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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

FEATURES OF IMMUNE STATUS AND APOPTOTIC PROCESSES IN CHILDREN WITH CONGENITAL HEART DEFECTS

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Field of science:	Medical Sciences
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Baku – 2026

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Tibb üzrə fəlsəfə doktoru, dosent
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20.10.26

GENERAL DESCRIPTION OF THE WORK

Relevance of the topic. Congenital heart defects (CHD) are among the most common congenital anomalies in children and remain an important problem of modern pediatrics and pediatric cardiology[1]. Their clinical significance is determined not only by the nature of the anatomical defect and the severity of hemodynamic disturbances, but also by the development of systemic changes that may affect the child's condition, the course of the disease, and the adaptive capacity of the body. Despite significant advances in diagnosis, cardiac surgical correction, and postoperative management, assessment of the baseline condition of a child with CHD before surgical intervention remains important. The clinical picture of the disease is formed not only as a result of disturbed intracardiac hemodynamics, but also under the influence of changes in tissue perfusion, gas exchange, cellular metabolism, and systemic regulatory mechanisms. Prolonged hemodynamic overload, impaired tissue perfusion, and chronic hypoxemia, particularly characteristic of cyanotic forms of CHD, may be accompanied by changes in the inflammatory response and immune regulation[2]. In the setting of a long-standing defect, the child's body adapts to altered parameters of circulation and oxygenation; however, the possibilities of such adaptation are not identical in different clinical and hemodynamic variants of the disease. In acyanotic forms, the leading role may be played by the characteristics of intracardiac shunting and hemodynamic load, whereas in cyanotic defects systemic hypoxemia acquires additional significance. Therefore, comparison of these two groups is of interest not only from the standpoint of the anatomical classification of CHD, but also for assessing differences in the systemic response of the child's body. The relationship between hypoxia, immunity, and inflammation is mediated

[1] Liu Y., Chen S., Zühlke L., Black G.C., Choy M.K., Li N. et al. Global birth prevalence of congenital heart defects 1970–2017: updated systematic review and meta-analysis of 260 studies. International Journal of Epidemiology. 2019;48(2):455–463. doi:10.1093/ije/dyz009. https://doi.org/10.1093/ije/dyz009

[2] Wienecke L.M., Cohen S., Bauersachs J., Mebazaa A., Chousterman B.G. Immunity and inflammation: the neglected key players in congenital heart disease? Heart Failure Reviews. 2022;27(5):1957–1971. doi:10.1007/s10741-021-10187-6. https://link.springer.com/article/10.1007/s10741-021-10187-6

by the involvement of oxygen-dependent signaling mechanisms in the regulation of immunocompetent-cell activity[3]. Changes in tissue oxygen supply may affect the functional state of immune-system cells, their interactions, and regulatory mechanisms. In pediatric practice, the study of these processes is of interest for the comprehensive assessment of children with different clinical and hemodynamic variants of CHD. It is fundamentally important to consider immunological parameters not in isolation, but in the context of the underlying disease, hemodynamic characteristics, and the presence or absence of chronic hypoxemia. Such an approach makes it possible to preserve the clinical orientation of the study and to evaluate the identified laboratory changes as part of the overall characteristics of a child with a congenital heart defect. An important direction in the study of systemic changes in CHD is the assessment of leukocyte and lymphocyte counts, as well as the main subpopulations of immunocompetent cells. Studies of the T-cell compartment in children with CHD indicate the presence of specific features of the immune response in this condition[4]. T lymphocytes participate in the coordination of cellular immune reactions; therefore, assessment of their major subpopulations provides information about the state of cellular immunity. At the same time, interpretation of these parameters in childhood requires caution, because the absolute number of circulating cells and their percentage distribution may change differently. In this regard, the simultaneous use of absolute and relative parameters is more informative than assessment of only one of these characteristics. Determination of the absolute and relative counts of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes, and $CD16/56^+$ natural killer cells broadens the characterization of immune status [5]. $CD3^+$ cells

[3] McGettrick A.F., O'Neill L.A.J. The role of HIF in immunity and inflammation. Cell Metabolism. 2020;32(4):524–536. doi:10.1016/j.cmet.2020.08.002. https://pubmed.ncbi.nlm.nih.gov/32853548/

[4] Логинова Н.П., Четвертных В.А., Лебединская О.В., Шилов Ю.И., Годовалов А.П., Шилов Д.Ю., Суханцева И.В. Функциональные особенности Т-лимфоцитов у детей с врождёнными пороками сердца. Российский иммунологический журнал. 2015;9(18)(2):216–221.

[5] Singampalli K.L., Jui E., Shani K., Ning Y., Connell J.P., Birla R.K. et al. Congenital heart disease: an immunological perspective. Frontiers in Cardiovascular Medicine. 2021;8:701375. doi:10.3389/fcvm.2021.701375. https://www.frontiersin.org/journals/cardiovascular-medicine/articles/10.3389/fcvm.2021.701375/full

reflect the total T-cell pool, whereas $CD4^+$ and $CD8^+$ subpopulations characterize the main functional directions of the T-cell immune response. $CD19^+$ cells allow assessment of the B-cell compartment, and $CD16/56^+$ cells characterize the natural killer cell population. Combined investigation of these parameters makes it possible to assess immune status not by an isolated marker, but as a set of interrelated cellular components.

Comparison of absolute and relative parameters is of particular importance, because changes in the number of immune cells are not always accompanied by unidirectional changes in their percentage distribution. An increase in the absolute number of a certain subpopulation while its relative proportion remains unchanged may reflect general changes in the cellular pool, whereas a change in percentage may indicate rearrangement of the proportions between individual cell populations. Therefore, simultaneous assessment of both types of parameters helps avoid oversimplified interpretation of immunological data and is especially important when comparing children with CHD and practically healthy children. Particular attention should be paid to HLA-DR, a major histocompatibility complex class II molecule involved in antigen presentation and used to characterize the activation state of certain cell populations. Features of HLA-DR expression on lymphocytes have been studied in children with septal CHD [6]. Assessment of HLA-DR is of interest as an additional component of immunological characterization, especially when comparing different clinical and hemodynamic forms of CHD. It is fundamentally important to distinguish between the absolute number of $HLA\text{-}DR^+$ cells and their relative proportion, because these parameters may change differently and reflect different aspects of the cellular response. Further interest lies in assessing the absolute and relative counts of $HLA\text{-}DR^+$ cells in combination with other immunological parameters in acyanotic and cyanotic forms of the disease. Such an approach makes it possible to determine whether the identified changes are isolated or form part of a

[6] Шабалдин А.В., Синицкая А.В., Шмулевич С.А., Гришачева Е.О. Особенности экспрессии молекул HLA-G и HLA-DR на лимфоцитах женщин и их детей с септальными врождёнными пороками сердца. Медицинская иммунология. 2024;26(1):89–106.

broader complex of immunological features. Comparison of HLA-DR with T-, B-, and NK-cell indices is particularly important because it allows immune status to be considered as an integrated system rather than as a set of independent laboratory parameters. Cytokine assessment plays an important role in characterizing the inflammatory response. Their involvement in the regulation of immune reactions and the development of pathological processes, as well as the significance of their clinical investigation, has been discussed in the works of national authors[7,8]. Cytokines mediate intercellular communication and participate in the regulation of inflammatory and immune reactions. In CHD, their study is of interest because hemodynamic load, impaired tissue perfusion, and hypoxemia may create conditions for alterations in the systemic inflammatory response even before surgical correction of the defect. In CHD, particular attention is paid to tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). Changes in the levels of these cytokines reported in children with CHD support the need to investigate the inflammatory component of systemic alterations in this condition [9]. TNF-α is of interest not only as a pro-inflammatory mediator, but also as a factor associated with the regulation of cell death and intercellular interactions. IL-6 also participates in the formation of the inflammatory response and may complement its laboratory characterization. At the same time, interpretation of cytokine parameters requires consideration of the study design, the size of the examined sample, and the clinical condition of the children. In the present study, IL-6 is assessed as an additional indicator of the inflammatory component in the examined subgroup and is not used for independent assessment of differences between all main groups. This approach makes it possible to avoid overinterpretation of limited data and to maintain consistency between the findings actually obtained and the

[7] Poluxova A.Ə., Rəhimova N.C., Quliyev N.C. Müxtəlif patologiyaların yaranmasında, gedişində və proqnozlaşdırılmasında sitokinlərin öyrənilməsinin əhəmiyyəti. Azərbaycan Perinatologiya və Pediatriya Jurnalı. 2013;1(1):169–178.

[8] Tağıyev N.Y., Poluxova A.Ə., Musayev İ.M. Sitokinlər haqqında müasir təsəvvürlər, onların norma və müxtəlif patologiyaların inkişafında rolu. Sağlamlıq Jurnalı. 2006;(7):13–20.

[9] Wang D., Fang J., Wang R., Sun D., Xia K., Yin W., Zhang S., Sun L. Elevated serum ghrelin, tumor necrosis factor-α and interleukin-6 in congenital heart disease. Pediatrics International. 2016;58(4):259–264. doi:10.1111/ped.12773.

conclusions formulated. Immune and inflammatory changes are closely related to the processes of programmed cell death. Studies of the thymus in children with CHD indicate an association between hypoxia and changes in apoptotic processes in an immunocompetent organ [10]. Apoptosis is a physiologically regulated mechanism for maintaining cellular homeostasis; however, changes in its regulation may accompany chronic pathological conditions. In CHD, interest in this process is primarily related to the possible relationship between chronic hypoxemia, inflammatory mediators, and the state of immunocompetent cells. The membrane receptor CD95 (Fas), which participates in the extrinsic apoptotic pathway, is of particular interest. Assessment of the relative and absolute counts of $CD95^+$ cells allows characterization of the Fas/CD95-dependent component of apoptotic regulation and the apoptotic readiness of immunocompetent cells; however, this parameter itself is not a direct measure of the intensity of cell death. This is fundamentally important for correct interpretation of the results: an increase in $CD95^+$ cell counts should be regarded as a change in the state of Fas/CD95-dependent regulation rather than as direct evidence of increased cell death. Combined investigation of CD95 and TNF-α is also of interest because it allows comparison of apoptotic-regulation and inflammatory-response parameters within the same clinical sample. Combined investigation of inflammatory and apoptotic markers in children with CHD has already been reported in the literature [11]. Nevertheless, there remains a need to compare these markers with indices of cellular immunity, HLA-DR expression, and the leukocyte–lymphocyte compartment within a single examined cohort. Such comparison is particularly relevant when children are divided into acyanotic and cyanotic forms of CHD, because it allows assessment of which changes are common to congenital heart defects as

[10] Ceyran A.B., Şenol S., Güzelmeriç F., Tunçer E., Tongut A., Özbek B. et al. Effects of hypoxia and its relationship with apoptosis, stem cells, and angiogenesis on the thymus of children with congenital heart defects: a morphological and immunohistochemical study. International Journal of Clinical and Experimental Pathology. 2015;8(7):8038–8047

[11] Nassef Y.E., Hamed M.A., Aly H.F. Inflammatory cytokines, apoptotic, tissue injury and remodeling biomarkers in children with congenital heart disease. Indian Journal of Clinical Biochemistry. 2014;29(2):145–149. doi:10.1007/s12291-013-0341-0. https://link.springer.com/ article/10.1007/s12291-013-0341-0

a whole and which may be more pronounced in the presence of chronic hypoxemia.

Thus, comprehensive investigation of immune status, inflammatory activity, and apoptotic-regulation processes in children with CHD is relevant. The obtained data may complement the comprehensive clinical and laboratory assessment of the baseline condition of children with CHD at the preoperative stage and contribute to a more complete understanding of the immunological, inflammatory, and apoptotic changes associated with different clinical and hemodynamic forms of the disease.

Object and subject of research. The object of the study was children with congenital heart defects examined before surgical correction and practically healthy children in the control group. The subject of the study was the pattern of cellular immunity, Fas/CD95-dependent apoptotic regulation and inflammatory response, and their relationships in acyanotic and cyanotic forms of CHD.

Purpose of the study. To determine the features of immune status, apoptotic regulation and inflammatory changes in children with CHD and assess the relationships between the studied parameters with consideration of the clinical and hemodynamic form of the disease.

The tasks of the research:

1. To assess the state of cellular immunity in children with CHD based on the absolute and relative values of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD16/56^+$ and $HLA\text{-}DR^+$ cells, as well as the CD4/CD8 ratio, in comparison with practically healthy children.
2. To investigate the features of apoptotic regulation in children with CHD based on $CD95^+$ cell levels and TNF-α levels, taking into account the acyanotic and cyanotic forms of the disease.
3. To characterize inflammatory changes in children with CHD based on leukocyte count, absolute and relative lymphocyte counts, and IL-6 levels in the examined subgroup.
4. To compare immunological, apoptotic and inflammatory parameters in acyanotic and cyanotic CHD and assess the frequency of their combined changes.
5. To determine the nature and strength of correlations between the studied parameters of cellular immunity, apoptotic regulation,

and inflammatory response in children with congenital heart defects using correlation analysis.

Research methods: Clinical, instrumental, laboratory, immunological, and statistical methods were used. CHD was assessed clinically, by echocardiography, and by pulse oximetry; immunological indices were determined by flow cytometry and enzyme-linked immunosorbent assay. Comparative and correlation analyses were performed.

Main provisions of the dissertation submitted for defense:

1. In children with CHD, the absolute counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and $CD16/56^+$ cells are increased, whereas most relative parameters and the CD4/CD8 ratio remain substantially unchanged.
2. Apoptotic regulation in CHD is characterized by increased relative and absolute counts of $CD95^+$ cells and elevated TNF-α levels.
3. The inflammatory response in children with CHD is accompanied by changes in leukocyte–lymphocyte parameters; determination of IL-6 in the examined subgroup complements its characterization.
4. Changes in acyanotic and cyanotic CHD are predominantly unidirectional; the cyanotic form is characterized by a lower relative count of $HLA\text{-}DR^+$ cells and a more frequent combination of several altered parameters.
5. Relationships between immunological, apoptotic and inflammatory parameters reflect the interrelated nature of systemic changes in children with CHD.

Scientific novelty of the work: A comprehensive assessment of cellular immunity, apoptotic regulation, and inflammatory response was performed in children with acyanotic and cyanotic CHD. Higher absolute counts of the major lymphocyte subpopulations, increased $CD95^+$ cell counts and TNF-α values, and a lower relative $HLA\text{-}DR^+$ cell count in cyanotic CHD were identified. Relationships between $CD95^+$ cells and cellular indices, as well as a higher frequency of combined abnormalities in cyanotic CHD, were established.

Practical significance: The obtained results complement the clinical and laboratory assessment of children with CHD at the preoperative stage. Joint evaluation of absolute and relative lymphocyte-subpopulation indices, CD95, and TNF-α is advisable with

consideration of the clinical and hemodynamic form of CHD. The studied parameters have additional value and do not replace standard clinical and cardiological examination.

Approval of research work: The main provisions and results of the dissertation were presented and discussed at national and international scientific events: the International Conference on Immunopathology (Baku, 2019); the scientific-practical congress dedicated to the 80th anniversary of the Department of Pediatric Surgery of Azerbaijan Medical University (Baku, 2019); the scientific-practical seminar dedicated to the 100th anniversary of National Leader Heydar Aliyev (Baku, 2023); the 1st International Congress of the Azerbaijan Pediatric Society (Baku, 1–3 June 2023); the X International Scientific and Practical Conference "Challenges and Problems of Modern Science" (London, United Kingdom, 19–20 October 2023); and the International Scientific and Practical Conference "Current Issues of Medicine, Pharmacology, Therapy and Rehabilitation" (Ivano-Frankivsk–Kherson, Ukraine, 23–24 May 2025).

Application of the research: The study results were incorporated into the educational and clinical process of the Educational-Therapeutic Clinic of Azerbaijan Medical University and into the practical activities of the "Uniklinika" clinic, as confirmed by the corresponding implementation acts.

The organization in which the dissertation work was carried out: The dissertation research was conducted at the clinical and laboratory facilities of Azerbaijan Medical University, including the Educational Surgical Clinic, the Educational Therapeutic Clinic, and the Immunology Laboratory of AMU, as well as at Uniklinika. The comparative study included 113 children: 90 children with CHD and 23 practically healthy controls. The CHD group included 59 patients with acyanotic and 31 patients with cyanotic defects. The main investigations were performed before surgical correction. A comprehensive assessment of combined changes in six parameters was performed in 68 children who had the complete required laboratory dataset. The study was conducted in accordance with ethical principles and after informed consent had been obtained from parents or legal representatives.

Published scientific works: Fifteen scientific works were published on the topic of the dissertation, including 8 scientific

articles, 6 abstracts, and 1 material of an international scientific conference, reflecting the main results of the study.

Structure and Scope of the Dissertation. The dissertation is presented on 181 pages (223.381 characters) and consists of an introduction (19.818 characters), six chapters, a general conclusion (17.760 characters), conclusions (3.078 characters), practical recommendations (1.515 characters), a list of references, and a list of abbreviations. Chapter I (51.259 characters) contains the literature review; Chapter II (19.356 characters) presents the materials and methods. Chapters III (26.136 characters) and IV (20.639 characters) present the results of studies of cellular immunity, apoptotic regulation, and the inflammatory response in children with CHD. Chapter V (25.440 characters) presents the analysis of relationships and the comprehensive assessment of combined abnormalities, and Chapter VI (38.390 characters) discusses the obtained results. The dissertation is illustrated with 14 tables and 21 figures. The bibliography includes 170 sources.

MATERIALS AND METHODS OF THE RESEARCH

The study was designed as a comparative clinical-immunological study with a single assessment of the parameters at the preoperative stage. A total of 113 children were included: 90 patients with congenital heart defects and 23 practically healthy children in the control group. Among the children with CHD, 59 had acyanotic and 31 had cyanotic defects. All children with CHD were examined before surgical correction and had not previously undergone operative treatment.

The inclusion criteria for the study were:

- presence of a congenital heart defect confirmed by clinical and instrumental methods;
- no previous surgical correction of CHD;
- stable clinical condition at the time of examination;
- absence of an acute infectious-inflammatory disease;
- informed consent of parents or legal representatives.

The criteria for exclusion from the study were:

- previous surgical correction of CHD;
- acute infectious disease at the time of examination;

- established primary immunodeficiency, systemic autoimmune, or oncological disease;
- other severe concomitant diseases capable of affecting the studied parameters;
- absence of informed consent from parents or legal representatives;
- inability to obtain biological material meeting the study requirements.

The diagnosis of CHD and its clinical-hemodynamic variant were determined on the basis of clinical examination and echocardiography. The presence and severity of systemic hypoxemia were assessed by pulse oximetry. Electrocardiography was performed according to clinical indications.

The clinical and laboratory assessment included leukocyte count and the absolute and relative lymphocyte counts in peripheral blood. Flow cytometry on a BD FACSCalibur instrument (Becton Dickinson, USA) was used to determine the absolute and relative counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and $CD16^+/CD56^+$ cells, as well as $HLA\text{-}DR^+$ and $CD95^+$ cells. The CD4/CD8 immunoregulatory index was additionally calculated. Serum TNF-α concentration was determined by enzyme-linked immunosorbent assay and expressed in pg/mL. TNF-α was evaluated together with CD95 expression to characterize the relationship between inflammatory activity and Fas/CD95-dependent apoptotic regulation.

IL-6 concentration was determined by enzyme-linked immunosorbent assay in 20 children with CHD. The results were analyzed descriptively in the overall examined subgroup. Comparisons of IL-6 with the control group and between acyanotic and cyanotic CHD were not performed.

Quantitative variables were presented as mean and standard deviation (M±SD). One-way analysis of variance (ANOVA) was used for simultaneous comparison of three groups; parametric tests were used for pairwise comparisons, and Welch's t-test was applied when variances were unequal. Frequencies of categorical variables were compared using Fisher's exact test. Relationships among the studied parameters were assessed by Spearman rank correlation analysis. Differences were considered statistically significant at $p<0.05$.

The study was approved by the Ethics Committee of Azerbaijan Medical University, Protocol No. 19. Children participated after

informed consent had been obtained from their parents or legal representatives.

Table 1.

Demographic characteristics of the examined children by study group

Parameter	Control group (n=23)	Acyanotic CHD (n=59)	Cyanotic CHD (n=31)	Total (n=113)
Age, years, M±SD	8.84±3.85	5.51±5.02	4.46±4.81	5.90±4.96
Boys, n (%)	16 (69.6)	34 (57.6)	19 (61.3)	69 (61.1)
Girls, n (%)	7 (30.4)	25 (42.4)	12 (38.7)	44 (38.9)

Note. Quantitative data are presented as arithmetic mean and standard deviation (M±SD); categorical variables are presented as the absolute number of observations and the proportion within the corresponding group, n (%). CHD — congenital heart defects; SD — standard deviation.

RESEARCH RESULTS AND THEIR DISCUSSION

Features of cellular immunity in children with congenital heart defects

Investigation of cellular immunity in children with CHD at the preoperative stage revealed differences predominantly in the absolute counts of the major lymphocyte subpopulations. Comparative analysis was performed among practically healthy children (n=23), patients with acyanotic CHD (n=59), and patients with cyanotic CHD (n=31).

The absolute $CD3^+$ T-lymphocyte count was 1831.80±460.03 cells/μL in the control group, 2716.72±1147.24 cells/μL in acyanotic CHD, and 2780.86±1621.87 cells/μL in cyanotic CHD. The overall intergroup difference was statistically significant (p=0.0130). A similar pattern was observed for $CD4^+$ and $CD8^+$ cells: their absolute counts were 728.40±271.32 and 569.40±192.88 cells/μL in controls, 1135.30±567.96 and 875.54±439.34 cells/μL in acyanotic CHD, and 1201.00±895.77 and 887.91±627.55 cells/μL in cyanotic CHD, respectively. Overall intergroup differences were significant for $CD4^+$ (p=0.0273) and $CD8^+$ (p=0.0321).

Table 2.

T-cell immunity indices in children with congenital heart defects, M±SD

Parameter	Control (n=23)	Acyanotic CHD (n=59)	Cyanotic CHD (n=31)	p
$CD3^+$, cells/μL	1831.80± 460.03	2716.72± 1147.24*	2780.86± 1621.87*	0.0130
$CD3^+$, %	66.30±4.11	63.39±8.40	61.32±15.05	0.2649
$CD4^+$, cells/μL	728.40± 271.32	1135.30± 567.96*	1201.00± 895.77*	0.0273
$CD4^+$, %	37.95±6.39	40.93±6.70	40.41±10.09	0.3412
$CD8^+$, cells/μL	569.40± 192.88	875.54± 439.34*	887.91± 627.55	0.0321
$CD8^+$, %	30.05±4.31	32.50±8.75	30.77±8.34	0.4503
IRI (CD4/ CD8)	1.31±0.36	1.39±0.62	1.38±0.46	0.8450

Note. Data are presented as M±SD. p — significance level of the overall intergroup comparison (one-way ANOVA). * — statistically significant difference from the control group in pairwise comparison ($p<0.05$). IRI — CD4/CD8 immunoregulatory index; CHD — congenital heart defects.

The relative count of $CD3^+$ cells progressively decreased from 66.30±4.11% in the control group to 63.39±8.40% in acyanotic CHD and 61.32±15.05% in cyanotic CHD; however, the overall difference did not reach statistical significance (p=0.2649). Intergroup differences in the relative counts of $CD4^+$ and $CD8^+$ cells were also absent (p=0.3412 and p=0.4503, respectively). The CD4/CD8 immunoregulatory index was 1.31±0.36, 1.39±0.62, and 1.38±0.46, respectively (p=0.8450). Thus, higher absolute T-lymphocyte counts in children with CHD were not accompanied by statistically significant differences in their relative counts or in the CD4/CD8 ratio.

Similar features were found for B lymphocytes and NK cells. The absolute $CD19^+$ cell count was 526.45±179.44 cells/μL in the control group, 827.33±450.98 cells/μL in acyanotic CHD, and 918.45±485.13 cells/μL in cyanotic CHD (overall p=0.0072). Pairwise comparisons showed significant differences from control for both forms of CHD

(p=0.00026 and p=0.00149, respectively), whereas no difference was found between the two patient groups (p=0.463). The absolute $CD16^+/56^+$ NK-cell counts were 397.10±165.64, 663.09±399.68, and 640.59±374.92 cells/μL, respectively (overall p=0.0190). The parameter was significantly higher than control in both acyanotic (p=0.00030) and cyanotic CHD (p=0.00974), with no difference between the two forms (p=0.822).

Table 3.

Absolute and relative indices of $CD19^+$ B lymphocytes and $CD16^+/56^+$ NK cells in the study groups, M±SD

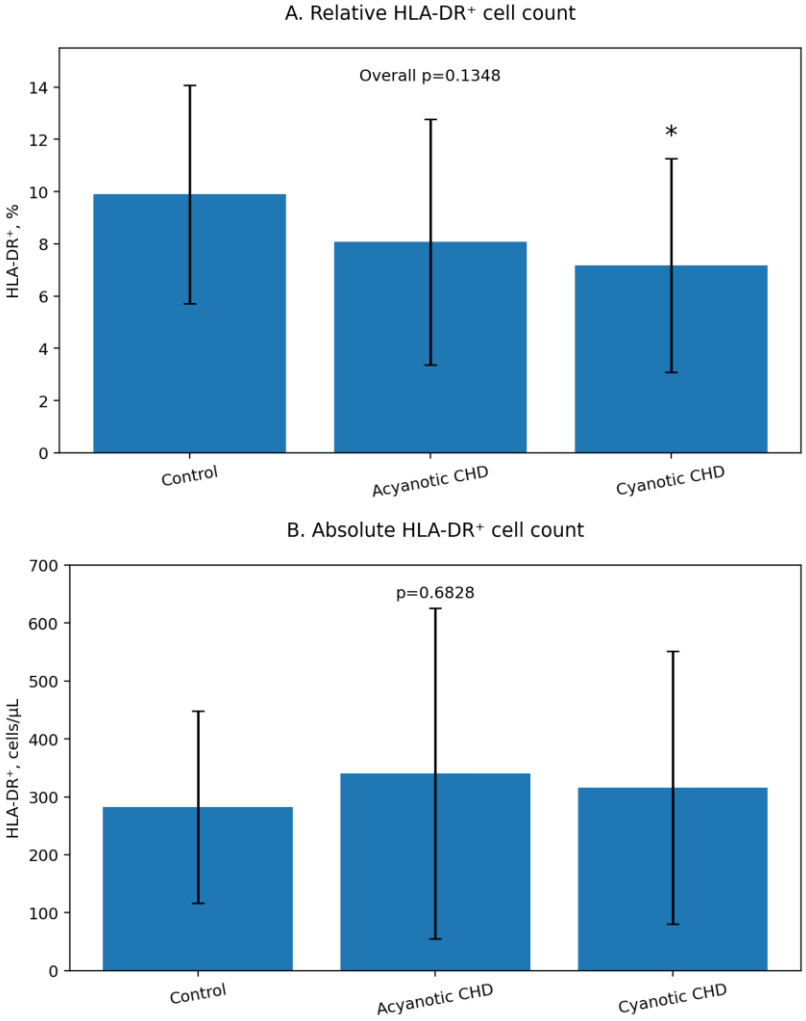
Parameter	Control (n=23)	Acyanotic CHD (n=59)	Cyanotic CHD (n=31)	p
$CD19^+$, cells/μL	526.45± 179.44	827.33± 450.98*	918.45± 485.13*	0.0072
$CD19^+$, %	19.40±4.37	19.15±6.63	21.95±12.53	0.3978
$CD16^+/56^+$, cells/μL	397.10± 165.64	663.09± 399.68*	640.59± 374.92*	0.0190
$CD16^+/56^+$, %	14.20±4.31	15.98±8.02	15.73±8.04	0.6589

Note. Data are presented as M±SD. p — significance level of the overall intergroup comparison (one-way ANOVA). * — statistically significant difference from the control group in pairwise comparison (p<0.05). CHD — congenital heart defects.

The relative counts of $CD19^+$ B lymphocytes and $CD16^+/56^+$ NK cells did not differ significantly between the groups (p=0.3978 and p=0.6589, respectively). Thus, as with T lymphocytes, changes in these cell populations were manifested predominantly in absolute indices. HLA-DR showed a different pattern from the major lymphocyte subpopulations.

The relative count of $HLA\text{-}DR^+$ cells was 9.90±4.18% in the control group, 8.07±4.71% in acyanotic CHD, and 7.18±4.09% in cyanotic CHD. The overall intergroup difference was not statistically significant (p=0.1348); however, pairwise comparison revealed a decrease in children with cyanotic CHD relative to control (p=0.0397). Differences between the control and acyanotic groups (p=0.1227), as well as between the two CHD forms (p=0.4319), did not reach statistical significance. The

absolute HLA-DR⁺ cell counts were 282.10±165.70, 340.48±285.23, and 316.23±235.50 cells/μL, respectively. No statistically significant differences were found in either the overall comparison (p=0.6828) or pairwise analyses (p>0.05).



Note. * — statistically significant difference from the control group (p=0.0397).

Figure 1. Relative (A) and absolute (B) HLA-DR⁺ cell indices in the study groups (M±SD)

Thus, in the examined cohort of children with CHD, higher absolute counts of the major T-, B-, and NK-cell populations were found in the absence of statistically significant differences in their relative counts and in the immunoregulatory index. An additional feature was a lower proportion of HLA-DR$^+$ cells in children with cyanotic CHD in pairwise comparison with control. These data emphasize the importance of joint evaluation of absolute and relative indices when characterizing immune status in children at the preoperative stage.

Features of apoptotic regulation and inflammatory response in children with congenital heart defects

Investigation of the Fas/CD95-dependent component of apoptotic regulation showed increased relative and absolute counts of CD95$^+$ cells in children with both forms of CHD. The relative CD95$^+$ cell count was 8.70±2.23% in the control group, 19.70±9.52% in acyanotic CHD, and 20.23±10.12% in cyanotic CHD. The overall intergroup difference and the pairwise differences of each CHD group from control were statistically significant ($p<0.001$). No difference was found between acyanotic and cyanotic CHD ($p=0.837$). The absolute CD95$^+$ cell counts were 242.50±95.89, 780.78±414.16, and 862.82±594.65 cells/μL, respectively. The overall intergroup difference and the differences of both CHD groups from control were statistically significant ($p<0.001$). Compared with control, the relative CD95$^+$ cell count was approximately 2.3-fold higher in both forms of CHD; the absolute count was 3.2-fold higher in acyanotic and 3.6-fold higher in cyanotic CHD.

The findings characterize altered Fas/CD95-dependent apoptotic regulation and increased apoptotic readiness of immunocompetent cells. At the same time, the CD95$^+$ cell count is not a direct quantitative measure of completed apoptosis. Assessment of TNF-α revealed higher mean cytokine concentrations in children with CHD. The value was 7.89±2.38 pg/mL in the control group, 34.60±52.84 pg/mL in acyanotic CHD, and 33.30±61.99 pg/mL in cyanotic CHD. Marked individual variability was reflected by the high standard deviations.

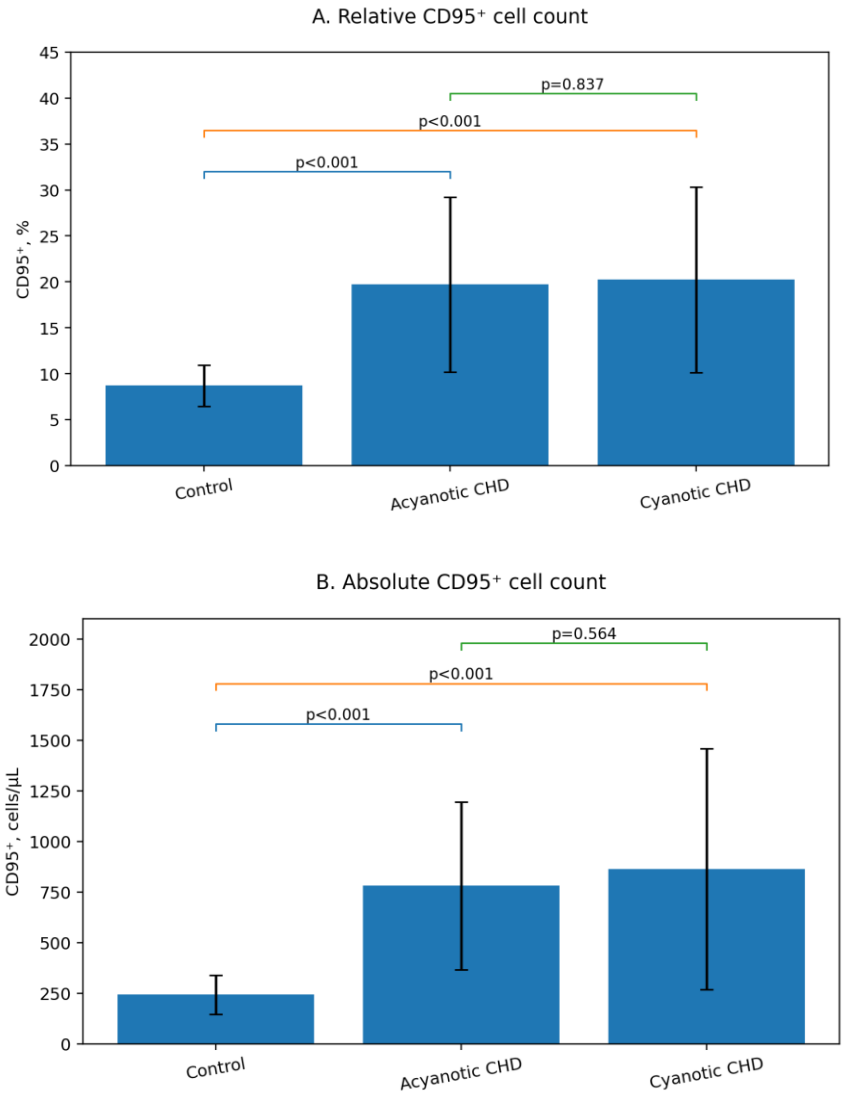


Figure 2. Relative (A) and absolute (B) CD95⁺ cell counts in the study groups (M±SD)

Note. * — statistically significant difference from the control group in pairwise comparison (p<0.001).

Table 4.

TNF-α concentration in the study groups (M±SD)

Parameter	Control (n=23)	Acyanotic CHD (n=59)	Cyanotic CHD (n=31)	p
TNF-α, pg/mL	7.89±2.38	34.60±52.84*	33.30±61.99*	0.0857

Note. Data are presented as M±SD. p — significance level of the overall intergroup comparison (one-way ANOVA). Pairwise comparison by Welch's test: control vs acyanotic CHD, p=0.00027; control vs cyanotic CHD, p=0.0298; acyanotic vs cyanotic CHD, p=0.9215. * — statistically significant difference from the control group (p<0.05).

The overall intergroup comparison for TNF-α did not reach statistical significance (p=0.0857). Nevertheless, pairwise analysis using Welch's test showed higher cytokine concentrations than control in both acyanotic (p=0.00027) and cyanotic CHD (p=0.0298). No difference was found between the two forms of the disease (p=0.9215). Thus, higher TNF-α values and increased $CD95^+$ cell counts were observed in both forms of CHD. Statistical relationships between individual parameters were assessed separately.

Analysis of the leukocyte–lymphocyte compartment showed that the most consistent differences concerned the absolute lymphocyte count. The value was 2723.90±694.25 cells/μL in the control group, 4346.11±1708.71 cells/μL in acyanotic CHD, and 4433.14±1961.30 cells/μL in cyanotic CHD (overall p=0.0006). In pairwise comparisons, the value was higher than control in both CHD groups (p<0.001 and p=0.0007, respectively), with no difference between the two forms (p=0.8595).

The relative lymphocyte count was 39.55±9.82% in the control group, 52.15±13.43% in acyanotic CHD, and 45.09±15.52% in cyanotic CHD (overall p=0.0019). A statistically significant increase relative to control was found in acyanotic CHD (p<0.001). Leukocyte counts were 7.08±1.65, 9.99±11.18, and $9.80\pm2.79\times10^9$/L, respectively. The overall intergroup difference was not statistically signi-

ficant (p=0.4034). Pairwise analysis showed a higher value in children with cyanotic CHD than in controls (p=0.0004). Differences between the acyanotic group and control (p=0.0910) and between the two CHD forms (p=0.9132) did not reach statistical significance.

Table 5.

Leukocyte–lymphocyte indices in the study groups (M±SD)

Parameter	Control (n=23)	Acyanotic CHD (n=59)	Cyanotic CHD (n=31)	p
Leukocytes, $\times 10^9$/L	7.08±1.65	9.99±11.18	9.80±2.79*	0.4034
Lymphocytes, %	39.55±9.82	52.15±13.43*	45.09±15.52	0.0019
Lymphocytes, cells/μL	2723.90± 694.25	4346.11± 1708.71*	4433.14± 1961.30*	0.0006

Note. Data are presented as M±SD. p — significance level of the overall intergroup comparison. * — statistically significant difference from the control group in pairwise comparison (p<0.05).

In addition, IL-6 concentration was determined in 20 children with CHD. The mean value was 9.99±8.01 pg/mL, the median was 10.10 pg/mL, and the range of individual values was 1.1–29.4 pg/mL. The results showed marked variability and were analyzed descriptively, without comparison with the control group and without stratification by clinical-hemodynamic form of CHD.

Thus, the examined children with CHD showed a combination of increased relative and absolute $CD95^+$ cell counts, higher TNF-α concentrations in pairwise comparison with control, and an increased absolute lymphocyte count. Most of these changes had the same direction in both forms of the disease. The findings provided the basis for subsequent analysis of relationships among the studied parameters and the frequency of their combined abnormalities.

Relationships among immunological, apoptotic, and inflammatory indices in children with congenital heart defects

Spearman rank correlation analysis was performed to investigate relationships among the studied parameters. Analysis of CD95

associations was conducted in 68 children with CHD for whom paired data were available. The absolute $CD95^+$ cell count positively correlated with the absolute lymphocyte count (r_s=0.481; p<0.001), leukocyte count (r_s=0.469; p<0.001), and absolute $HLA\text{-}DR^+$ cell count (r_s=0.466; p<0.001). Positive relationships were also identified with the absolute indices of $CD8^+$ (r_s=0.383; p=0.001), $CD3^+$ (r_s=0.377; p=0.002), $CD16/56^+$ (r_s=0.333; p=0.006), $CD19^+$ (r_s=0.298; p=0.014), and $CD4^+$ cells (r_s=0.281; p=0.020). Thus, the absolute $CD95^+$ cell count was related to quantitative characteristics of various lymphocyte subpopulations and the cellular composition of peripheral blood.

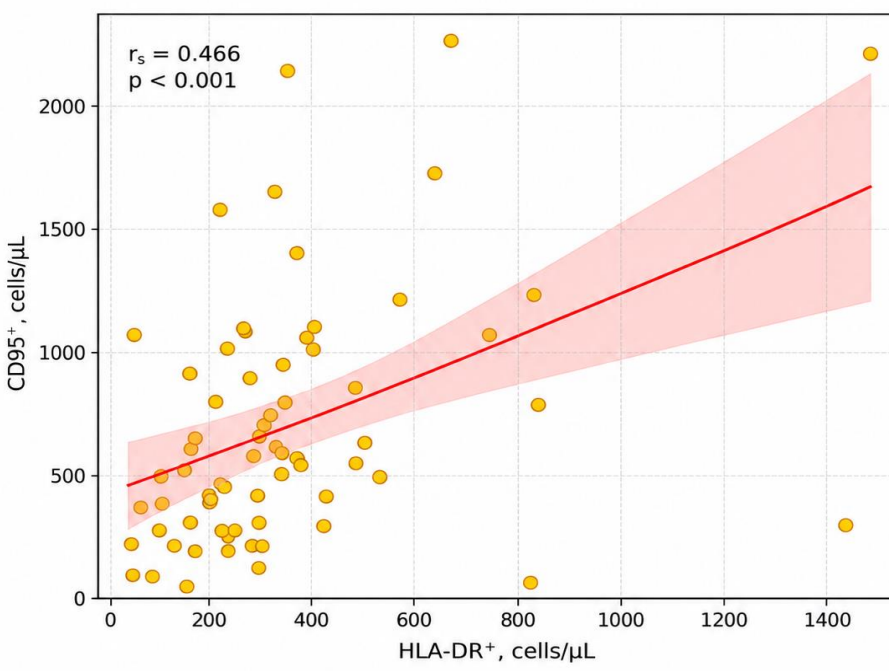


Figure 3. Correlation between the absolute counts of $CD95^+$ and $HLA\text{-}DR^+$ cells in children with CHD (r_s=0.466; p<0.001)

The relative $CD95^+$ cell count showed a different direction of individual associations. Negative correlations were found with the relative lymphocyte count ($r_s=-0.430$; $p<0.001$), the absolute $CD4^+$ ($r_s=-0.419$; $p<0.001$), $CD3^+$ ($r_s=-0.377$; $p=0.002$), and $CD19^+$ cell counts ($r_s=-0.326$; $p=0.007$), and the absolute lymphocyte count ($r_s=-0.309$; $p=0.010$). Positive associations were found with the absolute $CD95^+$ cell count ($r_s=0.618$; $p<0.001$) and the relative HLA-DR^+ cell count ($r_s=0.250$; $p=0.040$). The different directions of these correlations emphasize the need for separate interpretation of absolute and relative indices.

The strongest relationships within the T-cell compartment were found between the absolute counts of $CD3^+$ and $CD4^+$ cells ($r_s=0.946$), $CD3^+$ and $CD8^+$ cells ($r_s=0.844$), and $CD4^+$ and $CD8^+$ cells ($r_s=0.766$); $p<0.001$ in all cases. The absolute HLA-DR^+ cell count positively correlated with $CD8^+$ cells ($r_s=0.587$), lymphocytes ($r_s=0.581$), $CD3^+$ cells ($r_s=0.523$), and $CD4^+$ cells ($r_s=0.438$); $p<0.001$ in all cases. No statistically significant correlation between CD95 and TNF-α was found in the final sample ($p>0.05$). Therefore, although both parameters were increased relative to control, this was not accompanied by statistically confirmed concordance of their individual values in children with CHD. The identified cellular correlations characterize statistical associations and do not establish causal relationships.

Comprehensive assessment of combined abnormalities in the studied parameters

The comprehensive analysis was performed in 68 children with CHD who had a complete set of six parameters: relative and absolute $CD95^+$ cell counts, TNF-α, relative HLA-DR^+ cell count, leukocyte count, and absolute lymphocyte count. Research thresholds were determined from the distribution of control-group values. The 95th percentile was used for CD95%, absolute $CD95^+$ count, TNF-α, leukocyte count, and absolute lymphocyte count; the 5th percentile was used for HLA-DR%. These thresholds were applied exclusively for comparative characterization of combined abnormalities.

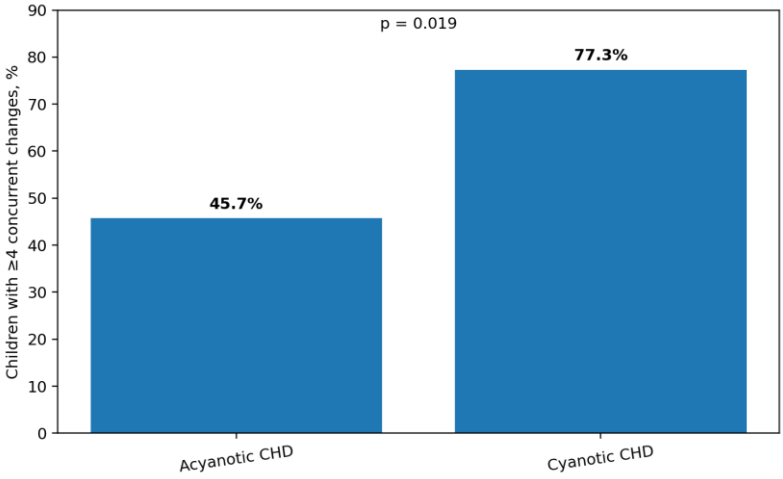
Table 6.

Frequency of altered immunological, inflammatory, and apoptotic indices in children with CHD

Parameter	Criterion of change	n	%
$CD95^+$, %	>12.0%	49	72.1
$CD95^+$, absolute	>376.85 cells/μL	62	91.2
TNF-α	>11.6 pg/mL	60	88.2
HLA-DR^+, %	<5.0%	14	20.6
Leukocytes	$>9.91\times10^9$/L	22	32.4
Lymphocytes, absolute	>3438 cells/μL	44	64.7

Values above the research threshold were most frequently observed for the absolute $CD95^+$ cell count — in 62 children (91.2%) — and TNF-α — in 60 children (88.2%). An increased relative $CD95^+$ cell count was found in 49 children (72.1%), an increased absolute lymphocyte count in 44 (64.7%), and an increased leukocyte count in 22 (32.4%). A relative HLA-DR^+ cell count below the established threshold was identified in 14 children (20.6%). Simultaneous alteration of four or more of the six parameters was found in 55.9% of the examined children. The frequency of this combination was 45.7% in acyanotic CHD and 77.3% in cyanotic CHD. The difference was statistically significant ($p=0.019$; Fisher's exact test).

Thus, the comprehensive analysis showed that a substantial proportion of children with CHD had simultaneous alterations in several immunological, inflammatory, and apoptotic parameters. The combined abnormalities were more frequent in cyanotic CHD. These findings complement the clinical and laboratory characterization of the baseline condition of the examined children; however, the applied research thresholds and the criterion of combined abnormalities are not independent diagnostic or prognostic tools.



Note. p=0.019; Fisher's exact test.

Figure 4. Frequency of combined alteration of four or more of the six studied parameters in children with acyanotic and cyanotic CHD

Conclusions

1. In children with CHD, the absolute counts of the major lymphocyte subpopulations were predominantly increased: $CD3^+$ (p=0.0130), $CD4^+$ (p=0.0273), $CD8^+$ (p=0.0321), $CD19^+$ (p=0.0072) and $CD16/56^+$ (p=0.0190), whereas most relative parameters and the CD4/CD8 ratio showed no significant changes. In cyanotic CHD, the relative count of $HLA\text{-}DR^+$ cells was lower than in controls — 7.18±4.09% versus 9.90±4.18% (p=0.0397) [9, 11, 13, 14].
2. In both forms of CHD, the relative and absolute counts of $CD95^+$ cells were increased (p<0.001). TNF-α levels were also higher than control values in pairwise comparisons: 34.60±52.84 and 33.30±61.99 versus 7.89±2.38 pg/mL (p=0.00027 and p=0.0298, respectively). No significant differences were found between the acyanotic and cyanotic forms. [1–3, 7–9, 11, 12].

3. In children with CHD, an increase in the absolute lymphocyte count (p=0.0006) and differences in the relative lymphocyte count (p=0.0019) were observed. In cyanotic CHD, the leukocyte count was higher than in controls (p=0.0004). In 20 children with CHD, the IL-6 level was 9.99±8.01 pg/mL and was assessed descriptively. [5, 8, 10, 13, 14].
4. Acyanotic and cyanotic CHD were characterized predominantly by changes in the same direction. Changes in ≥4 of 6 parameters were detected in 77.3% of children with cyanotic CHD and in 45.7% of those with acyanotic CHD (p=0.019). [9–11, 14,15].
5. Strong positive correlations were found between $CD3^+$ and $CD4^+$ (r_s=0.946), $CD3^+$ and $CD8^+$ (r_s=0.844), and $CD4^+$ and $CD8^+$ (r_s=0.766; p<0.001). The absolute $CD95^+$ cell count correlated with lymphocytes, leukocytes and HLA-DR^+ cells (r_s=0.466–0.481; p<0.001); no significant relationship was found between $CD95^+$ and TNF-α. [9, 11, 12].

PRACTICAL RECOMMENDATIONS

1. When assessing immune status in children with CHD, it is advisable to consider together the absolute and relative counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and $CD16/56^+$ cells, the CD4/CD8 index, and HLA-DR indices, because the identified differences predominantly involve absolute cell counts.
2. Within an expanded clinical and laboratory assessment, determination of CD95 and TNF-α may complement the characterization of apoptotic regulation and the inflammatory response. The results should be interpreted together with clinical data and should not be used as independent diagnostic or prognostic criteria.
3. Interpretation of the studied parameters should take into account the clinical-hemodynamic form of CHD and the possibility of combined abnormalities, which were more frequently identified in cyanotic defects. The applied parameter thresholds and the criterion of ≥4 of 6 abnormalities should be used exclusively for research comparison.

PUBLISHED SCIENTIFIC WORKS ON THE TOPIC OF THE DISSERTATION

1. Мамедова Л., Рзаев Э. Изменение мембранных маркеров апоптоза при врожденных пороках сердца у детей // Journal of Azerbaijan Allergy and Clinical Immunology. — 2019. — Vol. 7, № 2, специальный выпуск: материалы I Международной конференции «Immune System Disorders», Баку, 17–18 мая 2019 г. — С. 41.
2. Məmmədova L.V., Həsənov Ə.Q. Uşaqlarda anadangəlmə ürək qüsurları zamanı apoptoz // Azərbaycan Tibb Universitetinin Uşaq cərrahlığı kafedrasının yaranmasının 80 illiyinə həsr olunmuş uşaq cərrahiyyəsi üzrə elmi-praktik konqres materialları. — Bakı, 2019.
3. Мамедова Л.В., Гасанов А.Г. Апоптоз при врожденных пороках сердца // Journal of Azerbaijan Perinatology and Pediatrics. — 2020. — Vol. 6, № 1.
4. Hasanov A.Q., Mammadova L.V., Nasrullayeva G.M., Mammadova V.R. Cardiovascular system defects in children with primary immunodeficiency // Ulu Öndər Heydər Əliyevin 100 illik yubileyinə həsr edilmiş elmi-praktik seminarın materialları. — Bakı, 2023.
5. Məmmədova L. Anadangəlmə ürək qüsurları olan uşaqlarda TNF-α və İL 6-nın serum səviyyəsinin qiymətləndirilməsi // Azərbaycan Pediatriya Cəmiyyətinin I Beynəlxalq konqresi: qəbul olunmuş tezislər. — Bakı, 1–3 iyun 2023.
6. Nasrullayeva G.M., Mammadova L.V., Mammadova V.R. Frequency of congenital defects in children with primary immunodeficiency // Challenges and problems of modern science: Proceedings of the X International Scientific and Practical Conference, 19–20 October 2023. — London, United Kingdom: World of Conferences, 2023. — P. 90.
7. Hasanov A., Nasrullayeva G., Mammadova L., Mammadova V. Changes in membrane markers of apoptosis in congenital heart defects in children // Journal of Theoretical and Clinical Medicine. — 2023. — № 4. — P. 309.
8. Mammadova L.V. Tumor Necrosis Factor-A and Interleukin-6 in Blood Serum of Infants and Children with Congenital Heart

Diseases // Актуальні проблеми сучасної медицини: Вісник Української медичної стоматологічної академії. — 2024. — Т. 24, вип. 1(85). — DOI: 10.31718/2077-1096.24.1.17.
9. Məmmədova L.V. Anadangəlmə ürək qüsurlarında immun sistem pozuntuları və apoptoz markerlərinin qarşılıqlı əlaqəsi: klinik və biomolekulyar təhlil // Azərbaycan təbabətinin müasir nailiyyətləri. — 2025. — № 1. — S. 104–109.
10. Мамедова Л.В., Султанова Н.Г., Гасанов А.Г., Сулейманлі А.А. Маркери запалення у дітей із вродженими вадами серця: порівняльний аналіз за рівнем імунної активації // Світ медицини та біології. — 2025. — № 2(92).
11. Mammadova L.V., Sultanova N.H., Hasanov A.G., Suleymanli A.A. Immunological and Apoptotic Disturbances in Children with Congenital Heart Defects: Pathogenetic Aspects and Diagnostic Significance // Modern Pediatrics. Ukraine. — 2025. — № 3(147).
12. Mamedova L.V. Apoptosis and inflammatory markers in children with congenital heart defects: the role of CD95 and TNF-α // Current Issues of Medicine, Pharmacology, Therapy and Rehabilitation: International Scientific and Practical Conference. — Ivano-Frankivsk–Kherson, Ukraine, May 23–24, 2025.
13. Мамедова Л.В. Иммунный профиль и воспалительные маркеры у детей с врождёнными пороками сердца // Современная медицина: новые подходы и актуальные исследования: сборник статей по материалам XCVI Международной научно-практической конференции. — Москва: Интернаука, 2025. — № 5(87).
14. Мамедова Л.В., Султанова Н.Г., Гасанов А.Г., Сулейманлы А.А. Иммунная недостаточность и цитокиновая активность: сравнительный анализ врожденных цианотических и ацианотических пороков сердца // Медицинские новости. — 2025. — № 6.
15. Qasımov A.Q., Məmmədova L.V., Səfərova İ.A., Eynalova M.K. Uşaqlarda anadangəlmə ürək qüsurları zamanı xronik hipoksiya və immunopatoloji profil // Elmi Məqalələr. — 2025. — Cild 5, № 2. — DOI: 10.28942/atuj.v5i2y2025.130.

LIST OF ABBREVIATIONS

CHD	— congenital heart defects
CD	— Cluster of Differentiation
CD3	— general T-lymphocyte marker
CD4	— T-helper cells
CD8	— cytotoxic T lymphocytes
CD16/56	— marker of natural killer (NK) cells
CD19	— B-lymphocyte marker
CD95 (Fas)	— apoptosis receptor
ELISA	— Enzyme-Linked Immunosorbent Assay
HLA-DR	— Human Leukocyte Antigen-DR
IL-6	— interleukin-6
IRI	— immunoregulatory index
Leu	— leukocytes
NK	— Natural Killer cells
SD	— Standard Deviation
SPSS	— Statistical Package for the Social Sciences
TNF-α	— Tumor Necrosis Factor-alpha
WHO	— World Health Organization



The defense will be held on 25 November 2026 at 14:00 at the meeting of the Dissertation council FD 2.27 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Azerbaijan Medical University.

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The dissertation is accessible at the Library of the Azerbaijan Medical University.

Electronic version of the abstract is available on the official website of the Azerbaijan Medical University (http://www.amu.edu.az).

Abstract was sent to the required addresses on
“20” October 2026.

Signed for printing: 21.09.2026
Paper format: 60×84 1/16
Volume: 36.890 characters
Order: 306
Number of hard copies: 20
“Tabib” publishing house