



THE STATE OF CELLULAR AND HUMORAL IMMUNE IN EXAMINED PATIENTS WITH POVH

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✓ Resume

Hernioplasty currently ranks first in terms of the number of operations in abdominal surgery. Over 5% of all laparotomies are complicated by the formation of postoperative ventral hernias. A fundamentally important condition for the successful treatment of patients with postoperative ventral hernias is the need to assess immunological reactivity both before surgery and in the postoperative period. An immunological examination of patients with POVH was carried out and the state of cellular and humoral immunity was studied, as well as correlations were studied.

Keywords: postoperative ventral hernias, cytokines, correlation

СОСТОЯНИЕ КЛЕТОЧНОГО И ГУМОРАЛЬНОГО ИММУНИТЕТА У ОБСЛЕДОВАННЫХ БОЛЬНЫХ С ПОВГ

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✓ Резюме

Герниопластика в настоящее время занимает первое место по количеству операций в абдоминальной хирургии. Свыше 5% всех лапаротомий осложняются формированием послеоперационных вентральных грыж. Принципиально важным условием успешного лечения больных послеоперационными вентральными грыжами признается необходимость оценки иммунологической реактивности как до операции, так и в послеоперационном периоде. Проведено иммунологическое обследование пациентов с ПОВГ и изучены состояние клеточного и гуморального иммунитета, а также изучены корреляционные связи.

Ключевые слова: после операционные вентральные грыжи, цитокины, корреляция

ПОВЧ БИЛАН ОҒРИГАН БЕМОРЛАРДА ҲУЖАЙРА ВА ГУМОРАЛ ИММУНИТЕТНИНГ ҲОЛАТИ

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Герниопластика ҳозирда қорин бўшлиғи жарроҳлиғида операциялар сони бўйича биринчи ўринда туради. Барча лапаротомияларнинг 5% дан ортиғи операциядан кейинги вентрал чурра шаклланиши билан мураккаблашади. Операциядан кейинги вентрал чурраси бўлган беморларни муваффақиятли даволашнинг асосий шартини - бу операциядан олдин ҳам, операциядан кейинги даврда ҳам иммунологик реактивликни баҳолаш ҳисобланади. ПОВЧ билан озриган беморларнинг иммунологик текшируви ўтказилди, ҳужайра ва гуморал иммунитет ҳолати текширилди, шунингдек корреляцион боғлиқликлар ўрганилди.

Калит сўзлар: операциядан кейинги вентрал чурралар, цитокинлар, корреляция

Relevance

The problem of treatment of incisional ventral hernias (POVH) remains relevant even when using laparoscopic techniques [2, 5, 8, 14, 19].

In more than 20% of cases, laparotomy leads to the formation of POVH. Until recently, autohernioplasty was considered the most commonly used method of plastic surgery, a significant drawback of which is the use of own compromised tissues, sewn with tension, which inevitably leads to relapses. The practice of herniology has proven that in the treatment of patients with large and recurrent POVH, only endoprosthesis are effective [1, 4, 9, 16, 20].

Despite the good biocompatibility and inertness of modern mesh implants, in parallel with the increase in the number of performed operations, the number of "implant-associated" complications is growing. In this regard, it is important to clarify what the resulting complications are related to.

Immunological reactivity of the body to various structures shape, thickness, rigidity and chemical structure of the endoprosthesis is the most pressing issue of modern herniology [11, 15, 17, 18]. A complex of interacting immunocompetent cells is involved in the formation of the immune response, resulting in the production of immune system mediators involved in intercellular interactions, in the body's resistance to various exogenous and endogenous factors [3, 6, 7, 10, 13].

Purpose of the study: to identify the most significant immunological changes in patients with POVH in the preoperative period when using esfil heavy and esfil easy.

Materials and methods

In connection with the foregoing, we conducted studies to study the state of the immune system in the examined patients with POVH, who subsequently underwent surgical treatment using a classic mesh endoprosthesis (Esfil-heavy and Esfil-light). 137 people were examined, including 33 men (26%) with an average age of 52.9 ± 2.73 years and 94 women (74%), whose average age was 56.2 ± 1.3 years

Immunological studies in the examined patients were carried out in several stages. The first stage included the determination of a standard immunogram in the peripheral blood, where the parameters of the cellular and humoral immunity (CD3+, CD4+, CD20+, CD16+, IgM, IgG, IgA) were determined, as well as acute phase proteins and cytokine synthesis.

Blood sampling was carried out on the day of admission to the hospital, i.e. in the preoperative period.

Determination of the concentration of cytokines (IL-1 β , IL-4, IL-6, TNF α) and acute phase proteins (lactoferrin, SRP) was carried out using a set of reagents from Vector-Best (Novosibirsk). The determination method was based on a solid-phase "sandwich" - a variant of enzyme immunoassay.

The level of superoxide dismutase (SOD) was determined by enzyme immunoassay using a Human Reader HS analyzer (Germany) using ELISA-SOD test systems (LLC Cytokine, St. Petersburg, Russia).

Result and discussion

Analysis of relative indicators of cellular immunity revealed a significant decrease in the total number of T-cells and their subpopulations. As can be seen from the data in Figure 1, a pronounced deficiency was observed in patients with POVH compared with the data of the control group ($42.5 \pm 1.2\%$ versus $56.4 \pm 1.9\%$ in control, $P < 0.01$). A low level of T-lymphocytes is an unfavorable prognostic sign that indicates a decrease in the effector function of T-cells [2, 7, 12, 15].

Analysis of the obtained data on the study of CD4+ lymphocytes showed their significant decrease in comparison with the control group ($21.3 \pm 1.1\%$ vs. $34.3 \pm 1.7\%$, respectively, $P < 0.05$).

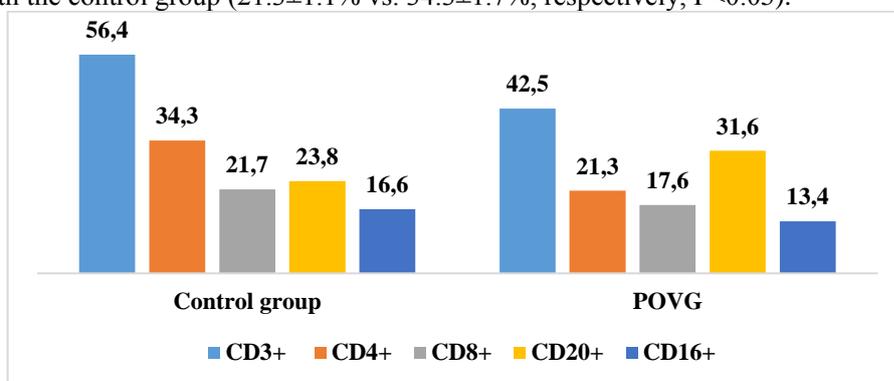


Fig.1. Relative values of T and B indicators - immune systems in examined patients

At the same time, suppressor activity was also significantly lower in the group of subjects ($17.6 \pm 0.8\%$ versus $21.7 \pm 1.62\%$ in control, $P < 0.05$). It should be noted that the absolute values are labile values, as they depend on the level of leukocytes and lymphocytes. The level of CD3+ and CD4+ positive lymphocytes, as well as the index of suppressor activity, was significantly higher in the group of examined patients ($P < 0.05$).

The level of T-lymphocytes in patients with POVH was 1.23 times higher than the control values ($P < 0.05$). The level of absolute values of T-helpers in patients were also higher than the values of the control group ($P < 0.05$).

The humoral link of immunity is represented by the content of CD20 + - lymphocytes, as well as the immunoglobulins A, M, G synthesized by them.

The level of B-lymphocyte values in patients was significantly increased compared to the data of the control group, both relative and absolute values. ($P < 0.01$).

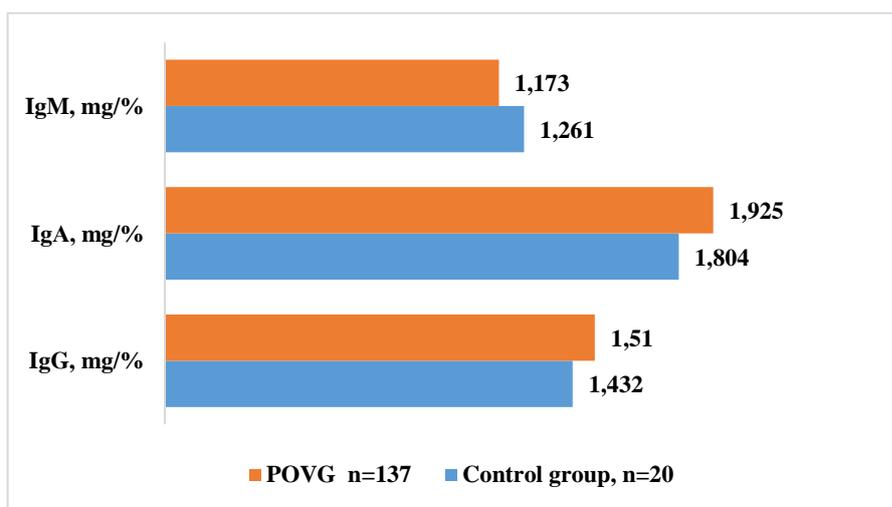


Fig 2. The level of immunoglobulins in the examined

The role of immunoglobulins of various classes in the immune response and protective functions of the immune system is different. And depending on this role, their synthesis occurs, and the concentration depends on the switching of C-genes, as a result of which immunoglobulins appear on the surface of macrophages [6, 18, 20].

Immunoglobulin G is the main serum immunoglobulin, its concentration is the highest among all immunoglobulins in the body. The results of the study of the content of serum immunoglobulins in the blood of patients showed an increased synthesis of Ig G by 2.1 times ($P < 0.05$) compared with the control group, a tendency to increase IgA and decrease IgM (Fig. 2).

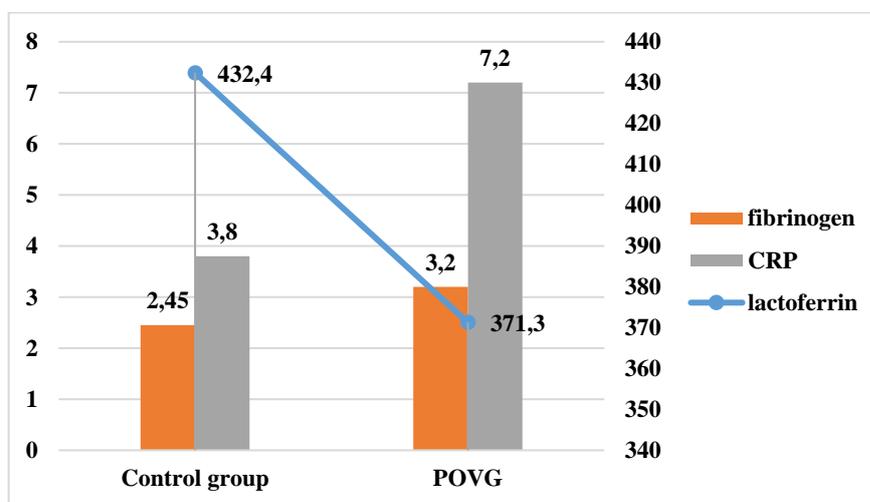


Fig. 3. Indicators of acute phase proteins in the examined

Our studies show the factors of innate immunity - the level of killer activity, which is represented

by the quantitative content of CD16⁺-lymphocytes and the concentration acute phase proteins.

Analysis of the results of our studies revealed an increased level of killer activity of absolute values in the group of examined by 1.3 times (588.1±31.2; against 448.6±24.1 in control) and a reduced level of its relative number (P<0, 05).

The main function of the acute phase protein system is the excretion (elimination) of foreign cells and the regulation of the immune response.

In response to any damage to the body, protective physiological reactions develop in the body, aimed at localizing the focus of damage and restoring impaired functions, that is, inflammation. The process of their formation is intensified by catecholamines, that is, stimulation of the sympathetic-adrenal system, as well as the release of a number of peptide factors from leukocytes during phagocytosis, which contribute to the biosynthesis of messenger RNA molecules in the cell nuclei and, consequently, protein. In addition, BOF synthesis is activated by pro-inflammatory cytokines - interleukin (IL)-1, IL-6, IL-11, tumor necrosis factor (TNF), and interferon- γ [4, 9, 15].

In addition to CRP, acute phase proteins also includes lactoferrin, the level of which is reduced in the group with POVH by 1.16 times compared to the control group (432.4±5.1 ng/ml; against 371.3±1.9 ng/ml in control) (P<0.05).

The obtained results suggest that the reduced level of serum lactoferrin is associated with the fact that it keeps neutrophils in the focus of inflammation, probably in connection with this, its content in the blood is reduced.

The level of fibrinogen did not reveal significant differences in the group of examined patients and in the control group.

C-reactive protein (CRP) is also an acute phase protein related to non-specific protective factors produced by liver cells. It is a predictor of complications, inhibits the anti-inflammatory properties of IL-4 and leads to a systemic inflammatory response. In the group of patients with POVH, CRP reached an average of 7.2 ± 0.6 mg/l, which is 1.8 times higher than in the control group (P<0.05). (Fig.3.)

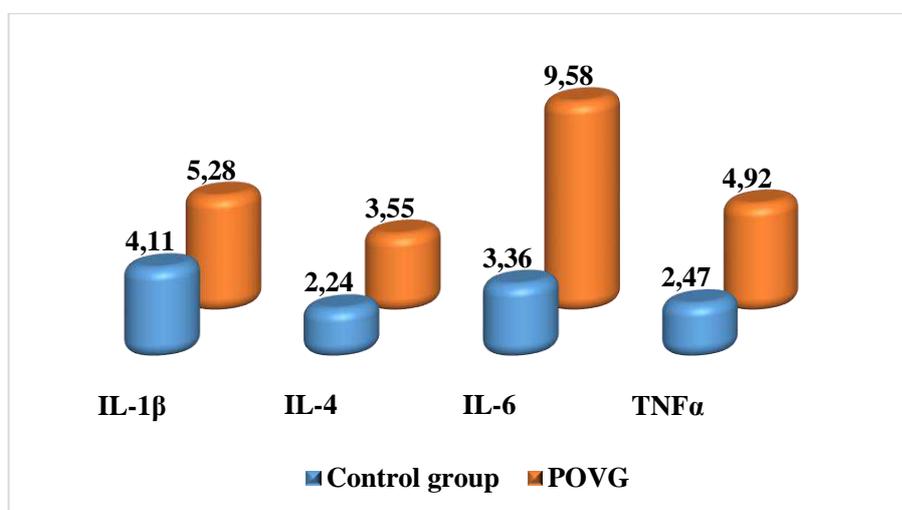


Fig. 4. The level of pro- and anti-inflammatory cytokines in the peripheral blood serum of the examined (pg/ml)

Transmission of signals between cells in the immune system is carried out by direct contact interaction of cells or with the help of mediators of intercellular interactions-molecules-mediators-cytokines [3, 17, 19].

The study of pro- and anti-inflammatory cytokines provides important information in the diagnosis and monitoring of various diseases [8, 16].

In this connection, the next stage of our research was to study the synthesis of pro- and anti-inflammatory cytokines (IL-1 β , IL-4, IL-6, TNF α) in the blood serum of patients with POVH before surgical treatment in comparison with the control group.

A study was conducted to determine the level of production of IL-1 β as an important mediator, which is one of the most universal regulators of immunity and inflammatory reactions with a wide range of

biological effects, which include the proliferation of T- and B-lymphocytes, antibody production, induction of the synthesis of other cytokines, etc. [5, 17, 19].

Analysis of the results showed that in patients with POVH, the level of IL-1 β increased by 1.3 times compared with the data of the control group.

Interleukin-4 (IL-4) is produced by Th2 cells and acts predominantly on B cells, T cells, macrophages, and mast cells. IL-4 promotes the proliferation and differentiation of B cells, as well as their costimulatory activation. It is known that it is a factor in switching the synthesis of immunoglobulins from IgG to IgE. IL-4 is a key cytokine of the Th2-type response - it induces the activation and maturation of B-cells, as well as the differentiation of Th0 into Th2-type. In addition, IL-4 inhibits Th1 differentiation and function. Our studies revealed a significant increase in the level of IL-4 in the examined - 3.55 ± 0.43 pg/ml versus 2.24 ± 0.7 pg/ml in the control ($P < 0.01$).

IL-6 is considered the most important mediator of the acute phase of inflammation, since it induces the synthesis of acute phase proteins: fibrinogen, alpha1-antichemotrypsin, C-reactive protein, haptoglobin, serum amyloid A. IL-6 is constantly present in the blood, so its concentration is more consistent with the severity inflammatory process [23, 24].

In our studies, in patients with POVH, the level of IL-6 was 2.8 times higher than the control values, averaging 9.58 ± 1.8 pg/ml ($P < 0.01$).

It is known that TNF α is a product of monocytes/macrophages, endothelial, mast and myeloid cells, LAK cells, neuroglial cells, and, in special cases, activated T-lymphocytes. As a result of TNF release, capillary permeability increases, vascular endothelium is damaged, and intravascular thrombosis occurs [22].

The level of TNF α in our study averaged 4.92 ± 1.1 pg/ml, which is 1.99 times higher than the control values.

Among AOS enzymes, first of all, superoxide dismutase (SOD) should be singled out - an antioxidant, which is the first link of protection. This enzyme is found in all cells that consume oxygen. The role of superoxide dismutase is to accelerate the reaction of the transformation of the oxygen radical toxic to the body - superoxide into hydrogen peroxide and molecular oxygen.

Determination of the total antioxidant status helps the clinician to better assess the state of the organism of the observed and its potential protective reserves under certain physiological and pathological conditions.

Based on the foregoing, we investigated the level of SOD in the blood serum in the preoperative period

Correlation analysis (Fig. 5.) of clinical and immunological parameters in patients with POVH before surgical treatment in blood serum revealed 53 relationships, of which 37 ($r = 0.3-0.8$) are positive and 16 are negative values (from $r = -0.3$ to -0.6).

Analysis of the results of the study of the concentration of SOD in patients with POVH in the blood serum showed that it was 138.3 ± 11.8 pg/ml, in contrast to the level of SOD in the control group of 269.4 ± 21.9 pc/ml.

Next, a correlation analysis (Fig. 5.) of clinical and immunological parameters was carried out in patients with POVH before surgical treatment in blood serum, which revealed 53 relationships, of which 37 ($r = 0.3-0.8$) were positive and 16 were negative values (from $r = -0.3$ to -0.6).

Thus, leukocytes have mainly 9 inverse correlations ($r =$ from -0.3 to 0.52) with CD4+, CD16+, CD25+, CD20+, lactoferrin, CRP, IL-1 β , IL-6 and TNF α . Lymphocytes have 1 direct ($r = 0.47$) relationship with IL-6. CD3+ has 4 direct links with CD4+ ($r = 0.62$), CD8+ ($r = 0.42$), CD95+ ($r = 0.70$), SOD ($r = 0.33$) and 1 feedback with IL-6 ($r = -0.36$). CD4+ has 7 direct relationships with such indicators as CD8+, CD16+, CD25+, CD95+, CD20+, lactoferrin, TNF α (from $r = 0.32$ to $r = 0.58$) and 1 inverse relationship with CRP ($r = -0.3$). CD8+ also has 7 direct links with CD25+ ($r = 0.37$), CD95+ ($r = 0.38$), IgA ($r = 0.35$), IgM ($r = 0.36$), lactoferrin ($r = 0.37$), TNF α ($r = 0.42$), SOD ($r = 0.37$). CD16+ has 3 direct relationships with CD25+ ($r = 0.48$), CD20+ ($r = 0.37$) and IgG ($r = 0.54$). CD25+ has 2 direct links with CD20+ ($r = 0.79$) and lactoferrin ($r = 0.46$). CD95+ also has 2 direct links with lactoferrin ($r = 0.31$) and SOD ($r = 0.44$). CD20+ has 1 direct association with IgG ($r = 0.3$). IgA has 1 direct relationship with TNF α ($r = 0.32$) and 2 feedbacks with IgM ($r = -0.3$) and CRP ($r = -0.32$). IgM has 1 direct link to IgG. In turn, it has one direct relationship with IL-4 ($r = 0.37$). Lactoferrin has 1 direct association with IL-1 β . CRP has 3 direct relationships with IL-1 β , IL-6, TNF α ($r = 0.44-0.55$) and 1 inverse relationship with IL-4. IL-1 β has one direct relationship with TNF α ($r = 0.32$) and one inverse relationship with IL-4 ($r = -0.49$). And the latter, in turn, has a feedback with TNF α ($r = -0.46$). IL-6 and TNF α are directly related ($r = 0.58$), as are SOD and TNF α ($r = 0.34$). IgM has 1 direct link to IgG. In turn, it has one direct relationship with IL-4 ($r = 0.37$). Lactoferrin has 1 direct association with IL-1 β . CRP has 3 direct relationships with IL-1 β , IL-6, TNF α ($r = 0.44-0.55$) and 1 inverse relationship

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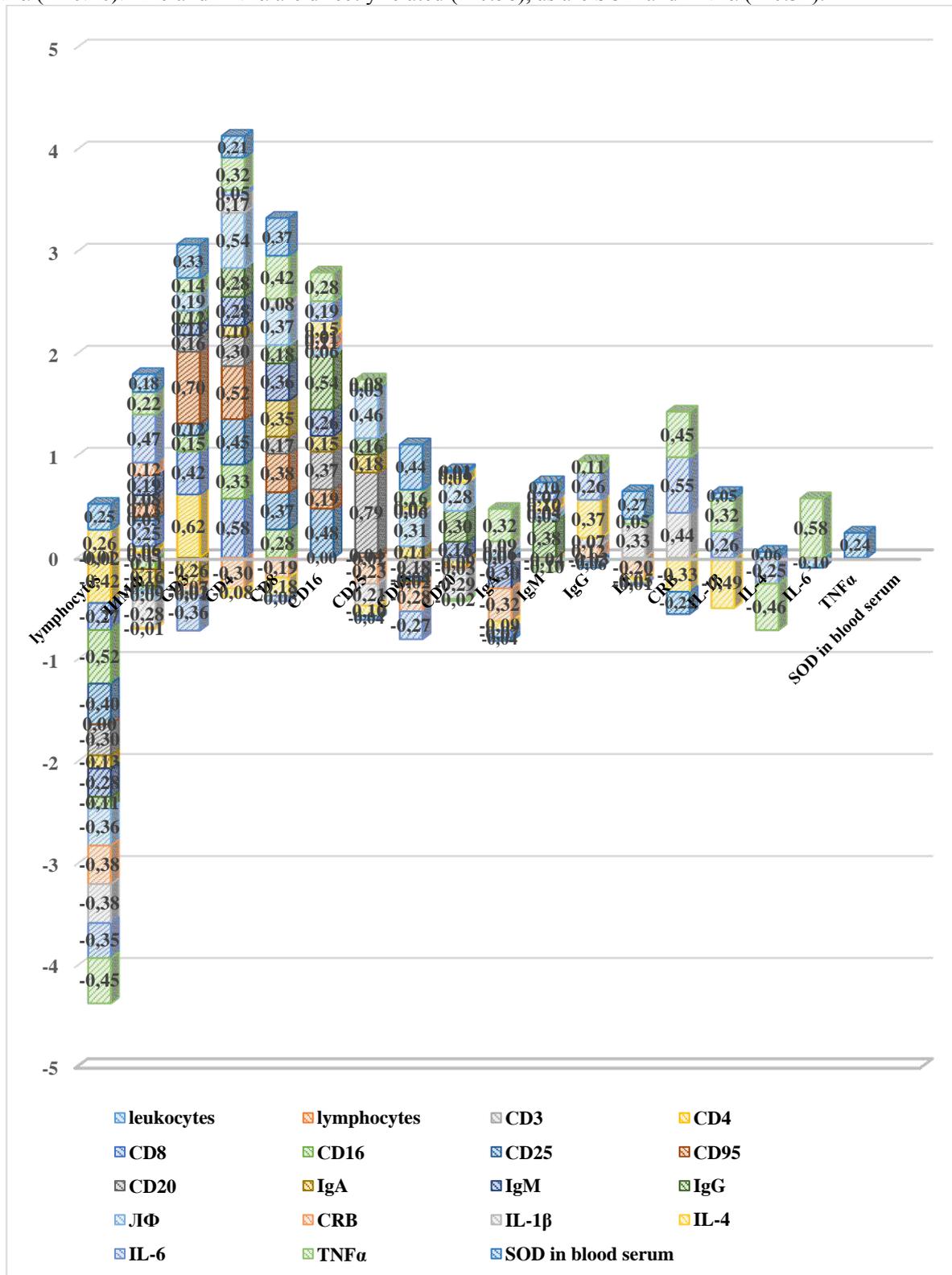


Fig. 4.14. Correlation parameters of patients with POVH

Conclusions

Thus, our studies allowed us to note that the characteristic features of the state of systemic immunity in the examined patients before surgery are:

- increased levels of CD20+, IgG, IgA, IL-1 β , IL-6, IL-4, TNF α , CRP
- decrease in the level of CD3+, CD4+, CD16+, LF

These changes in the state of the immune system in patients with POVH are classified as secondary immune deficiency. This is a state of impaired immunological reactivity due to a change in one or more components of the immune system or non-specific protective factors that closely interact with them. These facts indicate that in patients with POVH, when the T-cell composition of the immune system is suppressed, a pronounced imbalance in the humoral link of immunity and hyperactivation of cytokines are revealed. Changes in overall immunoreactivity were manifested by a decrease in the number of effector lymphocytes and the predominance of Th2 type. However, it is necessary to take into account the presence of concomitant pathology, in particular hormonal disorders, the predominance of women in the group of subjects, their age, etc. It may also have influenced changes in the overall immune response.

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