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LIVER DYSFUNCTION IN CHILDREN WITH COMMUNITY-ACQUIRED PNEUMONIA: THE ROLE OF INFECTIOUS AND INFLAMMATORY MARKERS

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Abstract

Markers of infectious-inflammatory process were studied by determining the levels of pro-inflammatory cytokines - interleukin (IL) 1 and IL-6 and proteins of the acute phase of inflammation - C-reactive protein (CRP) and fibrinogen in the serum of children with community-acquired pneumonia.

It was found that the course of community-acquired pneumonia is accompanied by an increase in serum concentrations of IL-1 and IL-6 in children in parallel with the disease severity. The synthesis of pro-inflammatory cytokines stimulates the production of acute CRP, but reduces the concentration of fibrinogen in the serum of sick children. The revealed connections between the content of the studied cytokines at the systemic level and multidirectional changes in the indicators of the acute phase of inflammation indicate a violation of the liver, where proteins are synthesized in the study.

It is shown that with increasing severity of pneumonia, the enzyme activity of aminotransferases - alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of children increases with a simultaneous decrease in the de Ritis coefficient, indicating "hepatic" genesis. High levels of aminotransferase is closely correlated with the

activity of the infectious-inflammatory process, as indicated by the positive correlation between the level of IL-1 and ALT (r = 0.047) and AST (r = 0.111). At the same time, there is a negative correlation between the levels of IL-1, CRP and the activity of aminotransferases in blood serum.

Key words: cytokines; C-reactive protein; fibrinogen; alanine aminotransferase; aspartate aminotransferase; liver; community-acquired pneumonia in children; early age.

Introduction. Pneumonia remains the leading cause of infant mortality and morbidity worldwide [1, 2]. In patients with this pathology, in addition to clinical signs, inflammatory processes are intensively increased on the basis of the body's immune response, which is accompanied by an increase in inflammatory mediators and can provoke significant metabolic disorders in the body [3]. As a component of the body's reactions to infectious inflammation, changes in metabolism are pathological, leading to irreversible damage to cellular structures and disruption of individual organs and systems [4]. In this case, biochemical markers of inflammation and functional status of organs, in particular the liver, often precede morphological changes in tissues and organs, so they can be considered as early criteria for the development of the pathological process in various diseases [5].

Inflammation, which occurs in community-acquired pneumonia, along with hypoxia, is one of the most common typical pathological processes [2]. It should be noted that the protective role of inflammation is undeniable, but this reaction is also pathological, as the mechanisms of inflammation lead to secondary self-damage of tissues [6]. It is the severity of inflammation, along with other factors that determines the severity and prognosis of pneumonia, especially in childhood [2]. Pneumonia is accompanied by a systemic response of the body to inflammation in the lung tissue, and the components involved determine the pathogenetic mechanisms of the disease and play an important role in the course of pneumonia [7].

The liver occupies a special place in the development of inflammatory reactions, as it is an organ that ensures homeostasis of the whole organism, and is directly involved in the detoxification and elimination of products of infectious agents, is central to the regulation of acute inflammation, metabolism of biologically active and antibacterial substances [8]. Therefore, the development of community-acquired pneumonia can affect the condition of the liver, the defeat of which will reduce the metabolism of drugs, which will contribute to greater intoxication of the body.

The aim of the study was to study the dynamics of changes in markers of infectious-inflammatory process in the serum of children with community-acquired pneumonia, and to assess their impact on liver dysfunction.

Materials and methods of research

The results of clinical and laboratory examination of 338 children aged from one month to three years with community-acquired pneumonia were analyzed. Among the patients there were 171 (50.6%) boys and 167 (49.4%) girls.

The children were hospitalized in the infectious diseases department for children of the Vinnytsia Regional Children's Clinical Hospital. Verification of the diagnosis was performed by identifying clinical signs, medical history, chest X-ray, laboratory tests. The severity of pneumonia was determined by a scale that takes into account the severity of clinical and laboratory-functional manifestations of the disease [9]. Prior to admission to the hospital, patients did not receive prior antibacterial treatment.

All patients had pneumonia of varying severity, depending on which they were divided into two groups: Group I - 129 children with community-acquired pneumonia of moderate severity; Group II - 209 patients with severe pneumonia. The study did not include children with combined pathologies. Comparison group - 40 healthy children.

Markers of infectious-inflammatory process were determined in serum- pro-inflammatory cytokines (IL-1, IL-6) and proteins of the acute phase of inflammation (C-reactive protein (CRP), fibrinogen).

The content of IL-1 and IL-6 in serum was determined by enzyme-linked immunosorbent assay using a diagnostic test system from IMMUNOTECH (France). Serum CRP levels were determined by enzyme-linked immunosorbent assay using the High Sensitivity CRP Enzyme Immunoassay Test (hsCRP-DA-USA). The concentration of fibrinogen in serum was determined by the method of Klauss using a set of reagents FIBRINOGEN-TEST (LLC "Laboratory Granum", Ukraine).

The functional state of the liver was assessed by the enzymatic activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which form the basis of the "liver panel". The activity of these aminotransferases in serum was determined by Reitman and Fraskel method [10].

To evaluate the quantitative results of the study, the values of the arithmetic mean (M) and the error of the arithmetic mean (m) - M ± m were calculated. Statistical processing of the obtained results was performed using statistical analysis packages Microsoft Excel and

Statistica. Student's t-test was used to assess the significance of the difference in averages between groups. The difference was considered significant at a probability factor of $p<0.05$

Correlations between the samples were calculated using the Pearson parametric correlation method. Verification of the relationship between the samples was evaluated using correlation tables using Pearson's criterion $\chi 2$.

Research results

Inflammation is a non-specific protective reaction of the body to tissue damage and the basis of most pathological processes. One of the groups of modulators of inflammation and immune response are cytokines, which have endocrine, paracrine and autocrine activity and are a key element of the immune system in the development of inflammation in pneumonia [11].

Cytokines are produced in the first minutes of the inflammatory reaction and can serve as a reliable diagnostic criterion for infectious-inflammatory process in young children with community-acquired pneumonia [12]. The leading place among proinflammatory cytokines belongs to IL-1, which is produced by macrophages, to a lesser extent by fibroblasts, dendritic cells, endothelium and stimulates the emigration of nuclear leukocytes from the bone marrow [13].

The results of the studies showed that in young children with pneumonia in the serum significantly increased the level of IL-1, which reached a value of 28.3 ± 0.74 pg / ml, which is 2.2 times higher than in healthy children ($p<0$, 05) (Fig. 1).

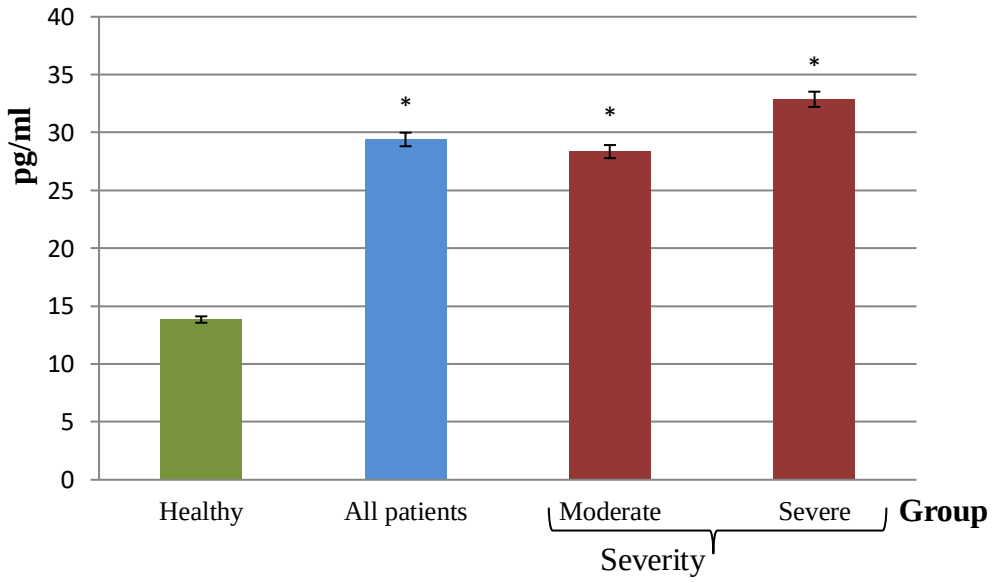

Fig.1. The content of interleukin 1 in the serum of children with community-acquired pneumonia depending on the disease severity

** - statistically significant difference compared with healthy children, p <0,05*

A study of the IL-1 level in serum of patients with varying degrees of pneumonia showed that the studied rate increased in parallel with the disease severity. It was found that in patients with moderate pneumonia, the level of IL-1 was 28.35 ± 0.641 pg / ml, and in patients with severe - 32.87 ± 0.472 pg / ml (Fig. 1), which may be accompanied by complications. other bodies. Thus, the target cells of IL-1 are hepatocytes, bone cells, myocytes, lymphocytes, neurons [14]. IL-1 can cause exocytosis of lysosomal enzymes and free radicals by phagocytes; stimulates the degranulation of mast cells with the release of inflammatory mediators, activates the production of prostacyclin and stimulates the formation of acute phase proteins by hepatocytes, resulting in pro-inflammatory and pyrogenic effects [13].

Elevated levels of IL-1 may induce the synthesis of other "pro-inflammatory" cytokines, in particular IL-6. Analyzing the level of IL-6 in the serum of young children with pneumonia, we found an increase compared with that characteristic of healthy children (Fig. 2). As the disease severity increased, the level of IL-6 in the serum of children increased with maximum values in severe pneumonia (24.9 ± 0.981 pg / ml), which is 10.3 times higher than the corresponding rate of healthy children (Fig. 2).

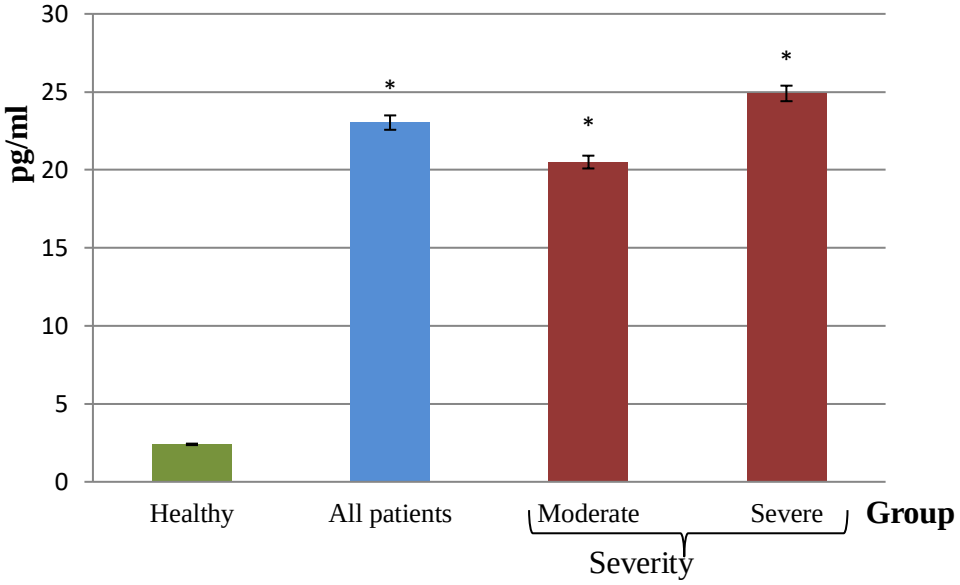

Fig.2. The content of interleukin 6 in the serum of children with community-acquired pneumonia depending on the disease severity

** - statistically significant difference compared with healthy children, p <0,05*

Elevated levels of IL-6 produced by leukocytes induce an inflammatory response in the body, which is most pronounced in children with severe pneumonia. The studied interleukin stimulates the differentiation of T-lymphocytes into pro-inflammatory Th-

lymphocytes and inhibits the differentiation into anti-inflammatory regulatory T-lymphocytes. Due to its pro-inflammatory properties, IL-6 triggers the synthesis of acute phase proteins in the liver [15].

Proteins of the acute phase of inflammation are characterized by nonspecificity in relation to the root cause of inflammation, but at the same time show high sensitivity of blood concentrations and massiveness of the infectious process, which determines their diagnostic and prognostic value [16]. One of the most studied acute phase proteins is CRP, the definition of which is widely used in clinical practice. This is due to the availability of its definition, including quantitatively, in almost any medical institution.

During the analysis of the obtained results, it was found that pneumonia in the serum of children increased the level of CRP, which depended on the disease severity (Fig. 3). Thus, it was found that in patients with moderate pneumonia, the content of CRP in serum was 34.6 ± 2.73 mg / l, which is 10.6 times higher than in healthy children, and in the group of patients with severe pneumonia, this figure was 35.7 ± 2.17 mg / l, which is 11 times higher than the control group (p <0,05) (Fig. 3).

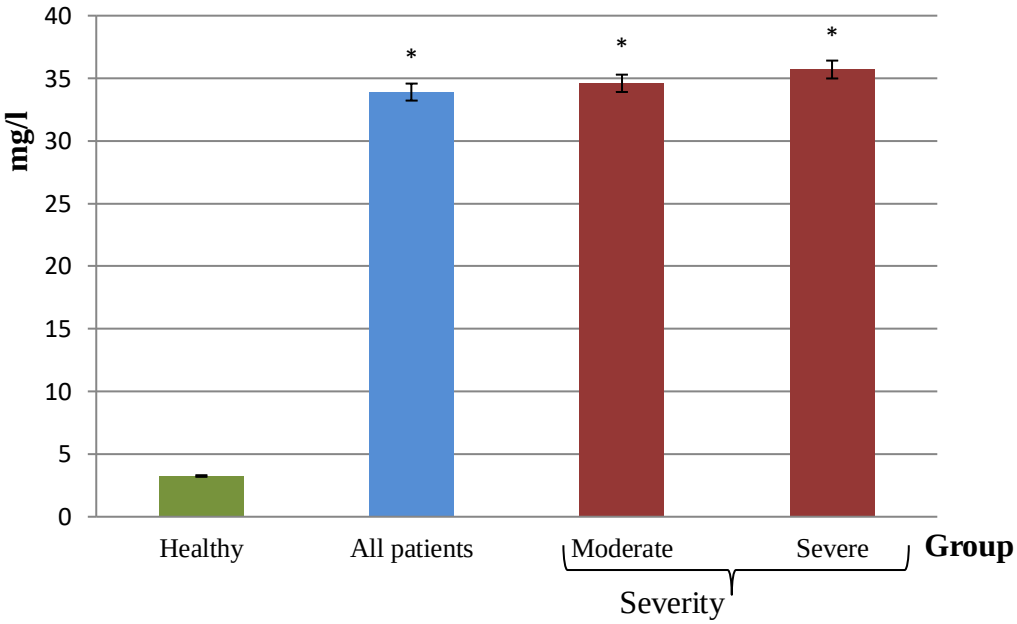

Fig.3. The content of C-reactive protein in the serum of children with community-acquired pneumonia depending on the disease severity

** - statistically significant difference compared with healthy children, p <0,05*

The established fact is an important diagnostic aspect of the use of CRP in pneumonia, because the protein of the acute phase of inflammation appears in the blood in significant concentrations much earlier before the appearance of antibodies. Moreover, it is critical to

change the content of CRP in the blood when the inflammatory process subsides, when its concentration decreases significantly within 4-9 hours [17].

An increase in the concentration of CRP in the blood may be accompanied by its adsorption on the surface of erythrocytes, which will reduce their charge and repulsion from each other. Such changes in the patient's body will promote the formation of "coin columns" and the rapid deposition of erythrocytes. In addition, the rate of erythrocyte sedimentation may be affected by another acute phase protein, fibrinogen.

However, the results of the studies showed a decrease of fibrinogen level in the serum depending on the disease severity (Fig. 4).

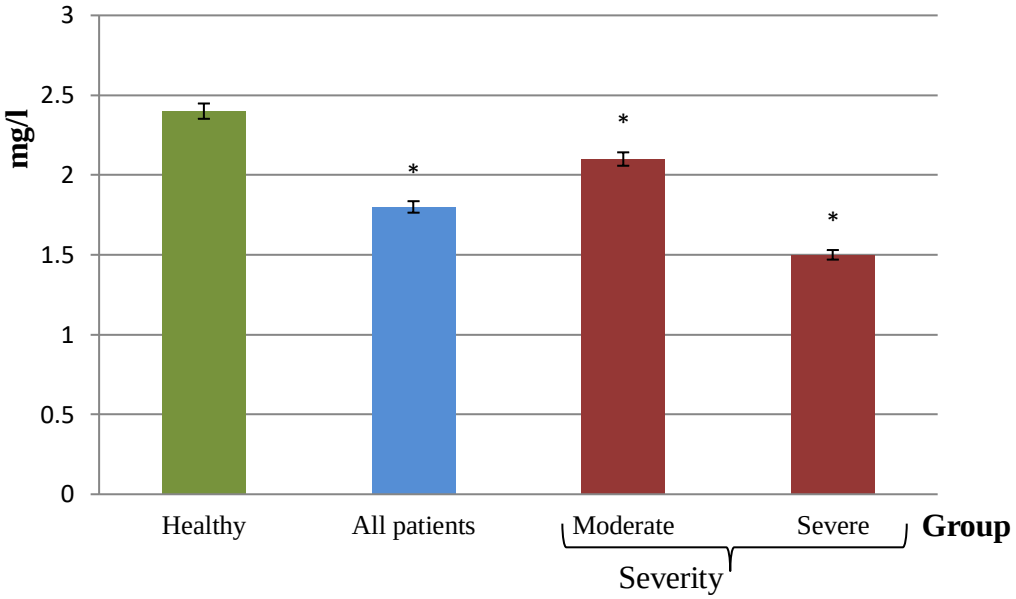

Fig.4. The concentration of fibrinogen in the serum of children with community-acquired pneumonia, depending on the disease severity

** - statistically significant difference compared with healthy children, p <0,05*

A decrease in the concentration of fibrinogen in the serum may indicate a violation of the protein-synthesizing function of the liver, because it is in this organ that fibrinogen is synthesized

Thus, as the severity of community-acquired pneumonia, there is a marked increase in the level of CRP in blood serum, which indicates the development of an inflammatory process in the body. However, in the serum decreases the concentration of another acute phase protein - fibrinogen. Presumably, the pathogen that causes pneumonia in young children in the body stimulates the development of complex cascading inflammatory and immune reactions, which lead to the gradual development of pathological manifestations, exacerbated by severe disease

[16]. These cascade reactions can be manifested as follows: IL-1 stimulates the production of IL-6, and the latter functions as a regulator of various "normal" and pathological biological processes associated with local and systemic inflammation, with metabolism. The production of cytokines is the starting point for the start of CRP synthesis in response to the inflammatory process. However, this may impair liver function, as the concentration of fibrinogen in serum decreases. To verify this omission, we determined the enzymatic activity of ALT and AST.

Analysis of the study results showed an increase in the enzymatic activity of ALT in the serum of patients with pneumonia as the disease severity. Thus, in patients with severe pneumonia, this figure was the highest and amounted to 28.04 ± 1.65 U / l, which is 3.2 times higher than the group of healthy children - 8.84 ± 0.575 U / l ($p<0, 05$) (table 1). At the same time moderate and average activity of inflammatory process prevailed. In patients with moderate pneumonia, the activity of ALT in serum was 24.7 ± 1.93 U / l, which is 2.8 times higher than in healthy children ($p<0.05$) (Fig. 5a).

A similar trend was determined by the activity of ACT: its increase was found in young children with pneumonia, as the disease severity (table 1). It was found that in moderate pneumonia AST was 40.48 ± 2.213 IU / l, in severe - 48.9 ± 3.13 IU / l, which is 2.2 times and 2.7 times higher than healthy children - 18 , 45 ± 1,723 ($p<0.05$) (Fig. 5b).

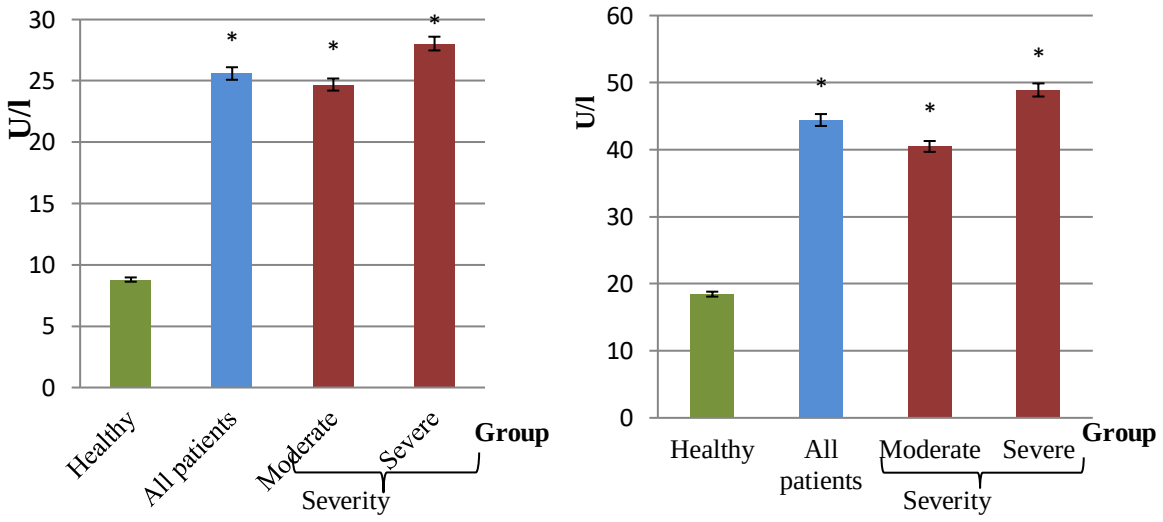

Fig.5. Enzymatic activity of ALT (a) and AST (b) in the serum of children with pneumonia, depending on the disease severity

** - statistically significant difference compared with healthy children, p <0,05*

In addition to determining the activity of aminotransferases, the indicator of the ratio of AST / ALT - de Ritis coefficient - is important for diagnosis. It is known [18] that a decrease in the de Ritis coefficient confirms the "hepatic" genesis of hyperenzymemia, and an increase - indicates, in most cases, the "non-hepatic" origin of this phenomenon. The calculation of the de Ritis coefficient showed that in moderate and severe pneumonia, this figure decreased compared to healthy children, which indicates the hepatic genesis of increased ALT and AST in blood serum.

Presumably, hyperproduction of cytokines may damage the liver. A large number of cytokines are formed in the liver by various stimulus reactions [19]. Interferon γ (IFN-γ) is produced by hepatocytes during viral infection. Tumor necrosis factor (TNF-α) is synthesized by Kupffer cells under the action of a number of hepatotropic damaging agents [20]. Proinflammatory cytokines TNF-α, IL-1 and IL-6 are secreted by Kupffer cells during infectious-inflammatory processes in the body [21]. These effects are associated with the synthesis of acute phase proteins, in particular CRP, and increased adhesion of sinusoids.

Therefore, cytokines that circulate in the systemic circulation in patients with pneumonia, or that are produced locally in the liver, may play an important role in liver cell damage. To test this assumption, we determined a correlation between the indicators of the activity of the infectious-inflammatory process and the indicators of the cytolysis syndrome in children with community-acquired pneumonia.

As a result of research, the relationship between the indicators of the activity of the infectious-inflammatory process - IL-1, IL-6 and CRP and the indicators of the cytolysis syndrome - ALT and AST in serum was revealed. Thus, calculating the Pearson correlation coefficient, a positive correlation was found between the level of IL-1 and the activity of ALT and AST, and the correlation coefficient is higher for AST - r = 0,111 compared with ALT - r = 0,047 ($p < 0,05$) Coefficient correlation of IL-1 and GGT activity was r = 0.054 (table 1).

Table 1

Relationship between indicators of activity of infectious-inflammatory process and indicators of cytolysis syndrome in children with community-acquired pneumonia

Indexes	ALT	AST	GGT
IL-1	0.047	0.111	0.054
IL-6	-0.262	-0.324	-0.068
C-reactive protein	-0.281	-0.288	-0.124

Presumably, under conditions of increased IL-1 in the circulating blood in the liver, destructive processes occur, which is manifested by the release of ALT, AST and GGT into the bloodstream as a result of cytolysis of liver cells. Regarding IL-6, between this indicator and indicators of enzymatic activity of ALT, AST there is a high degree of negative correlation, because the correlation coefficient between IL-6 and ALT is r = -0.262, IL-6 and AST is equal to r = -0.324 and IL-6 and GGT is equal to r = -0,068 (table 1). It should be noted that the degree of relationship between IL-6 and ACT is higher than IL-6 and ALT (p <0,05). A more pronounced correlation between cytokines and AST may be due to the fact that the studied enzyme is localized not only in the liver, but also present in large quantities in the myocardial muscle tissue [18]. The negative correlation between IL-6 and the activity of liver enzymes in the blood indicates that an increase in the level of IL-6 in the blood is accompanied by a decrease in the activity of ALT and AST. This may indicate destructive processes in the liver, as IL-6 accelerates the process of blood coagulation by triggering a focal inflammatory response and accumulation of reactive proteins in the hearth [15]. IL-6 causes exacerbation of chronic diseases and translates acute diseases into a chronic form, which can be observed in the case of the liver. In addition, this cytokine is also a major inducer of CRP.

Analysis of the results of the relationship between CRP and indicators of cytolysis syndrome showed the presence of a negative correlation. Thus, the correlation coefficient between CRP content and ALT activity in serum is equal to r = -0.281, and the correlation coefficient between CRP content and AST activity and GGT in serum is equal to r = -0.281 and r = -0.124, respectively (Table 1). The established fact indicates that the increase in CRP in the serum of patients with pneumonia may indicate certain destructive changes in the liver.

Thus, in the development of community-acquired pneumonia, the synthesis of proinflammatory cytokines underlies the action of bacterial toxins, and Kupffer cells, which produce proinflammatory cytokines, play an important role in the development of liver damage. The following mechanism of liver damage can be described in young pneumonias: endotoxin through the portal vein increases its concentration in the liver, followed by activation of Kupffer cells and their release of chemoattractants, including proinflammatory IL-1 and IL-6. Next are activated neutrophils with receptors for adhesion molecules that adhere to sinusoidal endothelial cells. Adhesion molecules promote the migration of leukocytes into the liver parenchyma. Activated neutrophils produce free radical forms of oxygen, which cause liver damage [21].

Conclusions

1. Evaluation of the level of inflammatory markers in the serum of young children with community-acquired pneumonia showed that the disease is accompanied by the development of infectious-inflammatory process, which is manifested by increased serum levels of proinflammatory cytokines - IL-1, IL-6 and acute protein concentration - CRP. In severe disease, cytokine and CRP levels increase to a greater extent.

2. Decreased serum concentrations of fibrinogen indicate a violation of protein-synthesizing function due to liver damage, which is confirmed by hyperenzymemia ALT, AST and GGT. Liver dysfunction is caused by both local and general infectious-inflammatory reactions of the body of young children with community-acquired pneumonia, as evidenced by the correlation between indicators of activity of infectious-inflammatory process and indicators of cytolysis syndrome.

Prospects for further research. The study of the nature of the imbalance of metabolic changes in the liver and the cellular barrier of lung protection in young children with community-acquired pneumonia may be an additional criterion for the diagnosis of hepatocellular lesions and underlie the development of new treatment regimens for pneumonia in young children.

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