day10, its level dramatically decreased, accounting for only 57% of the level of the AHI group. No significant changes in the mean red blood cell volume were noted for 10 days. However, on days 3 and 5, the mean red blood cell volume ($RBCV_m$) was slightly reduced and only on day 10 it was restored to the level of the AHI group.

The mean content of hemoglobin in red blood cells ($HbContRBC_m$) changed within 7 days in a wave-like manner: on day 1 this index decreased (by 12 % relative to the similar index of AHI group) and was equal to the index on day 5 of the investigation (by 10 %). On day 10, the mean content of hemoglobin in red blood cell was almost restored. The mean hemoglobin concentration in red blood cells had a similar progression, while the changes in this index were significant relative to the indices of AHI group at all periods of observation. The findings of the study of red blood cells indices showed that in CAP, the average volume of the red blood cell did not significantly change, while the mean content of hemoglobin in the red blood cell ($HbContRBC_m$) and the mean concentration of hemoglobin in the red blood cells ($HbConcRBC_m$) decreased on day 1, and subsequently increased (on day 5 and 6) (table 2). Notably, on day 10, $HbContRBC_m$ reaches the values of the AHI group level, whereas $HbConsRBC_m$ remains reduced.

With a decrease in the volume of red blood cells, an increase in $HbContRBC_m$ and $HbConsRBC_m$ occurs, while the concentration of hemoglobin in the blood decreases. Subsequently, a decrease in the volume of cells shifts to increase, caused by plasmolysis due to deep membrane-destructive changes. The hemolysis of red blood cells is indicated by the dramatic decrease in their number and hemoglobin concentration, starting from day 5 of the observation. Thus, we believe that such progression of red blood cells indices is caused by the membrane-destructive processes in red blood cells, changes in their absolute number due to hemolysis, as well as changes in hematocrit due to blood redistribution.

Table 2

The progression of changes in red blood cells indices in patients with community-acquired pneumonia (X±Sx)

Period of observation	Index		
	$RBCV_m$	$HbContRBC_m$	$HbConsRBC_m$
AHI	54.1±1.2	17.2±0.1	317.0±2.7
Day 1	55.9±1.6	15.2±0.3*	271.0±6.8*
Day 2	55.1±1.9	17.1±0.4	309.0±10.1
Day 3	52.4±1.5	15.5±0.4*	297.0±7.9*
Day 4	55.9±1.2	15.0±0.4*	268.0±7.9*
Day 5	56.0±1.3	19.8±0.6*	352.0±6.6*
Day 6	52.5±1.2	22.0±0.2*	419.0±6.0*
Day 10	58.0±1.3*	17.0±0.1*	292.0±6.3*

Note: * – $p<0.05$ compared to the AHI group.

We have established a positive progression of the level of high molecular weight CICs in the blood serum of patients with CAP: a decrease in their concentration to the upper limit of the reference value and normalization of the molecular composition to the level of the AHI group (1.94±0.08 g/l).

We hypothesize that the insignificant changes in the volume of red blood cells towards a decrease in their size, noted in CAP during the first six days, are due to partial cell dehydration and shrinkage due to the opening of calcium-dependent potassium channels (Gardos effect), which occurs under the effect of oxidants that are peroxidation products. The authors believe that the activation of Gardos channels is a common characteristic of the cellular response to oxidative effects [8, 9].

However, the absolute and relative concentration of medium- and small molecular weight fractions of the CICs decreased simultaneously relative to the upper limit of the indices of the AHI group and remained elevated. The findings of our study provide evidence that the standard antibacterial therapy used in patients with CAP is pathogenetically determined, but insufficient to normalize the concentration of CICs and their molecular composition in blood serum, and also should be supplemented with vitamin therapy and medications that improve blood oxygenation [6, 7, 8, 9].

The findings of our studies are consistent with the literature data that under various effects on red blood cells, in particular, hydrogen peroxide, oxidation and denaturation of hemoglobin (the formation of the so-called Heinz bodies) is observed, which is accompanied by the release of heme/hemin-ferriprotoporphyrin IX [9, 10].

Conclusions

1. The marked disorders in immunological indices of severity in patients with community-acquired pneumonia have been established, which must be taken into account when diagnosing the disease: an increase in the CICs level in the blood serum of patients and an increase in the total CICs level, mainly due to an increase in the most toxigenic small- (<11 S) and medium molecular weight (11 S–19 S) fractions of immune complexes: the higher the level of CICs, especially small molecular weight ones, the more likely a severe course of community-acquired pneumonia is.

2. In community-acquired pneumonia, oxidative stress and intensified generation of reactive oxygen species develops. Changes in the quantity and quality of blood corpuscles necessitate the inclusion of medications that improve blood oxygenation into the standard antibacterial therapy.

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