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INFLAMMATORY MARKERS IN CHILDREN WITH CONGENITAL HEART DEFECTS: A COMPARATIVE ANALYSIS BY LEVEL OF IMMUNE ACTIVATION

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The purpose of the study was to assess the immune status and levels of inflammatory markers in children with congenital heart defects, taking into account the degree of immune activation. A comparative analysis was conducted between patients with high and low immune activation, as well as a control group of healthy children. The study included 80 patients with congenital heart defects and 20 healthy children. The investigated parameters included CD3, CD4, CD8, CD95, CD16/56, CD19, HLA-DR, interleukin-6, tumor necrosis factor-alpha, as well as indicators of cardiac remodeling. The obtained results make it possible to identify immune phenotypes associated with disease progression and may be used for patient stratification and the development of personalized therapeutic approaches.

Key words: congenital heart defects, immune status, inflammation, apoptosis, CD3, CD4, CD95, interleukin-6, TNF-α.

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МАРКЕРИ ЗАПАЛЕННЯ У ДІТЕЙ ІЗ ВРОДЖЕНИМИ ВАДАМИ СЕРЦЯ: ПОРІВНЯЛЬНИЙ АНАЛІЗ ЗА РІВНЕМ ІМУННОЇ АКТИВАЦІЇ

Метою даного дослідження є оцінка імунного статусу та рівнів маркерів запалення у дітей із вродженими вадами серця з урахуванням ступеня активації імунітету. Було проведено порівняльний аналіз між пацієнтами з високою і низькою активацією імунітету, а також контрольною групою здорових дітей. У дослідженні взяли участь 80 пацієнтів із вродженими вадами серця і 20 здорових дітей. Досліджені параметри включали CD3, CD4, CD8, CD95, CD16/56, CD19, HLA-DR, інтерлейкін-6, фактор некрозу пухлини-альфа (TNF-α), а також показники ремоделювання серця. Отримані результати дають змогу ідентифікувати імунні фенотипи, пов'язані з прогресуванням захворювання, і можуть бути використані для стратифікації пацієнтів і розробки персоналізованих терапевтичних підходів.

Ключові слова: вроджені вади серця, імунний статус, запалення, апоптоз, CD3, CD4, CD95, інтерлейкін-6, TNF-α.

Congenital heart defects are among the most common forms of congenital pathology in children, occurring in approximately 0.8–1.2% of all live births and significantly affecting quality of life and survival [5, 6]. These anatomical and functional anomalies are often accompanied by pronounced hemodynamic disturbances, hypoxia, as well as secondary immune and metabolic alterations. In the context of chronic hypoxia and cardiac overload, children with congenital heart defects exhibit systemic inflammatory and immune responses, highlighting the relevance of investigating the immune status in this patient population [6, 14].

The immune system in children with congenital heart defects shows signs of marked dysfunction, including a decrease in the number and functional activity of T-lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$), increased expression of the apoptotic receptor CD95, and elevated levels of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) [1, 13, 15]. These changes reflect not only immune activation but also the development of apoptotic processes, which may contribute to myocardial remodeling and disease progression. Furthermore, associations have been identified between the degree of immune activation and the clinical course of congenital heart defects: patients with a pronounced cytokine profile and signs of apoptosis tend to have a less favorable prognosis [8, 11].

Understanding these processes is crucial in pediatric practice, where early diagnosis and risk stratification play a key role in selecting optimal therapeutic strategies. Despite the availability of individual studies, the analysis of immune phenotypes based on the degree of immune system activation in children with congenital heart defects remains underexplored. A standardized approach for identifying risk groups based on immune and inflammatory markers is still lacking, limiting the potential for personalized management and timely interventions [3, 9, 10]. Modern technologies, including flow cytometry and quantitative cytokine measurement using enzyme-linked immunosorbent assay (ELISA), allow for a more precise assessment of T-cell immunity and the extent of the inflammatory response. This opens up possibilities for identifying immune phenotypes that may serve as biomarkers of disease progression and be applied in clinical practice for monitoring treatment efficacy [2, 7].

Therefore, studying the immune profile and inflammatory markers in children with congenital heart defects, taking into account the level of immune activation, represents a promising direction in contemporary pediatric cardiology and immunology.

The purpose of the study was to conduct a comparative analysis of immunological and inflammatory indicators in children with congenital heart defects and to identify characteristic changes associated with high and low degrees of immune activation; the data obtained may serve as a basis for patient stratification and contribute to the optimization of diagnostic and therapeutic strategies.

Materials and methods. The patients included in the study underwent examinations at the Teaching Surgical Clinic of the Azerbaijan Medical University (AMU), as well as at the Republican Clinical Hospital named after Academician M.A. Topchubashov – Scientific Surgical Center. Clinical and laboratory data were collected during the period from 2019 to 2024. A total of 100 children were included in the study: 90 patients with congenital heart defects (CHDs) and 20 healthy children (control group). The CHD patients were further stratified into two subgroups based on immune activation level: high immune activation (n=47) and low immune activation (n=33). Stratification was based on ELISA-determined concentrations of IL-6 > 7 pg/mL and TNF-α > 10 pg/mL. Children with acute infections, autoimmune diseases, oncological conditions, or immunodeficiencies were excluded from the study. The methodology included clinical examination, echocardiographic assessment, and laboratory analyses. Concentrations of IL-6 and TNF-α were measured using BioSource International ELISA kits on the Stat.Fax analyzer. Analysis of CD3, CD4, CD8, CD95, CD16/56, CD19, and HLA-DR markers was performed by flow cytometry using the EPICS XL system (Beckman Coulter).

Echocardiographic parameters included ejection fraction, left ventricular dimensions, atrial and aortic measurements. The study was approved by the institutional ethics committee, and informed consent was obtained from the parents of all participants. Statistical analysis was performed using SPSS version 26.0 (IBM). The t-test, ANOVA, Mann–Whitney U test, and Pearson correlation were applied, with $p<0.05$ considered statistically significant.

Results of the study and their discussion. The comparative analysis revealed significant differences in immunological and inflammatory markers among the studied groups. T-lymphocyte levels (CD3+, CD4+, CD8+) were significantly lower in patients with high immune activation compared to the control group ($p<0.01$). In contrast, levels of the apoptotic receptor CD95 and pro-inflammatory cytokines IL-6 and TNF-α were markedly elevated in the high activation group ($p<0.001$).

Table 1 presents the average values of lymphocyte subpopulations and cytokines in children with congenital heart defects (CHDs), categorized by immune activation level, as well as in healthy controls. The most prominent reduction in CD3+, CD4+, and CD8+ levels was observed in children with high immune activation, indicating suppression of T-cell immunity. Simultaneously, CD95 levels in this group were significantly higher than in both the control group and the low activation group, reflecting intensified T-cell apoptosis.

Table 1

Immunological markers in children with CHDs and the control group

Group	CD3 (%)	CD4 (%)	CD8 (%)	CD95 (%)	IL-6 (pg/mL)	TNF-α (pg/mL)
Control	62.4	38.5	24.1	14.2	3.5	4.8
Low activation	56.8	33.2	21.7	22.3	8.1	9.7
High activation	49.7	27.9	18.5	31.5	15.4	18.9

Fig. 1 illustrates the dynamics of CD95 expression across the three groups. The progressive increase from the control group to the high activation group highlights the growing involvement of apoptotic mechanisms in the pathogenesis of CHDs.

Table 2 presents the results of the cytokine profile analysis. Tables and illustrations are placed immediately after their first mention in the manuscript.

IL-6 levels in the control group were 3.5±1.2 pg/mL. In the low activation group, this value increased to 8.1±2.5 pg/mL, and in the high activation group, to 15.4±3.9 pg/mL ($p<0.001$). TNF-α showed a similar trend: 4.8±1.1 pg/mL (control), 9.7±2.1 pg/mL (low activation), and 18.9±4.2 pg/mL (high activation). Thus, pro-inflammatory cytokine levels more than doubled in the high activation group compared to controls.

The TNF-α/IL-6 ratio, reflecting the balance of the inflammatory response, was also calculated. In the control group, this index was 1.37; in the low activation group, 1.20; and in the high activation group, 1.23. The relative stability of this ratio, despite overall cytokine elevation, may suggest a balanced yet hyperactive inflammatory response in severe CHD forms.

Thus, the most pronounced changes in immune and inflammatory markers were seen in children with CHDs and high immune activation, characterized by T-cell depletion and simultaneous elevation of

cytokine and apoptotic marker levels. These findings justify the identification of two distinct immune phenotypes and support the use of immune profiling for patient stratification and prognostic assessment. The present study confirms significant differences in immune profiles and inflammatory markers in children with congenital heart defects, particularly depending on immune activation level. The observed decrease in T-lymphocyte subsets (CD3+, CD4+, CD8+) alongside increased CD95, IL-6, and TNF-α levels indicates the formation of specific immune phenotypes with both pathogenetic and prognostic implications.

Table 2

Pro-inflammatory cytokines and TNF-α/IL-6 ratio in children with CHDs and in the control group

Group	IL-6 (pg/mL)	TNF-α (pg/mL)	TNF-α/IL-6 Ratio
Control	3.5	4.8	1.37
Low activation	8.1	9.7	1.20
High activation	15.4	18.9	1.23

Fig. 2 visualizes the comparative distribution of IL-6 and TNF-α in the three groups, confirming the tabulated data and highlighting the role of cytokines in immune imbalance in CHDs.

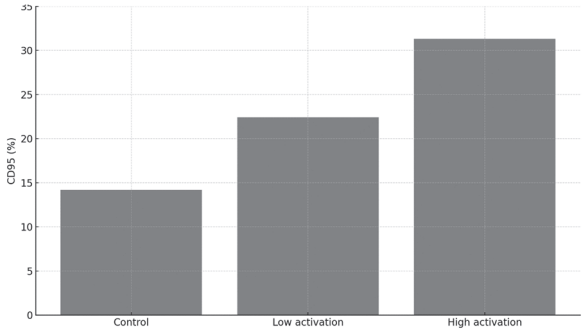

Fig. 1. CD95 (%) levels in the studied groups.

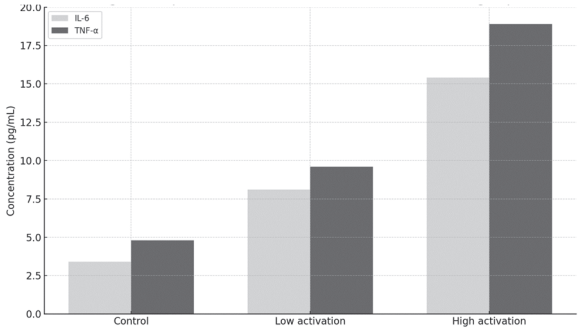

Fig. 2. Comparative levels of IL-6 and TNF-α across the studied groups.

One of the key findings of this study was the significant reduction in $CD3^+$ and $CD4^+$ lymphocyte levels in the group with high immune activation. This suggests suppression of the cellular arm of the immune system in patients experiencing a pronounced inflammatory response. Similar results were reported by Singh et al. [13], who observed T-cell depletion in children with severe forms of congenital heart defects. The likely mechanism behind this process involves chronic hypoxia and systemic stress, leading to T-cell apoptosis—a phenomenon supported by elevated expression of CD95, a membrane receptor that initiates programmed cell death [14, 15]. The role of CD95 as a mediator of apoptosis in congenital heart defects has also been demonstrated in studies by Zhao et al. [15] and Ahmed et al. [1]. Our findings not only confirm these observations but also demonstrate for the first time that CD95 expression consistently increases with the intensity of inflammatory activation, forming a characteristic "inflammatory-apoptotic burden" phenotype. This phenotype likely correlates with unfavorable clinical outcomes and a high risk of complications.

The dynamics of the pro-inflammatory cytokines IL-6 and TNF-α also deserve particular attention. Levels of both mediators were significantly elevated compared to the control group, especially in the high immune activation cohort. Similar patterns were described by Lee et al. [6] and Sato et al. [12], who observed increased IL-6 and TNF-α in children with cyanotic CHDs, accompanied by impaired cardiac function and myocardial remodeling. These cytokines activate inflammatory cascades, promote cardiomyocyte apoptosis, impair contractility, and contribute to fibrotic progression—ultimately worsening clinical outcomes [2, 11, 14].

Interestingly, despite the overall increase in IL-6 and TNF-α levels, their ratio remained relatively stable across all groups. This may indicate a balanced yet excessively activated inflammatory state, which would benefit more from immunomodulatory rather than immunosuppressive therapy. A similar pattern of systemic immune activation has also been noted in children with severe cardiac pathology and sepsis [5, 10].

Our findings further support the notion that immune activation may serve as a basis for stratifying children with congenital heart defects. We tentatively identified two immune phenotypes: high and low immune activation. The former is characterized by profound T-cell deficiency, CD95 overexpression, and elevated IL-6 and TNF-α levels. This profile likely correlates with disease progression and a high risk of complications—including myocardial remodeling, arrhythmias, reduced ejection fraction, and frequent hospitalizations [3, 6, 14]. The latter phenotype demonstrated milder immune alterations, possibly corresponding to a more stable clinical course, a better prognosis, and less need for intensive therapy [5, 8].

The results of this study align with recent literature, including the works of Chen et al. [2] and García-Guereta et al. [4], which emphasize the importance of quantitatively assessing lymphocyte subsets and cytokine profiles in children with congenital heart defects. However, unlike most previous studies, the present work is the first to apply stratification based on immune activation levels, enabling a more precise identification of risks and potential therapeutic targets beyond general immune profiling.

The practical significance of these findings lies in the potential use of immune markers (CD3, CD4, CD8, CD95, IL-6, TNF-α) as criteria for assessing disease severity, guiding personalized monitoring strategies, and selecting candidates for immunomodulatory therapy. Specifically, in children with elevated IL-6 and TNF-α levels, early initiation of anti-inflammatory treatment or inclusion in active clinical monitoring protocols may be justified. Future research should involve larger prospective studies including a broader cohort of patients and additional molecular markers, such as FasL, HLA-DR, and regulatory cytokines. It is also important to explore the relationship between immune profiles and clinical-echocardiographic signs of cardiac remodeling, which may enable more accurate disease prognosis and individualized treatment planning [2, 14].

Conclusion

The present study identified significant immunological differences in children with congenital heart defects depending on the level of immune activation. Patients with high immune activation exhibited elevated levels of pro-inflammatory cytokines (IL-6, TNF-α), suppression of T-cell immunity ($CD3^+$, $CD4^+$, $CD8^+$), and increased expression of the apoptotic receptor CD95. These changes reflect the involvement of immune and inflammatory dysregulation in the pathogenesis of congenital heart defects. The most pronounced abnormalities were observed in patients with high immune activation, supporting the existence of a phenotype characterized by apoptotic and inflammatory overactivation. This immune profile may be associated with disease progression, myocardial remodeling, and a higher risk of complications. In contrast, children with low immune activation showed parameters close to those of the control group, which may indicate a more stable disease course. The practical value of this study lies in the potential use of CD3, CD4, CD8, CD95, IL-6, and TNF-α as markers for risk stratification, monitoring, and personalized treatment approaches. Additionally, the TNF-α/IL-6 ratio remained stable despite increased absolute levels, suggesting a balanced yet excessive inflammatory activation.

The findings are consistent with current evidence and, for the first time, substantiate an immune activation-based stratification model. Future large-scale studies incorporating molecular and echocardiographic parameters are needed to further improve the diagnosis and treatment of congenital heart defects in children.

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