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# CYTOKINES AS MEDIATORS OF THE IMMUNE SYSTEM AND THEIR ROLE IN THE PATHOGENESIS OF COMMUNITY-ACQUIRED PNEUMONIA

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The clinical and immunological examination of patients with community-acquired pneumonia has been carried out that included the study of the IL-2, IL-4, IL-6, IL-8 and TNFα cytokines' system. Immune system disorders that determine the severity of pneumonia, the amount of inflammatory damage to the lung tissue, disrupted elimination of the immune complexes have been found. These tendencies to a decrease in the immunoglobulins M and G result in a moderate course of community-acquired pneumonia and lobar lesions of the lungs, and the immune system disorders are reliably associated with imbalance of cytokines, with a predominance of their pro-inflammatory activity and a decrease in regulatory functions. The findings of the immunological monitoring indicate that standard antibiotic therapy in patients with community-acquired pneumonia leads to its clinical and radiological resolution, though it is not accompanied by the normalization of immunity parameters. The imbalance of the cytokine component of immunity justifies the need for further development of pathogenetic, as well as immunocorrective therapy, in patients with community-acquired pneumonia.

**Keywords:** community-acquired pneumonia, pathogenesis, immunity, cytokines.

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# ЦИТОКІНИ ЯК ПОСЕРЕДНИКИ ІМУННОЇ СИСТЕМИ ТА ЇХНЯ РОЛЬ В ПАТОГЕНЕЗІ НЕГОСПІТАЛЬНОЇ ПНЕВМОНІЇ

Проведено клініко-іммунологічне дослідження хворих з негоспітальною пневмонією, що включає вивчення систем цитокінів-IL-2, IL-4, IL-6, IL-8 та TNFα. Виявлені порушення в системі імунітету. визначаючи ступінь важкості пневмонії, об'єм запального ураження легеневої тканини, порушення елімінації імунних комплексів. Ці тенденції до зменшення імуноглобулінів М і G обумовлюють середньо тяжкий перебіг протягом негоспітальної пневмонії і долевої поразки легень, порушення в системі імунітету, достовірно пов'язані з дисбалансом цитокінів, з переважанням їх провокуючої активності та зменшенням регуляторних функцій. Результати імунологічного моніторингу свідчать про те, що стандартна антибактеріальна терапія хворих з негоспітальною пневмонією призводить до її клініко-рентгенологічного врегулювання, але не спровокована нормалізація показників імунітету. Дисбаланс цитокінового зіркового імунітету визначає необхідність подальшої розробки патогенетичної та, у тому числі, імунокоригуючої терапії, у хворих із негоспітальною пневмонією.

**Ключові слова:** не госпітальна пневмонія, патогенез, імунітет, цитокіни.

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Currently, pneumonia is ranked 4th-5th in the structure of causes of death worldwide after cardiovascular and oncological diseases, cerebrovascular pathology, injuries and poisoning, and is ranked first among infectious diseases [7]. Mortality in hospital patients with severe form of the disease ranges from 14 to 40 % and increases among patients over 60 years of age [3].

Altered immunological reactivity of the body is currently considered as one of the leading causes of the complicated and protracted course of pneumonia [9]. However, the nature of immunity disorders at certain stages of the inflammatory process, the factors of intercellular interaction have not been fully studied and are interpreted ambiguously. In this regard, the study of cytokines acting as mediators of the immune system is crucial. They regulate the strength, duration of the immune response and the nature of the inflammatory process, providing positive and negative immunoregulation [5]. In lung diseases, cytokines are involved in the infectious-inflammatory process and the allergic response at the level of proper immune mechanisms and the effector component, largely determining the direction, severity and outcome of the pathological process [6].

**The purpose** of the study is aimed at identificating clinical and immunological disorders of changes in the cytokine system in patients with community-acquired pneumonia to evaluate their impact on the severity of the disease.

**Material and Methods.** We have examined 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. CAP was diagnosed on the basis of epidemiological, clinical, laboratory, and X-ray data. Patients with such pathologies as tuberculosis, bronchial asthma, Hepatitis B, C and D, HIV, blood diseases and oncological diseases were excluded from the study [1].

The control group was formed of 20 apparently healthy individuals (AHI) of the same age and gender.

To carry out statistical calculations, patients with mild CAP who have a low risk of mortality less than 5 % (according to the Pneumonia PORT scale, Fine M., 1997) have been assigned into Group I (n=83 (79.8 %)), and patients with moderate form of the disease with a higher mortality risk up to 30% have been assigned into Group II (n=21(20.2 %)).

According to the results of chest X-ray, patients with varying severity have been divided into 3 subgroups, depending on the amount of damage to the lung tissue. The first subgroup (with focal lesions) involved 63 patients (54.2 %), the second subgroup (with segmental lesions) involved 19 (21.7 %) patients and the third subgroup (with lobar pneumonia) involved 22 (24.2 %) people. During hospitalization, all patients, examined according to the standards of the International Society of Pulmonologists and the Recommendations of the F.G. Yanovsky National Institute of Phthisiology and Pulmonology (Kiev, 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. Etiological diagnostics of atypical pathogens of pneumonia included the enzyme-linked immunosorbent assay in progression (test systems of Proteinovyi KonturTest LLC, St. Petersburg), with the determination of specific immunoglobulins IgM and IgG to Mycoplasma and Chlamydia pneumoniae in the blood serum.

The complex of standardized immunological studies included the analysis of capillary blood leukogram data.

The study of the level of cytokines (IL-2, IL-4, IL-6, IL-8 and TNFα) in the blood serum of patients with CAP in progression during registration and following 10 days thereafter was carried out quantitatively using a set of reagents “Interleukin IFA-BEST” (VEKTOR- BEST, Russia) for determination in biological fluids and culture media. The method is based on a solid-phase “sandwich”, a variation of the enzyme immunoassay using the mono- and polyclonal antibodies.

Statistical processing of 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 the Student’s t-test. The level of reliability was taken at $p<0.05$.

**Results of the study and their discussion.** The analysis of the CAP clinical manifestations, depending on its severity, revealed more pronounced syndromes of intoxication and general inflammatory changes, clinical and radiological predominance of lobar lesions of the lung tissue with pleural effusion syndrome, and auscultation revealed moist bubbling rales and crepitus in patients with moderate pneumonia.

The findings of the microbiological study have revealed the following CAP etiological structure: mild and moderate pneumonia was caused, in most cases, by the intracellular pathogens, in particular Mycoplasma pneumoniae, gram-positive microflora Streptococcus pneumoniae; in 25 % of patients, the pathogen was not identified. The leading pathogens of moderate pneumonia were *Streptococcus*

*pneumoniae* and mixed cultures of bacteria, with an increase in the proportion of *Staphylococcus aureus*; in addition, gram-negative pathogens were identified (table 1).

Table 1

**The main variants of the association of pathogens of community-acquired pneumonia identified in patients of the Therapeutic Department of the Kharkiv Municipal Clinical Hospital No. 25. (n = 104)**

| Variants of associations | Frequency of identification | |
|---|---|---|
| | Abs. | % |
| S. pneumoniae – H. influenzae | 32 | 30.7±3.5 |
| S. pneumoniae – M. pneumoniae | 15 | 14.4±2.1 |
| S. pneumoniae – Adenovirus | 10 | 9.6±1.6 |
| S. pneumoniae – C. pneumoniae | 6 | 5.8±1.2 |
| S. pneumoniae – Herpes simplex I/II | 6 | 5,8±1,1 |
| S. pneumoniae – H. influenzae – M. pneumoniae | 8 | 7.7±1.3 |
| S. pneumoniae – H. influenzae – Herpes simplex I/II | 8 | 7.7±1.2 |
| S. pneumoniae – H. influenzae – Adenovirus | 8 | 7.7±1.2 |
| S. pneumoniae – M. pneumoniae – Adenovirus | 5 | 4.8±1.1 |
| S. pneumoniae – H. influenzae – M. pneumoniae – Adenovirus | 4 | 3.8±0.8 |
| S. pneumoniae – H. influenzae – M. pneumoniae – Herpes simplex I/II | 2 | 1.9±0.9 |

The analysis of capillary blood leukogram data in CAP patients revealed such nonspecific changes as leukocytosis with neutrophilia, left shift; monocytosis, increased ESR, which reflected different intensity of the inflammatory process. A relative lymphopenia in both groups of patients and absolute lymphocytosis in patients of Group I was noted. A tendency to a decrease in the absolute lymphocyte count in Group II (moderate forms) was established, which indicates an insufficient lymphocytic response in patients.

The NBT slide test showed the higher relative content of cells capable of reducing nitroblue tetrazolium in the peripheral blood in both groups of patients compared to controls. Possibly, this characterizes the activation of neutrophils in response to antigenic stimulation and/or bacterial sensitization.

Notably, the index of the functional reserve of microbiocidal activity of peripheral blood neutrophils (NBTst/NBTsp) was reduced in both groups, which can be evaluated as a criterion for insufficient reserve functions.

The findings of the study of immunoglobulins in patients with community-acquired pneumonia of varying severity has reveled the following (table 2): the IgA level tended to increase, while the IgM level tended to decrease in patients of both groups, though the indices did not differ statistically significantly from those in the AHI group. IgG level was significantly ($p<0.05$) decreased in patients of both groups.

Table 2

**The progression of cytokine indices in patients with CAP of varying severity (the Therapeutic Department of the Kharkiv Municipal Clinical Hospital No. 25)**

| Indices | Community-acquired pneumonia patients (n=104) | | | | AHI (control) group (n = 20) |
|---|---|---|---|---|---|
| | Group I (n=73) | | Group II (n=31) | | |
| | Day 1 | Day 10 | Day 1 | Day 10 | |
| IL-2, pg/ml | 86.80±19.15* | 87.26±27.60* | 55.26±17.48* | 73.60±37.47* | 22.90±4.70 |
| IL-4, pg/ml | 39.96±13.10 | 47.00±17.87 | 14.89±5.20* | 3.51±1.14* | 30.60±6.60 |
| IL-6, pg/ml | 6.07±3.24 | 2.42±2.03* | 64.20±48.65* | 59.16±30.8* | 5.50±2,30 |
| IL-8, pg/ml | 8.53±3.20 | 4.13±0.95* | 11.77±4.28* | 6.54±2.11 | 7.50±2.90 |
| TNFα, pg/ml | 1.88±0.70* | 0.95±0.40 | 2.24±1.46 | 4.09±2.62* | 1.50±0.35 |

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

The initial increase in the level of the inflammatory cytokine IL-2 ($p<0.05$) and a tendency to an increase in the TNFα value were common for patients with varying severity of CAP. In patients of Group I (with a mild course of CAP), the level of IL-4, the values of the regulatory cytokine IL-6 and the pro-inflammatory chemokine IL-8 were slightly increased. On the contrary, in patients with moderate course of the disease (Group II) the level of IL-4 lymphokine was significantly lower and the IL-8 value was

higher (p <0.05) compared to the control (p<0.05), and the IL-6 index was by dozens of times higher than the control value.

Immunological monitoring revealed that the level of IL-2 remained significantly elevated in both groups with the increase in patients with moderate course of the disease, while the value of IL-4 increased slightly in the group with mild and moderate course of the disease. In both groups, after 10 days of treatment with standard antibacterial therapy, normalization of the inflammatory activity of IL-8 was noted. However, in patients with moderate course of the disease, the value of regulatory IL-6 remained significantly high. The treatment showed that the level of TNFα, lymphokine with pronounced proinflammatory activity, which tended to increase in both groups at the beginning of the disease, decreased in the mild course of CAP and increased in moderate patients.

Changes in cytokine levels that are characteristic of varying degree of severity also corresponded to the amount of damage to the lung tissue. Notably, the activation of the chemokine IL-8 increased with the increasing volume of the inflammatory process in the lungs. The IL-8 index was by 1.9 times higher in the segmental lesions and by 2.7 times higher in the lobe lesions compared to the focal one, while the level of the anti-inflammatory cytokine IL-4 was significantly reduced. Its level was the lower the greater was the amounts of damage to the lung tissue, i.e., by 2 times lower compared to the AHI group in the segmental lesions and by 3.2 times lower in the lobar lesions. The value of the regulatory cytokine IL-6 was by 1.5 times higher in the segmental lesions and by 4 times higher in the lobar lesions compared to the focal one.

The study of the correlation between cytokine levels and other indices of the immune system (fig. 1) revealed the inducing role of these essential factors of intercellular interaction of different components of the immune system.

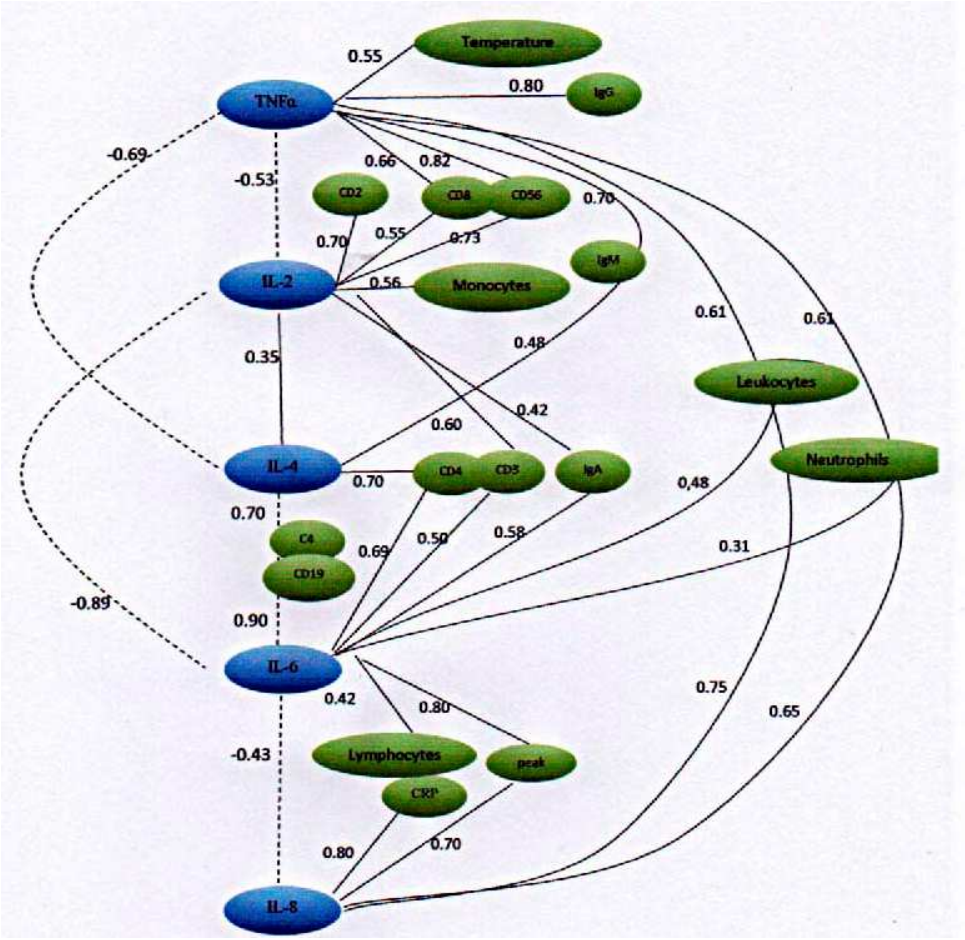


Fig. 1. Correlations between the indices of the immune system in patients with CAP (moderate course).

At the same time, in the nature of the immune response of patients with CAP, we noted the specific features of reactivity that determine the severity of the disease. At the early stages and peak of pneumonia, a relative lymphopenia in both groups of patients was detected, and it was more pronounced in moderate course of the disease, which, in our opinion, indicates an insufficient response of lymphocytic cells. This tendency is clearly observed in the analysis of subpopulations of lymphocytes in different categories of the pneumonia patients.

Indices of the primary classes of immunoglobulins in the initial period of CAP were characterized by the lower values compared to the control group, except for IgA.

Dysimmunoglobulinemia along with the detected enhanced immunocomplex mechanisms hypothesizes the presence of immunocomplex and autoimmune components in the pathogenesis of community-acquired pneumonia.

The findings of the studies of the cytokine system in patients with mild and focal course of the disease have been of greatest interest: the equivalent activation of the oppositional cytokine pools at the onset of the disease (IL-2, IL-4, IL-6, IL-8, TNFα) with an increase in IL-2, IL-4 and a decrease in IL-6, IL-8 and TNFα over time. On the contrary, moderate course and lobar lesions of the lung tissue is accompanied by the imbalance of the cytokine component in the form of an increase in the IL-6 content by 10.5 times, IL-8 by 1.4 times, TNFα by 1.3 times and a 1.6-fold decrease in IL- 2 and by 2.7 times in IL-4 (compared to the indices of patients with mild course). The findings of the analysis of the clinical course and etiological characteristics of community-acquired pneumonia of our patients revealed the general trends of the course of the disease that are consistent with the literature data [8].

Considering the most important regulatory role of the lymphokines IL-2, IL-4 (synthesized by Th1, Th2 lymphocytes, respectively) [2, 4], we can assume insufficient intercellular activation of specific factors of the cellular component of the immune system.

## Conclusions

1. In patients with community-acquired pneumonia with mild and moderate forms of the disease, an imbalance of the cytokine component was revealed, which determines the pathogenetic features of the course of the disease.

2. The findings of the immunological monitoring showed that standard antibiotic therapy in patients with community-acquired pneumonia leads to its clinical and radiological resolution, but is not accompanied by the normalization of the immunity indices.

3. The imbalance of the cytokine component of the immune system justifies the need for further development of pathogenetic and immunocorrective therapy in patients with community-acquired pneumonia.

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