

UDC: 616.24-002.5:615.015.8]-085.281-078.73-092-036

DOI: 10.15587/2706-5448.2023.291226

## IMMUNOPATHOLOGICAL RESPONSE OF THE BODY IN PATIENTS WITH CHEMOSENSITIVE AND DRUG-RESISTANT PULMONARY TUBERCULOSIS

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*Immunological methods are important for diagnosing tuberculosis, evaluating the process activity, and forecasting the course of the disease and recovery.*

**Materials and methods.** 47 patients with first diagnosed destructive sputum smear-positive pulmonary tuberculosis underwent a complex immunoassay. The patients were divided into two groups based on the sensitivity/resistance of mycobacterium tuberculosis to antimycobacterial agents. The first group consisted of 22 patients with first-diagnosed chemosensitive tuberculosis with preserved sensitivity to antimycobacterial agents. The second group consisted of 25 patients with multi-drug resistant tuberculosis pulmonary tuberculosis (MDR-TBP). The research was conducted during the 2018-2021 years.

**Results** Specific cell response disorders in patients with pulmonary tuberculosis are associated with the multi-structural T-cell protection imbalance caused by the quantitative changes of its components, the increase/decrease in the quantity of certain lymphocyte pools specifying the immune response vector.

*In cases of tuberculosis, phagocytosis plays an important role. Phagocytosis might release cells from the tuberculosis pathogen. To achieve this, the activation of cells should reach a certain level. However, the initial protective nature of cell activation might become aggressive.*

*The T-cell immunity disorders were more evident in patients with MDR-TBP versus donors and patients with chemosensitive tuberculosis. The apparent decrease in CD3+CD56+, CD3+CD4+ pools and the increase in CD3+CD8+ were revealed in cases of MDR-TBP tuberculosis versus chemosensitive tuberculosis. The difference in CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+, CD3+CD8+HLA-DR+, CD16/56+8+ between the study and observational groups was statistically confirmed. The evident specific cell immunity disorders in patients with MDR-TBP aggravate the clinical course of the disease, causing destructive changes and acute and extensive processes.*

**Conclusions** Changes in different components of the immune system might occur during pulmonary tuberculosis (in T- and B-cells, phagocytic cells), specific and enzymatic processes are activated, and autoimmunization is evident. The intensity of the changes varies at different stages of the disease. Most immune disorders caused by the specific inflammation process require immune correction

**Keywords:** immune responsiveness, phagocytosis, T- and B-cell immunity, multi-drug resistant and chemosensitive tuberculosis

### How to cite:

Platonova, I., Lapovets, L., Zubchenko, S., Sakhelashvili, M. (2023). Immunopathological response of the body in patients with chemosensitive and drug-resistant pulmonary tuberculosis. ScienceRise: Medical Science, 4 (55), 17–23. doi: <http://doi.org/10.15587/2706-5448.2023.291226>

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### 1. Introduction

The current situation with the tuberculosis epidemic in Ukraine has still been unfavourable. At present, the TB epidemic has two features. Firstly, the disease incidence rate has been interrelated with the spread of HIV/AIDS and drug addiction. Secondly, the rate of drug-resistant forms is high: up to 30 % and 75 % (primary and secondary cases correspondingly) in different regions. Multi-drug resistant strains prevail in the first diagnosed and recurrent cases, while mono-drug and poly-drug resistance occur much less frequently [1, 2].

The spread of tuberculosis in Ukraine, first of all, MDR-TBL and extensively drug-resistant (XDR-TB) forms as the most dangerous, results in the loss of labour capacity, poor health of the Ukrainians, increase in disability and mortality rates being interrelated with poverty and social inequality issues [3, 4].

Drug-resistant TB forms have been revealed in all countries all over the world. Consequently, drug resistance has been acknowledged as a global issue. MDR-TBP is one of the most severe forms of the disease that might cause a considerable epidemic danger. According to WHO data published in 2016, MDR-TBP forms were

revealed in 3.7 % and 21.0 % of patients with the first and recurrent diseases correspondingly. Extensive drug resistance of MTB and resistance to fluoroquinolones were evident in 9 % and 14.7 % of patients with MDR-TBP, correspondingly [3].

Favourable conditions for the selection of drug-resistant MBT (the lack of appropriate and monitored treatment, gaps in treatment, poor isolation of patients in hospitals, and nosocomial contamination) are some of the main factors causing the MDR tuberculosis epidemic. Along with that, there are specific conditions for the contamination of patients with drug-resistant MBT strains. Some foreign researchers emphasize that biological risk factors are one of the drivers of the global spread of MDR-TBP. The mycobacteria are more virulent and contagious. Histological research made by I. V. Liskina and coauthors [5], revealed a long-lasting progression in the lung tissue of patients with chronic TB. Typical rod-shaped MBT forms were revealed to be relatively rare, whereas cocci-shaped TB bacteria prevailed. This might be due to the therapeutic pathomorphosis of the pathogen and disease. Treatment gaps, the patients' attitude towards their health, low drug doses, poor implementation of the recommended treatment strategy, low quality of antitubercular medications are equally important triggers.

The low concentration of antitubercular medications in the specific inflammation site is one of the reasons for drug-resistant strains. The vascularization of affected lungs containing a considerable amount of MBT is poor. At the same time, mycobacteria are usually localized in caverns enforced with a fibrous tissue. Regular therapy with oral antitubercular medications cannot ensure the sufficient penetration of therapeutic concentrations in the affected site.

Immunological methods are important for diagnosing tuberculosis, evaluating the process activity, and forecasting the course of the disease and recovery. Tuberculosis belongs to the group of diseases with granulomatous chronic inflammation in the immune system caused by the long-lasting response to the persistent tuberculosis pathogen in organs and tissues. Immunocompetent cells play the main role in antituberculosis protection, making a specific granuloma which isolates the infection agent and prevents its impact [6]. The interrelation between the immune-competent cells with CD4+ (T-helpers/inductors – Th-1, tissue and wandering macrophages) and cytolytic CD8+ lymphocytes is the central point in antituberculosis protection. Stimulated CD4+ lymphocytes (Th-1) decree macrophage cytokines (gamma interferon, interleukins – IL-2 and IL-12) that activate phagocytosis of macrophages and monocytes [7, 8].

In cases of tuberculosis, phagocytosis plays an important role. Phagocytosis might release cells from the tuberculosis pathogen. To achieve this, the activation of cells should reach a certain level. However, the initial protective nature of cell activation might become aggressive.

Th-0 cell subpopulations generate the main cytokines of both Th-1 and Th-2 types and are considered to be Th-1 and Th-2 predecessors. The dynamic balance of Th-1 and Th-2 functions ensure a significant flexibility of the immune response. Types 1 and 2 immunity do not strictly correspond to cell-mediated and humoral immun-

ity, as far as Th-1 encourages a moderate antibody formation, while Th-2 actively suppresses phagocytosis [9].

Pulmonary tuberculosis causes changes in different components of the immune system (T- and B-cells, phagocytes), activates specific and enzymatic processes and leads to autoimmunization. The intensity of the changes varies at different stages of the disease. Most immune disorders caused by the specific inflammation process require immune correction.

TB belongs to the interleukin-related immune deficiency, with the evident changes in cytokines disturbing the quantitative balance of regulatory T-cells subpopulations. However, there have been few research papers about the immune system in cases of MDR-TBP so far. The interrelation between the course of disease and the immune system has been understudied.

**The aim of the study.** The study of the immune responsiveness of patients with chemosensitive and multi-drug resistant tuberculosis pulmonary (MDR-TBP).

## 2. Materials and methods

The immune response of 47 patients with first diagnosed destructive sputum smear-positive pulmonary tuberculosis was studied. The patients were divided into two groups based on the sensitivity/resistance of mycobacterium tuberculosis to antimycobacterial agents. The first group consisted of 22 patients with first-diagnosed chemosensitive tuberculosis with preserved sensitivity to antimycobacterial agents. The second group consisted of 25 patients with MDR-TBP. The examination was performed during the patient's admission to Lviv Regional Phthisiopulmonology Clinical Treatment and Diagnostic Center, Municipal Non-Commercial Enterprise within Lviv Regional Council. Research was conducted during 2018-2021 years.

At its meeting, the Commission on Bioethics at the Lviv National Medical University named after Danylo Halytskyi, reached a consensus that during the execution of the article submitted for examination by I. Platonova, et al. «Immunopathological response of the body in patients with chemosensitive and drug-resistant pulmonary tuberculosis» the safety rules for the health of the examinees were observed, the rights and canons were preserved the human dignity of patients, as well as moral and ethical norms in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and the relevant laws of Ukraine (protocol No. 2 dated February 22, 2021 and Protocol No. 8 dated August 31, 2023.)

Phagocytic protection was evaluated based on the total quantity of leukocytes, neutrophil phagocytic rate (phagocytic number and phagocytic index), oxidation-reduction of neutrophils (NBT test) and cationic lysosomal granulocyte proteins. The indicators mentioned above were evaluated by applying the standard procedures (Fig. 1).

The study of T-cell immunity in TB patients was made based on the lymphocyte populations and subpopulations (CD3+, CD3+CD56+, CD3+HLA-DR+, CD3+CD4+, CD4+45RA+, CD3+CD8+, CD4/CD8+, CD16/56+, CD16/56+CD8+) by means of the direct immunofluorescence with the use of anti-CD-monoclonal

antibodies with further identification of lymphocyte surface structures applying a fluorescence-based flow cytometry device FACScan BD Bioscience (the USA) in the licensed medical laboratory Dila involving erythrocyte diagnostic absorbed on the surface by the relevant anti-CD-monoclonal antibodies (Hranum, LLC, scientific and production laboratory, Kharkiv, Ukraine) microscopically. 28 (41.8 %) and 25 (32.1 %) patients from the study and observational groups correspondingly were examined. The level of cytokines IL-6, IL-10 i TNF- $\alpha$  was measured in the licensed medical laboratory Dila.

The B-cells were evaluated based on CD19+ and immunoglobulins IgA, IgM, IgG. The functions of B-cells were evaluated based on the level of serum immunoglobulins IgA, IgM and IgG by performing the enzyme-linked immunosorbent assay using Xema-Medica test systems (Ukraine) and fixing the results with the  $\mu$ Quant spectrophotometer (BioTek, the USA), measurement range is 200–999 nm, error  $\pm 1.0$  %.

The concentration of circulating immune complexes was measured by applying the

Polyethylene Glycol Precipitation and Xema-Medica test systems (Ukraine). The results were recorded with the  $\mu$ Quant spectrophotometer (BioTek, the USA), a measurement range of 200–999 nm, with an error  $\pm 1.0$  %.

Mantoux test (MT) with 2TE PPD-L was made in compliance with the guidelines for tuberculin tests. The positive tuberculin testing results: low-positive

(5–11 mm papule), high-positive (12–16 mm papule) and, hyperergic (17 mm and more papule), papule with vesicles and necrosis.

For the statistical analysis of the raw data, the software for mathematical calculations, their graphic representation and analysis results were used in Excel with the Microsoft Office application package [10]. For the statistical analysis of the raw data, the software for mathematical calculations, their graphic representation and analysis results were used in Excel with the Microsoft Office application package [10]. Statistical processing of the research results was performed using the methods of parametric (variational) statistics in compliance with the conditions for estimating the type of distribution. The results are presented in the form of the average statistical value of the indicator and the error of the average  $M \pm m$ . The probability of the results obtained was evaluated using Student's criterion and Mann-Whitney. The STATISTICA 2006 computer software package was used for statistical processing of the material.

### 3. Results

Phagocytic protection disorders in patients relative to donors differed in intensity and type of changes. The suppression of the neutrophil phagocytic rate was revealed in patients with the first diagnosed pulmonary tuberculosis (Fig. 1).

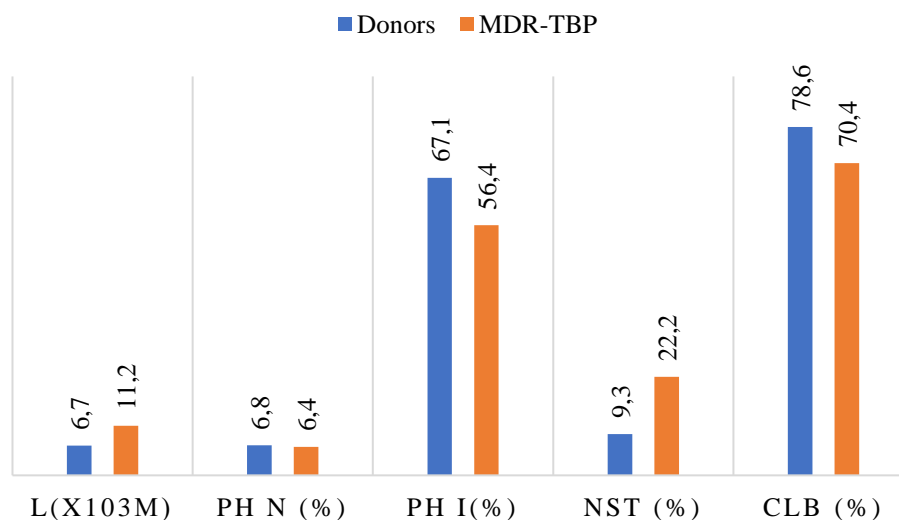


Fig. 1. Indicators of blood phagocytic activity

In patients with newly MDR-TBP, inhibition of the phagocytic activity of neutrophils was noted, while phagocytosis was activated in patients with chemosensitive tuberculosis. At the beginning of treatment, a number of significant disorders of immune hemostasis were found in patients with MDR-TB of the lungs. It was established that the specific inflammation caused by multi-drug resistant strains of MBT was accompanied by pronounced leukocytosis, which was  $(11.2 \pm 0.5) \times 10^3/\mu\text{l}$  versus  $(6.7 \pm 0.5) \times 10^3/\mu\text{l}$  in donors ( $p < 0,01$ ); functional disorders of phagocytic protection with a decrease in the number of FI to  $(56.4 \pm 2.3)$  % compared to the norm

$(67.1 \pm 3.1)$  % ( $p < 0.01$ ); CLB insufficiency –  $(70.4 \pm 1.5)$  % and  $(78.6 \pm 2.4)$  %, respectively ( $p < 0.05$ ); a sharp increase in the redox processes of neutrophil leukocytes, NST –  $(22.2 \pm 0.9)$  % against the norm  $(9.3 \pm 0.8)$  % ( $p < 0.01$ ).

The suppression of neutrophil phagocytic rate in cases of MDR-TBP was 1.2 times less versus chemosensitive tuberculosis, phagocytic index; the engulfing properties were 2.5 times lower, the quantity of cationic lysosomal proteins involved in the intracellular proteolysis and oxygen-dependent metabolism leading to the formation of hydrogen dioxide and superoxide anion, was by 1.3 times less.

Thus, the research revealed some immune pathogenetic features typical for MDR-TBP. In particular, the suppression of the phagocytic component of innate immunity manifested as the less quantity of phagocytic cells (phagocytic index), their suppressed engulfing properties (phagocytic number), the reduction of cationic lysosomal proteins, intensified oxygen-dependent metabolism as compared to the donors and patients with chemosensitive tuberculosis.

The T-cell immunity disorders were more evident in patients with MDR-TBP versus donors and patients with chemosensitive tuberculosis. CD3+CD56+, CD3+CD4+ pool was below target and CD3+CD8+ pool was higher in cases of MDR-TBP versus chemosensitive tuberculosis. The statistically confirmed difference in CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+, CD3+CD8+HLA-DR+, CD16/56+8+ between the study and observational groups was estimated. The difference rate varied from 1.2 to 1.5 (17.8 %–31.7 %).

The revealed difference between the groups pointed at the suppressed immune responses in cases of MDR-TBP (difference rate 27.1 % (by 1.4 times)) and the significant suppression of intercellular cooperation among antigen-presenting cells due to the decrease in populations with the expression of HLA-DR+antigens by 1.5 times and boost in non-specific protection caused by the increase in activated CD16/56+8+ populations by 1.2 times. The evident specific cell immunity disorders in patients with MDR-TBP aggravate the clinical course of the disease, causing destructive changes and acute and extensive processes.

This was due to the decrease in CD3+CD4+ fraction in the observational and study groups up to (33.8±2.1) % and (30.4±1.8) % versus (41.0±2.7) % in healthy people ( $p<0.05$ ) and the increase in CD8+ – (29.4±1.7) % ( $p>0.05$ ) and (36.3±1.5) % versus (28.5±1.4) % correspondingly ( $p<0.05$ ).

The quantity of T-cells was less in TB patients regardless of the pathogen sensitivity/resistance. The quantity of T-cells with CD3+ antigen expression was (63.4±2.2) % and (61.2±3.1) % in the observational and study groups versus (71.5±2.5) % in healthy people ( $p<0.05$ ).

The loss of dynamic balance between T-helpers CD4+ and suppressor/cytotoxic lymphocytes CD8+ with prevailing suppressed cell-mediated response was observed during the specific process.

The increase in T-suppressor/cytotoxic lymphocytes CD3+CD8+ was apparent for healthy people and for patients with MDR-TBP. The suppressed cell-mediated response was more evident in the study group as proved by IPI (CD4+/CD8+) being by 1.24 and 1.75 times less than in the observational group and healthy people correspondingly, comprising (0.92±0.07) versus (1.13±0.1) ( $p<0.05$ ) and (1.61±0.17) ( $p<0.05$ ).

The immunological assays made in both groups of patients showed that T-cell immune deficiency manifested as a decrease in T-cells and the suppression of their

functions. One should note that most evident T-cell immune deficiencies with the decrease in T-cells and the suppression of their functions were by 1.3 times more often revealed in the patients with MDR-TBP – 72.0 % (18) as compared to the patients affected by drug sensitive strains of mycobacterium tuberculosis – 55.0 % (11).

The results of the T cell-immunity assays in patients with chemosensitive and

MDR-TBP are available in Table 1. In most cases, T-cells are involved in the immune response to natural antigens, including contagious ones. T-helpers are involved in its initial stage – the antigen recognition. They encourage the humoral immune response. A decrease in the T-helper population was observed in TB patients. The apparent decrease in CD3+CD4+ was revealed only in the study group, being by 1.3 and 1.2 times less than in the donors and observational group correspondingly, comprising (28.5±2.0) % versus (36.5±1.8) % in healthy people ( $p<0.05$ ) and (34.1±1.4) % in the observational group,  $p<0.05$ . CD3+CD4+ in the patients from the observational group were within the confidence interval ( $p>0.05$ ).

Uncommitted T-helpers CD4+45RA+ make another subpopulation of

T-helpers involved in the antigen recognition. During activation, the uncommitted

T-helpers with CD4+45RA antigen expression migrate to the primary T-cell zone where they interrelate with antigen-presenting cells, recognize the antigen and are differentiated in effector memory T-helpers CD4+45RA–CD45R0+.

The study of the cell population revealed its decrease by 1.2 times below the target in both groups,  $p_1<0.01$ ,  $p_2<0.05$ .

The difference in CD4+45RA+ between the patients with chemosensitivity and MDR-TBP was not confirmed. The decrease in CD4+45RA+ population during the acute inflammation is not indicative of significant immune disorders and might be due to the migration of cells to the specific inflammation site.

T-cells with CD8+ antigen expression (suppressor/cytotoxic T-cells) are functional antagonists to CD3+CD4+. CD3+CD8+ subpopulation consists of a small fraction of cytotoxic T-cells and T-suppressors inhibiting the activity of T-helpers and slowing down humoral immune response. Misbalance with the prevailing suppressed immune response (based on CD3+CD8+ and especially IPI CD3+CD4+/CD3+CD8+) is evident in patients with chemosensitivity and MDR-TBP. These processes are much more active in patients with MDR-TBP. In particular, CD3+CD8+ was (36.5±1.4) % and (32.0±1.1) % ( $p<0.05$ ) in the patients from the study and observational groups correspondingly, versus (30.1±0.9) % in healthy people,  $p_1<0.05$ ,  $p_2>0.05$ . In cases of chemosensitive and MDR-TBP, IPI was 1.7 and 1.2 times lower as compared to the donors, comprising (0.78±0.10) and (1.07±0.08),  $p<0.05$  versus (1.32±0.10) correspondingly,  $p_1<0.01$ ,  $p_2<0.05$ .

Table 1

The population and subpopulation of lymphocytes in the blood of patients with chemosensitive and multi-drug resistant pulmonary tuberculosis

Groups/Indicators	Examined groups		
	Healthy people (n=15)	Observational group (n=13)	Study group (n=12)
Lymphocytes (%)	37.7±1.5	31.4±1.6*	32.1±1.8*
T-lymphocytes CD3+(%)	66.5±3.3	55.2±2.1*	52.8±2.7*
Activated T-lymphocytes CD3+HLA-DR+(%)	13.0±0.8	11.7±0.9	12.3±0.7
Cytotoxic T-lymphocytes CD3+CD56+(%)	5.0±0.2	4.5±0.5	3.7±0.3*
T-helpers CD3+CD4+(%)	36.5±1.8	34.1±1.4	28.5±2.0*#
Activated T-helpers CD3+CD4+HLA-DR+(%)	4.6±0.5	2.7±0.3*	3.1±0.4*
Uncommitted T-helpers CD4+45RA+(%)	61.0±3.1	52.5±2.4*	49.0±3.2*
T-suppressor/cytotoxic CD3+CD8+(%)	30.1±0.9	32.0±1.1	36.5±1.4*#
Activated T-cytotoxic lymphocytes CD3+CD8+HLA-DR+(%)	6.5±0.7	4.1±0.3*	2.8±0.5*#
The correlation of CD3+CD4+/CD3+CD8+(IPI Tx/Tc)	1.32±0.10	1.07±0.08*	0.78±0.10*#
Natural killers CD3-CD16/56+(%)	14.6±1.2	14.0±1.7	15.2±1.5
Activated natural killers CD16/56+8+(%)	22.5±2.3	39.3±2.0*	47.9±2.6*#

Note: \* – the difference is possible for the healthy people ( $p < 0.05-0.001$ ); # – the difference is possible for the observational group ( $p < 0.05-0.01$ )

The study of the cytotoxic T-cells fraction in CD3+CD8+ population structure, in particular, their functionally activated cells CD3+CD8+HLA-DR+ pointed at their smaller quantity as compared to healthy people, patients with chemosensitive and MDR-TBP,  $p_1 < 0.05$ ,  $p_2 < 0.01$ . However, the CD3+CD8+HLA-DR+ population was 1.5 times less in the study group as compared to the observational group,  $p < 0.05$ .

The insufficient mycobacterial antigen-specific cell response in TB patients was accompanied by the activation of antigen-independent cell defence mechanisms with the important role of natural killers, NK-cells with the expression of CD3-CD16/56+ receptor, their activated elements – activated natural killers CD16/56+8+. Along with normal findings of CD3+CD16/56+, the increase in the fraction of activated NK cells CD3+CD16/56+ was revealed in the TB patients. The CD3-CD16/56+ population significantly increased as compared to healthy people (by 1.8 times  $p < 0.05$  and 2.1 times  $p < 0.01$  in the observational and study groups correspondingly). The quantity of activated NK-cells in cases of MDR-TBP was 1.2 times larger than in patients with chemosensitive tuberculosis,  $p < 0.05$ .

The analysis of the humoral immunity (Table 2) showed that a more intensive antibody and immune complex formation was typical for patients with MDR-TBP with the statistically confirmed difference in IgA, IgG, and immune circulating complex. Consequently, IgA in the blood was (5.03±0.10) g/l in the study group versus (4.57±0.09) g/l in the observational group ( $p < 0.05$ ), and (1.88±0.11) g/l in donors ( $p < 0.01$ ) and (62.5±9.4) international units in donors ( $p < 0.01$ ). Im-

mune circulating complex (195.8±7.3) versus (171.6±8.1) optical density, ( $p < 0.05$ ) and (78.1±5.6) optical density correspondingly, ( $p < 0.01$ ). Moreover, IgM and IgG antibody formation was 1.3 times more intensive in patients with MDR-TBP as compared to the ones with chemosensitive tuberculosis – 66.7 % (16) versus 50.0 % (11) and 91.7 % (22) versus 68.2 % (15).

Thus, more intensive antibody and immune complex formation were evident in patients with MDR-TBP with the statistically confirmed difference as compared to chemosensitive tuberculosis patients (IgA, immune circulating complex). Different types of disimmunoglobulinemia, with the intense formation of certain immunoglobulin antibodies and the suppression of synthesis, were evident in 50.0 % and 45.5 % of patients from the study and observational groups respectively.

The analysis of the specific immune response proved that 52.4 % (11) and 33.3 % (8) patients from the observational and study groups correspondingly made a well-balanced immune response to the mycobacterial antigen triggering the growth of specific T-cells and intensifying their proliferative activity. The dysfunctional disorders of the specific protection were revealed in 38.1 % (8) and 54.2 % patients with chemosensitive and MDR-TBP correspondingly. 9.5 % of examined patients with an active specific process caused by mycobacterium strains sensitive to antimycobacterial agents had the normal quantity of lymphocytes sensitive to PPD-L provided that their functional activity was boosted. The number of T-cells sensitive to tuberculin increased in 28.6 % and 54.2 % of patients from the observational and study groups, respectively. However, their proliferative activity was not hardly observed.

Table 2

Humoral response indicators of patients with pulmonary tuberculosis caused by sensitive and multi-drug resistant strains of mycobacterium tuberculosis

Indicators	Groups		
	Donors, n=30 (M±m)	Observational, n=22 (M±m)	Study, n=25 (M±m)
Ig A(g/l)	1.88±0.11	4.57±0.09*	5.03±0.10*■
Ig M(g/l)	1.15±0.09	2.24±0.11*	2.41±0.09*
Ig G (g/l)	12.80±1.50	21.40±1.35*	22.73±1.52*
Circulating immune complex (optical-density units)	78.1±5.6	171.6±8.1*	195.8±7.3*■

Note: \* – the difference is reliable for the group of donors ( $p<0.05-0.01$ ); ■ – the difference is reliable for the patients with chemosensitive tuberculosis ( $p<0.05-0.01$ )

The suppression or total absence of lymphocyte proliferative response to PPD-L might point at the deficiency of antigenic cellular and membrane structures that become inert to mycobacterium tuberculosis, delaying the specific immune response and leading to specific T-cell anergy. The study proves the interrelation between the tuberculin anergy and a more severe tuberculous process [14, 95]. It is worth noting that absolute tuberculin anergy was by 1.3 times more often revealed in cases of MDR-TBP (12.5 %) as compared to chemosensitive tuberculosis (9.5 %).

The reveal of specific T-cell tuberculin anergy might not only specify the course of the specific inflammation but also predict the duration and efficiency of the specific treatment. T-cell mediated immunosuppression in patients with pulmonary tuberculosis was accompanied by the activation of B-cells with the intensified generation of immunoglobulins and increased circulating immune complex in blood, regardless of the pathogen sensitivity/resistance.

#### 4. Discussion of research results

The excessive activation of antigen-independent cell response and the insufficient specific cell response in cases of active tuberculosis suppress the formation of a specific granuloma. At the same time, natural killers, specific for the cytokine storm and lysis of infected cells, destroy the lung tissue. The hyperactive antigen-independent cell response aggravates the clinical course of the disease and provides a favourable environment for acute, progressing, destructive and extensive processes. The results of our research coincide with the ones of other authors [8, 9].

In cases of pulmonary tuberculosis, specific cell response disorders are due to the unbalanced MDR-TBP-structural T-cell protection system caused by the quantitative changes of its components, increase/decrease in some lymphocyte pools specifying the immune response vector. In general, tuberculosis was accompanied by T-cell immune deficiency with the predominant immunosuppressed responses, the activation of non-specific cell protection, and cellular regulation disorders of antigen-presenting cells.

The T-cell immunity deficiencies are more evident in patients with MDR-TBP versus donors and the ones with chemosensitive tuberculosis. CD3+CD56+, CD3+CD4+ pool was apparently below target, and CD3+CD8+ was higher in cases of MDR-TBP as compared to chemosensitive tuberculosis. The statistically confirmed difference in CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+, CD3+CD8+HLA-DR+, CD16/56+8+ was estimated be-

tween the study and observational groups. The difference rate of the indicators varied from 1.2 to 1.5 times (17.8 % – 31.7 %). The estimated difference between the groups pointed at the suppressed immune responses in cases of MDR-TBP (difference rate 27.1 % (by 1.4 times)), significant suppression of the intercellular cooperation among antigen-presenting cells due to the decrease in HLA-DR+ antigen expression by 1.5 times, the intensification of non-specific protection caused by the increase in activated CD16/56+8+ populations by 1.2 times. The evident specific cell immunity disorders in patients with MDR-TBP aggravate the clinical course of the disease, leading to destructive changes and acute and extensive processes.

The T-cell immune deficiency, misbalance among certain T-cell subpopulations, suppressed immune response, the decrease in antigen-presenting cells with the expression of HLA-DR+ receptor, the increase in activated NK-cells and uncommitted T-helpers that are most often revealed in cases of MDR-TBP, aggravate the clinical course of the disease leading to destructive changes, acute and extensive processes.

The increase in CD19+ with the intensified antibody formation was revealed in 54.5 % and 74.4 % of patients from the observational and study groups, respectively. It proved the initiation of Th2 or mixed immune response. The humoral response was activated at 2 times with the unfavourable forecast for the course of the specific process.

Cytokines, in particular, pro-inflammatory and anti-inflammatory interleukins, play an important role in the regulatory system and cell response to MTB. Our research focuses on

TNF- $\alpha$ , IL-6, IL-10 in patients with pulmonary tuberculosis caused by MTB strains with different sensitivity to antimycobacterial agents. The research proved an apparent increase in pro-inflammatory interleukins TNF- $\alpha$ , IL-6 i IL-10 in the blood of patients with pulmonary tuberculosis [6, 7].

T-cell immune deficiency, the misbalance among certain T-cell subpopulations, suppressed immune response, the decrease in antigen-presenting cells with the expression of HLA-DR+ receptor, the increase in activated NK-cells, uncommitted T-helpers, humoral immune response most often revealed in cases of MDR-TBP, the evident predominance of pro-inflammatory interleukins aggravate the clinical course of the disease, trigger progressive, destructive and generalized processes. Immune studies might be used to predict the clinical course of

tuberculosis and evaluate the process activity and treatment efficiency. A complex immunoassay for children and adolescents with MDR-TBP allows for the timely implementation of pathogenetic therapy to treat immune disorders and boost the efficiency of antimycobacterial agents.

**Study limitations.** The limitations of the study are related to the small cohort of examined patients.

**Prospects for further research.** Research to discover of immunological changes in pulmonary tuberculosis is planned even in children and adolescents.

## 5. Conclusions

1. The quantity of phagocytic heterophilic leukocytes, their engulfing properties and the amount of cationic lysosomal proteins were by 1.2, 2.5 and 1.3 times less and as compared to the observational group.

2. T-cell immune deficiency is caused by the combination of quantitative and functional disorders: the decrease in CD3+, suppression revealed in patients with first diagnosed MDR-TBP by 1.3 times more often (72.0 %) as compared to chemosensitive tuberculosis patients (55.0 %).

3. The full-value, well-balanced immunospecific response to mycobacterial antigen was evident in 33.3 % of patients with MDR-TBP and 52.4 % of patients with chemosensitive tuberculosis. Poor antituberculosis protection with dysfunctional disorders of the specific immune response was by 1.4 times more often revealed in

cases of MDR-TBP (54.2 %) versus chemosensitive tuberculosis (38.1 %). Tuberculin energy was evident in 12.5 % and 9.5 % of patients, correspondingly.

4. The functional failure of B-cells with the suppressed IgA, IgM, IgG immunoglobulin generation was revealed in 20.9 % patients with MDR-TBP (the suppression of IgA, IgG and IgM antibody formation was in 4.2 %, 4.2 % and 12.5 % cases correspondingly). In cases of chemosensitive tuberculosis, only IgM deficiency was evident in 13.6 % of cases.

## Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

## Funding

The study was performed without financial support.

## Data availability

Data will be made available on reasonable request.

## Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

## References

- Lytvynenko, N. A., Feshchenko, Yu. I., Pohrebna, M. V., Senko, Yu. O., Protsik, L. M., Grankina, N. V. (2020). Pathomorphosis of chemoresistant tuberculosis. *Tuberculosis, Lung Diseases, HIV Infection*, 3 (42), 48–56. <https://doi.org/10.30978/tb2020-3-48>
- Melnyk, V. M., Novozhylova, I. O., Matusyevych, V. H. (2013). Chemoresistant tuberculosis: the state of the problem in Ukraine. *Ukrainskyi medychnyi chasopys*, 5, 43–45. Available at: [http://nbuv.gov.ua/UJRN/UMCh\\_2013\\_5\\_14](http://nbuv.gov.ua/UJRN/UMCh_2013_5_14)
- Feshchenko, Y. I., Melnyk, V. M., Gumeniuk, M. I., Lynnyk, M. I. (2019). Tuberculosis epidemiological situation in Ukraine. *Infusion & Chemotherapy*, 4, 5–9. doi: <https://doi.org/10.32902/2663-0338-2019-4-5-9>
- Global Tuberculosis Report (2018). WHO. Geneva: World Health Organization. Available at: <https://www.who.int/publications/i/item/9789241565646>
- Liskina, I. V. (2016). Diagnostic possibilities of the immunohistochemistry at tuberculosis inflammatory process (UKR). *Tuberculosis, Lung Diseases, HIV Infection*, 3 (26), 93–100.
- Lapovets, N. Ye., Akimova, V. M., Lebed, H. B. et al. (2021). Laboratornaia imunolohiia. Lviv: Vydavets Marchenko T. V., 318.
- Petrenko, V. I., Varchenko, Yu. A. (2010). The role of Cytokines and their using in patients with tuberculosis. *Tuberculosis, Lung Diseases, HIV Infection*, 2 (2), 78–85.
- Platonova, I. L., Sakhelashvili, M. I., Tkach, O. A., Hrechukha, N. R., Komar, M. V. (2017). Osoblyvosti imunitetu u khvorykh na multyrezystentnyi tuberkuloz lehen z riznoiu efektyvnistiu khimioterapii. *Ukrainskyi pulmonolohichnyi zhurnal*, 2 (96), 116–117.
- Rekalova, O. M., Bilohortseva, O. I., Koval, N. H. (2017). Immunological methods of diagnosis of tuberculosis. *Tuberculosis, Lung Diseases, HIV Infection*, 1 (28), 75–83.
- Yunkerov, V. Y., Hryhorev, S. H. (2002). *Matematyko-statystycheskaia obrabotka dannikh medytsynskykh yssledovanyi*. Saint-Petersburg, 266.

*Received date 14.06.2023*

*Accepted date 26.07.2023*

*Published date 31.07.2023*

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