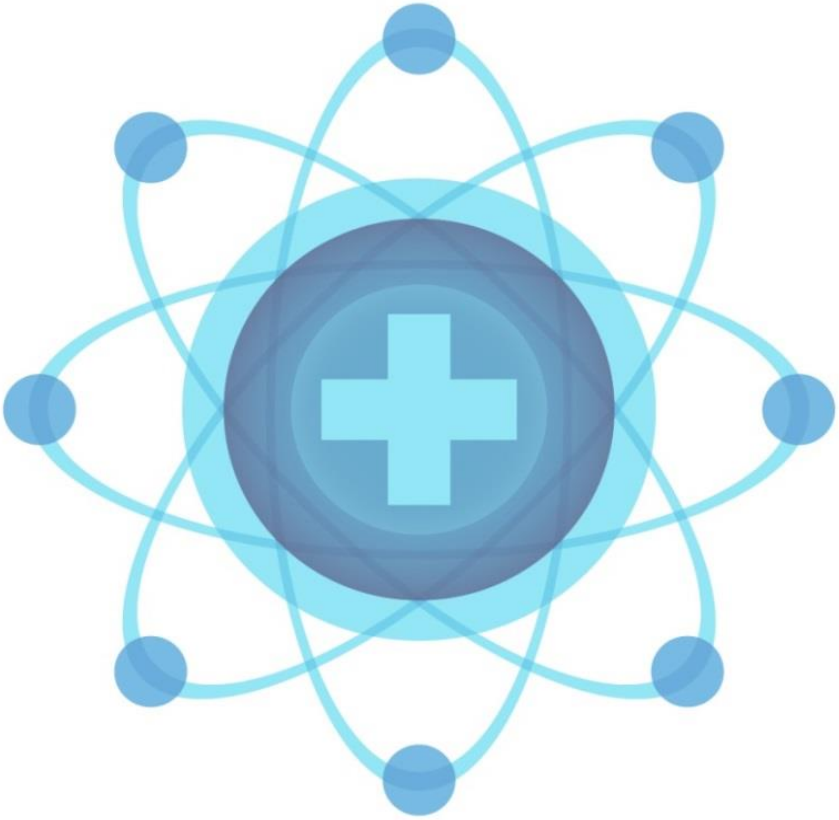

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THE EFFECT OF CYTOKINE GENE POLYMORPHISM ON THE COURSE OF CONGENITAL PNEUMONIA IN NEWBORNS.

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The most important factor in the adaptation of a newborn to extrauterine life is independent breathing. At this time, the newborn's lungs become a "critical organ" and the deterioration of the adaptive capabilities of the child's body can lead to the development of respiratory disorders [1,3,]. It is known that one of the common causes of respiratory disorders is congenital pneumonia. It develops in newborns in the first hours after birth as a result of transplacental infection, penetration of pathogens into the fetal lungs from amniotic fluid or when passing through the infected birth canal of the mother [2,4].

In recent years, among the large number of studied markers of the inflammatory response, the attention of researchers has been focused on cytokines, which are very sensitive markers for diagnosing the conditions of newborns during the entire neonatal period. The study of the balance of pro- and anti-inflammatory cytokines in newborns of various gestational ages with congenital pneumonia is an urgent task for the diagnosis, prognosis of the course and treatment of complications of the neonatal period. IL-6 is a multifunctional cytokine produced by fibroblasts, macrophages and other cells. According to the spectrum of biological action, it is close to IL-1 and TNF-α, participates in the development of inflammation, immune reactions, in the regulation of hematopoiesis, serves as a growth factor of plasma cells, participates in intersystem interactions. In the immune system, B-lymphocytes are the main target of IL-6. IL-6 is a cofactor of their proliferation and an independent differentiation factor. It evenly stimulates the production of immunoglobulins of all classes.

TNF-α is a key pro-inflammatory cytokine with multiple functions in the inflammatory network; its production is regulated at both transcriptional and post-transcriptional levels. TNF-α can stimulate the release of IL-1 and IL-6, and the latter enhances the response sensitivity of tissues to TNF-α. [5,11] TNF-α has an immunomodulatory effect - activates granulocytes, macrophages, hepatocytes

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(increases the production of acute phase proteins), stimulates the synthesis of other proinflammatory cytokines; stimulates the proliferation and differentiation of neutrophils, T - and B - lymphocytes, enhances their entry from the bone marrow into the blood and migration to the focus of inflammation. [7,8] TNF-α is a link in the pathogenesis of most infectious and immunopathological diseases, where it can perform various functions, mainly acting as a mediator of the development of innate immunity reactions [11]. However, increased TNFa production plays an important role in the pathogenesis of autoimmune diseases, graft rejection reactions and may cause septic shock and complications of acute inflammatory processes [12].

Congenital pneumonia refers to multifactorial diseases. One of the methods of studying the genetics of multifactorial diseases is the study of the association of polymorphic variants of genes, the products of which are presumably involved in the development of pathogenetic links of diseases. Currently, many authors note the significant role of cytokines in the pathogenesis of the development of congenital pneumonia of newborns. Cytokine production in response to various exogenous agents is genetically determined [5,6]. According to recent data, differences in the genes controlling the body's defense reactions can affect the level of cytokine production and thereby the nature of the development and course of the immune response [9]. In this regard, the functional polymorphism of cytokine genes is of particular interest, since it is these inflammatory mediator proteins that make a huge contribution to the regulation of immunity, therefore, the analysis of the regulation of their activity is very important for understanding the molecular basis of the pathogenesis of many diseases. [7,8, 10,11].

The aim of the study was to study the cytokine status (interleukin -6 (IL-6) and tumor necrosis factor - α (TNF-α)), as well as to analyze the association between IL-6 and TNF-α gene polymorphisms in newborn infants and the risk of congenital pneumonia.

Research methods: 106 newborns with confirmed congenital pneumonia (VP) who were in the neonatal intensive care unit (ICU) of the RSNPMC of Pediatrics and the Department of Pathology of newborns who were included in the main group were examined. Depending on gestational age and birth weight, the children of the main group were divided into 2 subgroups:

- I subgroup-55 premature newborns with a gestation period of -31.3±0.2 weeks, with an average body weight of - 1608.5±125.47 g.

- Subgroup II-51 full-term newborns with gestational age within 38.1±1.3weeks, with an average body weight of 3014.6±123.5g

The control group consisted of 20 healthy full-term newborns.

In addition to clinical laboratory and X-ray diagnostics, special biochemical research methods were carried out, which consisted in determining the content of IL-6, TNF-α in the blood by the ELISA method on a RANDOX (England) device using the BIOCHIP immunological technology.

Polymorphism of the genes of the IL- 6 promoter regions at position (-174) and TNF-α at position (-308) was determined. DNA extraction was carried out using a set of reagents "Ribot-prep" (AmpliSens, Russia). The detection of gene polymorphism

was carried out on the Rotor-Gene Q device (Quagen, Germany) using a commercial kit (Litech LLC, Russia) according to the manufacturer's instructions. Statistical processing of the obtained results was carried out using the applied programs of mathematical and statistical analysis Microsoft Excel Version 7.0 and Open Epi V 9.2.

Results and discussion.

Upon admission to the neonatal intensive care unit, the condition of all children of the main group was assessed as severe, due to severe respiratory failure. All newborns in this group needed intensive respiratory therapy. 34.6% of newborns of subgroup I and 21.6% of infants of subgroup II were on a ventilator, in the remaining newborns of both subgroups, the main method of respiratory support was the pSRAR method (32.7% and 35.3%) and oxygen therapy through nasal cannulas (Figure 1).

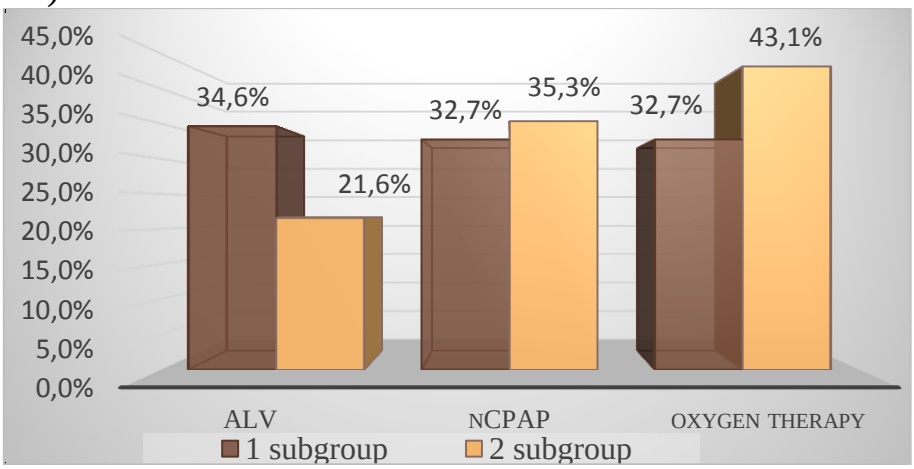

Fig.1. Respiratory support for newborns.

In addition to respiratory disorders, neurological disorders in the form of cerebral ischemia (CI) were observed in all children of the main group. In newborns of the 1st subgroup, QI of 2-3 degrees prevailed (49.1%) than in children of the 2nd subgroup (37.2%) in the form of CNS depression syndrome (muscle hypotension, hyporeflexia, decreased cerebral activity), in the remaining newborns of both subgroups, symptoms of QI of the 1st degree (neuroprotective excitability syndrome) were noted. Convulsive syndrome was more often observed in children of subgroup 1 with grade 2-3 QI (18.1% of cases) than in subgroup 2 (9.8% of cases).

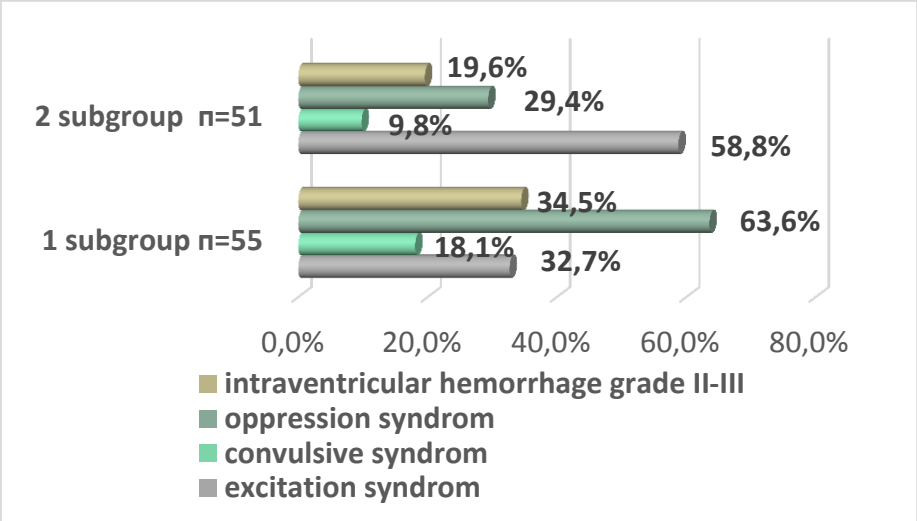

Fig.2. Neurological disorders

The analysis of neurosonographic data showed that in the subgroup of low-weight newborns, the frequency of hemorrhages in the ventricles of the brain was higher than in the subgroup of full-term newborns. Thus, the HFV of 2-3 art. was observed in 19 (34.5%) children of the 1st subgroup and in 10 (19.6%) children of the 2nd subgroup (Figure 2).

In our study, the level of IL-6 and TNF-α was determined on 3-5 days of newborn life. The results of the data obtained, presented in Table 1, indicate the presence of an imbalance in the cytokine profile.

Table 1.

The level of interleukins in the blood of newborns on 3-5 days of life

Cytokines (pg/ml)	**Main group N=106**		**Control N=20**	**P<**
	1 subgroup N=55	**2 subgroup N=51**		
IL-6	43,2±0,6	35,2±0,8	22,1±1,5	0,05
TNF-α	77,4±1,2	83,4±1,3	18,5±0,5	0,05

Note: P is the reliability of the difference between the main gr. and gr. control

According to our data, the content of IL-6 and TNF-α in the children of the main group was statistically significantly increased compared to the indicators of the control group (by 2 times and 1.6 times, respectively) (P<0.05). The persistence of higher concentrations of IL-6 in children of subgroup 1 compared with children of subgroup 2 indicates a more pronounced level of CVD from the moment of birth and indicates a low rate of relief of the inflammatory process in immature newborn children, despite intensive complex therapy. [9]

As a result of the study, we determined the frequency of occurrence of alleles and genotypes of polymorphic loci C174G of the IL-6 gene and G308A of the

TNF-α gene. As a control group, data on the frequency of occurrence of genes and genotypes obtained during the study in 83 newborns without congenital pneumonia were used. None of the compared groups revealed deviations from the Hardy–Weinberg equilibrium in terms of the genotype frequencies of the studied loci (P0.5).

During the comparative analysis, different frequencies of occurrence of alleles and genotypes of polymorphism C174G of the IL-6 gene were revealed in all groups and subgroups of newborns. In the main group, the incidence of the unfavorable G allele was 18.9%, in the control group – 19.9%, however, in the subgroup of premature newborns, its frequency was higher (23.6%), relative to the control. There was also a slight increase in the frequency of unfavorable homozygous genotype G/G in the subgroup of premature newborns (9.1%) compared with the control (6.0%). Heterozygous genotype C/G was also more common in this subgroup of patients (29.1% vs. 27.7% in the control). (Fig. 3).

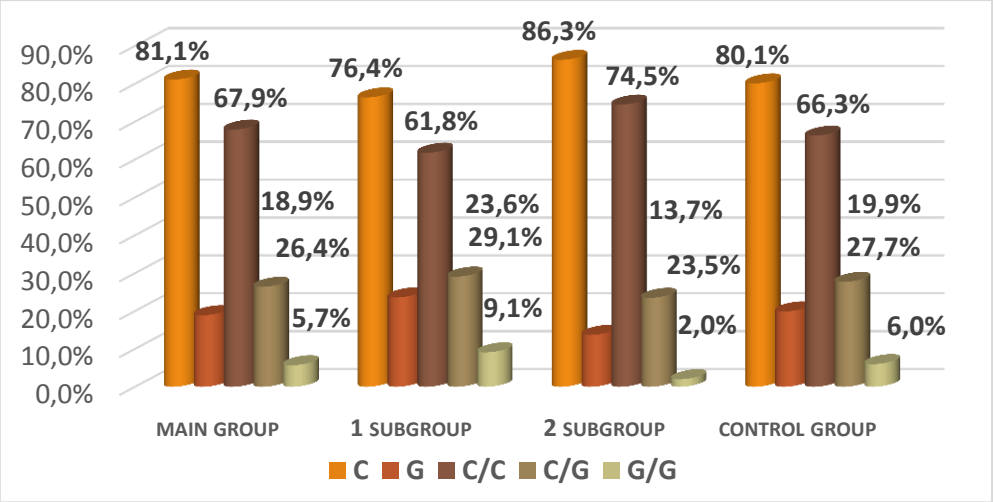

Fig.3. Frequency of distribution of alleles and genotypes of IL-6 gene polymorphism C174G in patient and control groups

Analysis of the frequency of distribution of alleles of the polymorphic locus C174G of the IL-6 gene in the patient and control groups revealed that the frequency of the unfavorable G allele determined in the main and control groups did not statistically significantly differ from each other ($\chi 2 = 0.06$: p=0.45; OR=0.93; 95% CI 0.55-1.56). The same can be said about heterozygous ($\chi 2 = 0.04$; p=0.56; OR=0.93; 95% CI 0.48-1.79) and homozygous ($\chi 2 = 0.01$; p=0.56; OR=0.93; 95%CI 0.27-3.18) genotypes, this suggests that there are significant differences in the distribution of genotypes and alleles between the main and control groups, but they are statistically insignificant. Nevertheless, there is a tendency to an almost 2-fold increase in the risk of developing congenital pneumonia in the subgroup of premature newborns compared with the subgroup of full-term newborns in the frequency of the unfavorable G allele ($\chi 2=3.4$; p=0.35; OR=1.94; 95% CI 0.95-3.95). The frequency of distribution of the pathological genotype G/G in the subgroup of premature newborns is 5 times higher than in the subgroup of full-term newborns ($\chi 2 = 2.51$; p=0.50; OR=5.0; 95% CI 0.68-36.48), but they are statistically insignificant, but there is a tendency to statistical difference.

We determined the frequency of occurrence and structure of alleles and genotypes of the TNF-α gene polymorphism G308A in the observation groups. When comparing groups of newborns with VP and healthy newborns, there is an increase in the frequency of occurrence of the unfavorable A allele in the main group (11.8%) relative to the control group (6.6%). The frequency of occurrence of heterozygous genotype G/A in the main group was 23.6%, in the control group-13.2%. When comparing two groups of newborns, as well as two subgroups of newborns, an unfavorable AA genotype was not found even once. (Figure 4.)

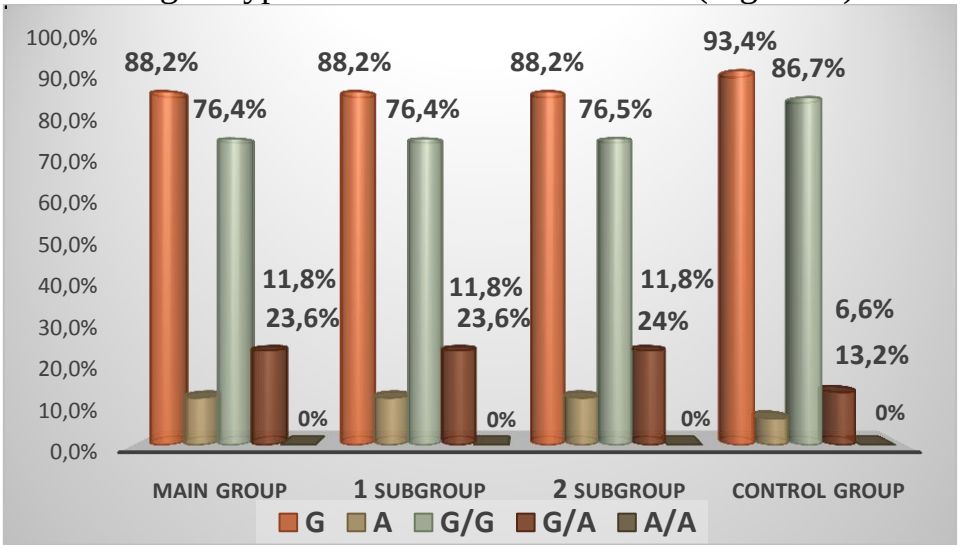

Fig.4. Frequency of distribution of alleles and genotypes of TNF-α gene polymorphism G308A in patient and control groups

A comparative analysis of the frequency distribution of alleles and genotypes of the polymorphic locus G308A of the TNF-α gene in the patient and control groups revealed that the frequency of the unfavorable A allele determined in the main and control groups did not significantly differ from each other ($\chi 2$ = 2.9: p=0.30; OR=1.88; 95%CI 0.91-3.91). Nevertheless, according to the frequency of the heterozygous genotype G/A ($\chi 2$ = 3.22: p=0.53; OR=2.02; 95% CI 0.94-4.35) there is a tendency to a statistical difference, leading to a 2-fold increase in the risk of severe congenital pneumonia in the group of sick newborns compared with children of the control group. It is possible that with the expansion of the number of groups of examined children, the differences will reach statistical significance and this genotype will be a genetic factor in the development of severe course in neonatal VP.

A whole cascade of genes may be responsible for the development of congenital infections, but even a small number of them may determine a key contribution to the genetic etiology of congenital pneumonia. Therefore, to predict the risk of developing this pathology, it is not enough to assess the influence of only individual polymorphic loci. Currently, there is no single universal model of "gene - gene" combinations of cytokine genes associated with the risk of severe congenital pneumonia. To search for genotypic combinations most associated with the risk of developing congenital pneumonia, an analysis of the "gene–gene" interaction in newborns with VP and in children of the control group was carried out.

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As a result of the analysis of the intergenic interaction of TNF-α and IL-6 cytokine genes involved in the regulation of the inflammatory process, no interacting genes determining the risk of developing VP and its severe course were identified.

Discussion.

Our study showed that the most significant single genetic markers that have the greatest risk for the development of congenital pneumonia are: the carrier of the G allele (1.9 times), the genotype G/G (5.0 times) of the polymorphic locus C174G of the IL-6 gene in premature newborns; the carrier of the genotype G/A of the polymorphic locus G308A TNF-α gene in newborns.

In the available sources, there are practically no studies devoted to the study of polymorphic loci of cytokine genes in newborns with RDS and VP. The TNF-α gene has a large locus variability. In particular, the promoter zone of the TNF-α gene includes 8 polymorphic sites with single nucleotide substitutions, but two of them are considered the most studied. These are single nucleotide substitutions of guanine to adenine at positions -308G/A and -238G/A, which cause changes in the level of TNF-α production, that is, they are functional. [12] However, in the work of Capasso M. with co-authors in the Italian population, the relationship of TNFa*-308G>A polymorphism with the development of VP in premature newborns was not revealed. Another study showed the association of a combination of TNFa and IL6 genotypes with an increased risk of acute kidney injury in children with infection [10].]

J.P. Mira et al. identified: in patients with the -308A allele, the risk of death from septic shock increases by 3.7 times, while the level of TNF-α circulating in the blood does not differ in patients of the control group and the group of patients with -308 TNF-α polymorphism [13]. Other researchers have shown that in the case of -308 TNF-α polymorphism, surgical patients have a higher risk of death due to septic shock [14]. In the study of children with meningococcal infection, a conclusion was made about the influence of genetic polymorphisms responsible for an increase in TNFa production on the outcome of the disease. The presence of at least one copy of the highly producing allele -308*A in the genotype of the child increased the probability of death from meningococcal infection by 2.5 times [15].

Harper et al. evaluated IL-6-174 polymorphisms, but the results were not quite as expected: there were no differences in the duration of pregnancy or the risk of premature birth in patients with IL-6-174 polymorphism. Other studies have also recently reported that IL-6 –174G>C and –572G>C polymorphisms are associated with complications in premature infants. Maternal polymorphism IL-6 -174 G>C is associated with chorioamnionitis, cerebral palsy and periventricular leukomalacia in premature infants [17-18].

Chen et al. The article studied the relationship between the single nucleotide polymorphism (SNP) of the interleukin-6 (IL-6) -174 G/C gene and the risk and/or mortality from sepsis by conducting an updated meta-analysis with experimental sequential analysis [20]. Meta-analysis by Chauhan M et al. In 2008, the association between -174 G/C polymorphism and the risk of sepsis in infants with very low birth weight (VLBW) was not confirmed [20]. After that, a meta-analysis in 2013 showed

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that IL-6 -174 G/C polymorphism was not associated with the risk and mortality from sepsis in any age and ethnic groups [21]. More research on this topic has been published in recent years. The study showed that the IL-6 -174 G allele was associated with the early onset of sepsis in Saudi infants [22]. However, Mao Z et al. and Feng B et al. It was believed that the risk of sepsis caused by pneumonia was associated with the allele with IL-6 -174, and not with G [23,24]. Regarding the mortality of patients with sepsis, Lorente L et al. The best survival rate of sepsis patients with the SS genotype was found [25].

Conclusions

Thus, the data obtained by us showed that the presence of an imbalance in the cytokine profile was revealed in newborns (full-term and premature) with VP, which is confirmed by an increase in the levels of proinflammatory cytokines IL-6 and TNF-α in both full-term and premature newborns.

The data obtained by us allow us to use the determination of cytokine gene polymorphism in newborns as a predictor of congenital pneumonia. Genetic variants of cytokines TNF-a, IL-6 may occupy a certain place not only in the development of variants of the course of congenital pneumonia in newborns, but also play an important role in the predisposition to the formation of this disease.

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