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THE IMPACT OF IMMUNE DISTURBANCES ON THE FAILURE OF ANTITUBERCULOSIS TREATMENT

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Abstract

Background and aim. *Tuberculosis is an infectious disease caused by Mycobacterium tuberculosis complex, with an evolution and treatment outcome determined by the interaction between the mycobacterial and human genotypes. Various deficiencies of innate immune response starting from the first encounter of M. tuberculosis with lung cells endanger host infection control due to decreased triggering of cellular immune resistance and disturbed humoral immunity. Disturbed cell mediated immunity, known as the basic immune response in tuberculous infection, contributes to the deficient generation of central necrosis granuloma, consequently being responsible for severe clinical aspects and low final outcome. The tuberculosis patient's immune assessment is important before treatment initiation, for establishing the risk reduction measures and increasing success rate.*

Material and methods. *The immune study was conducted on 54 new pulmonary tuberculosis cases with treatment failure, 34 new pulmonary tuberculosis cases that successfully ended the treatment and 50 healthy group individuals. Immune assays performed were: blastic transformation of lymphocytes induced by different antigens, quantitatitve assessment of cellular immunity through CD4+ T cell and CD8+ T cell phenotyping, humoral immunity - through immunoglobulin isotyping, innate resistance – through phagocyte activity of neutrophils, the titter of anti-tuberculosis antibodies and the serum level of circulating immune complexes. Investigations were performed at the onset the treatment and at the end of intensive phase of the standard anti-tuberculosis treatment.*

Results. *Immune disturbances evidenced in patients with treatment failure were: important deficiencies of cellular immunity, hyperactivity of humoral immunity and deficiencies of innate immunity. High predictive value for treatment failure showed the indices: deficiency of T lymphocytes count (OR=62.5) and T helper count (OR=12.5), high level of circulating immune complexes (OR=9.801), deficiency of innate resistance (decreased phagocytating index OR=2.875).*

Conclusions. *For increasing the treatment success rate, the study of immune disturbances must be performed before of antituberculosis treatment initiation, especially of cellular immunity for the early start of immune adaptive treatment.*

Keywords: tuberculosis, immune reactivity, treatment, failure, risk factors

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Background and aim

Immune studies have shown that development of active tuberculosis is caused by a multitude of associated exogenous and host-related risk factors. Disease evolution and treatment outcome are determined by the continuous interaction between *Mycobacterium tuberculosis* genetic diversity and human genotype [1]. It was well recognized that the degree of immune disturbances contributes to the development of pathogenesis, clinical expressiveness and final outcome of tuberculosis [2]. Innate immune response to *M. tuberculosis* infection starts with the activation of macrophage cells (neutrophils, dendritic cells, alveolar macrophages) that through the production of several cytokines (including TNF-α, Il-1β, Il-6, IL-12, IFN-γ, IL-10, TGF-β, IL-4) will initiate the granuloma formation [3]. Chemokine induction will be responsible for proinflammatory response and granulomatous inflammation, that ensures the infectious control at the alveolar level [4,5]. Caseous granuloma permits human organism to efficiently maintain latent the tuberculosis infection and enables its progression from latent form into active disease [4]. Various deficiencies of innate immune response and failure of granuloma constitution contribute to the spread of *M. tuberculosis* and development of generalized tuberculosis [3].

It is well recognized that innate immune response starts with the recognition of *M. tuberculosis* by macrophages due to Toll-like receptor 2 (TLR-2) activation [2,5]. Presentation of mycobacterial antigens by activated macrophages on their surfaces is performed through the association with histocompatibiliy classes I and II, and CD1 surface molecules [5]. Infected macrophages and CD8+ cells are recognized by CD4+ lymphocytes. The major role of CD4+ cells consists in the releasing of IFN-γ (the most important inducing interleukine responsible for antimycobacterial activity) and lysis of the infected macrophages. The failure in releasing of IFN-γ and TNF-α is responsible for the generalization of mycobacterial infection [3]. Humoral immunity is a noncellular response mediated by the antibody specific response. Its role in the protection against mycobacterial infection is less studied than the role of cellular resistance. The less expressed disturbances of B-cell response is due to intracellular residence of mycobacteria [2]. Despite this the high concentration of serium antibodies is correlated with extensibility of tissue lung destruction and endangers treatment outcomes.

The aim of the study was the assessment of immune disturbances responsible for antituberculosis treatment failure.

Highlighted objectives were: 1. Assessment of cellular immunity deficiencies responsible for anti-tuberculosis treatment failure; 2. Identification of innate deficiencies involved in the development of anti-tuberculosis treatment failure; 3. Evaluation of humoral immunity disturbances predictable for anti-tuberculosis treatment failure.

Material and methods

It was a selective, retrospective, laboratory case-control study on 88 new pulmonary tuberculosis cases, which underwent the intensive phase of anti-tuberculosis treatment in the Chiril Draganiuc Institute of Pneumophthisiology of Republic of Moldova (CDIFP). The diagnosis was established according National Tuberculosis Policy – 123, through the sputum microscopic examination at Ziehl-Neelson staining, culture on Lowenstein-Jensen medium and liquid BACTEC medium, and chest X-ray investigations. Immunological investigations were performed in the Laboratory of Immunology and Allergology of CDIFP. The patients were divided into two groups. Inclusion criteria in the study group were: age>18 years old, intensive phase of the anti-tuberculosis treatment performed in the CDIFP, signed consent form for enrolment. The intensive phase of drug-susceptible TB performed in hospital conditions during 2 months included isoniazid, rifampicin, pirazinamid and ethambutol. Treatment outcome defined as failure was appreciated as the microscopic smear positive patient after 5 and more months of antituberculosis treatment. The patients were divided into two groups: **study group (SG)** included 54 new pulmonary tuberculosis cases that failed the antituberculosis treatment and **control group (CG)** - 34 new pulmonary tuberculosis cases, that successfully ended the antituberculosis treatment, as well as a **laboratory sample group** - 50 healthy group individuals. Immune investigations were performed at the start and at the end of intensive phase, in average after 60 days of first line anti-TB treatment. Following assays were performed: lymphocyte blast transformation reaction of induced by phytohemagglutinin and other mitogens [6]), quantitatitve assessment of cellular immunity through CD4+ T cell and CD8+ T cell phenotyping, humoral immunity was assessed by immunoglobuline isotyping, innate resistance was studied using the phagocytic activity of neutrophils [7], the test of nitro-blue-tetrasolium reduction by neutrophils assessed their functional activity [8], antituberculosis IgG antibodies concentration used ELISA method and the serum level of circulating immune complexes was assessed according to the method Ghinda S [9]. Lymphocyte blast transformation reaction used as mitogens: 0.01 ml of phytohemagglutinin (1/10 dilution per 1.0 ml of cell culture medium), tuberculin (1/7 dilution per 0.04 – 1.0 ml of culture medium), staphylococcus, streptococcus and pneumococcus concentrates (1/7 dilution per 0.04 – 1.0 ml of culture medium). The serum levels of immune globulins were assessed by nephelometric method using Immunochemistry Systems ICS Analyzer (Beckman, USA).

The statistical analysis was performed using EpiInfo software. Data were appreciated as nominal or quantitative. The frequency and percentage were reported for nominal

data, and the mean and standard deviation were reported for continuous data [10]. The comparison between two groups of a continuous variable was performed using the independent sample t-test. The correlation between two variables was tested by using Pearson's correlation. A p value of <0.05 was considered statistically significant.

Results

Cellular immunity study established that functional activity of lymphocytes assessed through lymphocyte blast transformation reaction (LBTR) induced by phytohemagglutinin (PHA) at the start of antituberculosis treatment was lower in both groups of patients in comparison with healthy group (t=13.4; p<0.001 for CG and t=19.7; p<0.001 for SG), and was lower in SG in comparison with CG (t=4.86; p<0.001). The standard anti-tuberculosis regimen increased the activity of lymphocytes in both groups of patients, but more significant in CG (t=3.03; p<0.01 for CG and t=2.25; p<0.05 for SG), being established a significant difference between groups (t=4.24; p<0.001). The activity of lymphocytes induced by mycobacterial antigens (tuberculin) was similarly higher in both groups than in healthy group (t=3.0; p<0.01 for CG and t=3.7; p<0.001 for SG). The standard treatment increased the lymphocyte activity at the same level in both groups (t=3.27; p<0.01 for CG and t=2.89 and p<0.01 for SG) (Table I).

The T lymphocyte count before treatment was lower compared with the healthy group in SG (t=6.65; p<0.001) and higher in CG (t=2.1; p<0.05). The specific treatment increased the index in both groups, but more evident in CG (t=3.58; p<0.001 and t=2.68; p<0.01 for SG), being maintained statistical difference between groups (t=6.97; p<0.001). Helper T (CD4+) lymphocytes count was significantly lower in SG than in healthy group (t=8.18; p<0.001) and higher in CG than in healthy group, without achieving the statistical threshold. The anti-tuberculosis treatment increased T helper count, but the statistical threshold was achieved only by the SG (t=2.39; p<0.01), being maintained a conclusive difference between the groups of patients (t=4.71; p<0.001). The T suppressor (CD8+) lymphocytes count was superior in both groups than in healthy group (t=3.9; p<0.001 for CG and t=2.72; p<0.01 for SG). The anti-tuberculosis treatment increased the index in both groups, but more significantly in CG (t=2.33; p<0.05 and t=2.07; p<0.01 for SG), being maintained a lower level in SG than in CG (t=3.37; p<0.01). The findings evidenced more severe cellular deficiencies in the group of patients which failed the anti-tuberculosis treatment and a more evident rehabilitation in the group of patients successfully treated (Table I).

The B lymphocyte count before treatment was significantly higher than in healthy group in both groups (t=2.4; p<0.05 for CG and t=3.9; p<0.01 for SG), being established a higher level in CG (t=2.51; p<0.05). The specific treatment decreased the index in both groups, but more significantly in CG (t=5.4; p<0.001 and t=2.96; p<0.01 for SG), being maintained a statistical difference between groups (t=3.36; p<0.01). Data are shown in the table I.

The titer of IgG was significantly higher in both groups than in healthy group (t=11.0; p<0.001 for CG and t=14.0 p<0.001 for SG).) The specific treatment reduced the titer in both groups with the same statistical threshold (t=2.48; p<0.01 for CG and t=2.41; p<0.01 for SG), being established a significant difference between the groups (t=2.48; p<0.05). Serum titer of IgA was higher in both groups than in healthy group (t=4.0; p<0.001 for CG and t=5.8; p<0.001 for SG). The specific treatment reduced the serum level in both groups, but more evident in CG (t=3.58; p<0.001 for CG and t=2.41; p<0.01 for SG), being maintained a statistical difference between groups (t=3.55; p<0.001). Serum titer of IgM was higher in both groups than in healthy group, but the statistical significance was achieved only by the SG (t=6.4; p<0.001). The specific treatment reduced the serum titer in both groups, but more significantly in CG (t=3.19; p<0.001 for CG and t=2.11; p<0.01 for SG), being maintained a statistical difference between groups (t=3.55; p<0.001). By this way a higher level of IgM in SG than in CG before (t=3.96; p<0.001) was established, as well as at the end of the intensive phase of the specific treatment (t=5.11; p<0.001). Total serum IgE level was higher in both groups than in healthy group (t=3.3; p<0.001 for CG and t=7.4; p<0.001 for SG). The specific treatment reduced the serum level in both groups, but more evident in CG (t=3.67; p<0.001 for CG and t=2.68; p<0.01 for SG). Serum value of anti-mycobacteria antibodies (IgG) were significantly higher in both groups, than in healthy group (t=8.3; p<0.001 for CG and t=9.1; p<0.001 for SG), and evidently higher in SG compared with the CG (t=2.2; p<0.05). The treatment reduced the serum level of antibodies in both groups, but statistical threshold was achieved only in CG (t=2.94; p<0.01), being maintained a higher level in the SG than in CG (t=3.44; p<0.01).The data showed hyperactivation of humoral immunity in cases which failed the antituberculosis treatment and an optimal rehabilitation of humoral immunity of patients who successfully ended the treatment (Table II).

Serum levels of circulating immune complexes were higher in both groups than in healthy group, but the statistical threshold was achieved only by the SG (t=7.9; p<0.001). The specific treatment reduced at a similar statistical threshold the serum level of circulating immune complexes in both groups (t=3.35; p<0.01 for CG and t=2.94; p<0.01 for SG). Despite this fact, a higher concentration of circulating immune complexes was established in SG than in CG before treatment (t=8.2; p<0.001), as well as at the end of the standard anti-tuberculosis treatment(t=6.4; p<0.001).

Serum concentration of the complement C3

fragment was significantly reduced in both groups (t=7.01; p<0.001 for CG and t=7.39; p<0.001 for SG), without differences between groups. The treatment increased the index in both groups (t=3.97; p<0.001 for CG and t=2.77; p<0.01 for SG), being established a significant difference between groups only at the end of intensive phase (t=2.16; p<0.05). Complement fragment C4 serum concentration was significantly reduced in both groups (t=7.6; p<0.001 for CG and t=6.04; p<0.001 for SG), without differences between groups. The treatment increased the index in both groups (t=3.63; p<0.001 for CG and t=3.47; p<0.011 for SG), being established a significant difference between groups only at the end of intensive phase of the anti-TB treatment (t=2.47; p<0.05). Data are shown in the table III.

The functional activity of innate immune system was assessed through the nitro-blue-tetrasolium (NBT) salt reduction test by neutrophils, phagocytating number (PN) and phagocytating index (PI). The result of NBT reduction test was normal in CG and significantly lower in SG than in healthy group (t=2.51; p<0.05). The treatment determined the increase of this index in both groups (t=2.66; p<0.01 for CG and t=2.15; p<0.05 for SG), without achieving a significant difference between the groups of patients before and at the end of treatment. The total amount of neutrophils, capable for phagocytation (phagocytating number) was slightly increased in CG and decreased in SG. The specific treatment increased the index, more important in CG (t=4.24; p<0.001 for CG and t=4.48; p<0.05 for SG), being established a difference between the groups before (t=2.4; p<0.05) and at the end of intensive phase of the treatment (t=2.01; p<0.05). Phagocytating index was lower in SG and higher in CG than in healthy group, without achieving the statistical threshold. The specific treatment increased the index in both groups (t=3.5; p<0.001 for CG and t=2.86; p<0.01 for SG) being established a higher level in CG than in SG as well as before (t=2.41; p<0.05) and at the end of intensive phase of the treatment (t=4.93; p<0.001). Thus, innate resistance was decreased in the group of patients which failed the anti-tuberculosis treatment and showed a more relevant dynamics under the treatment only in the group successfully treated (Table III).

Statistically, the predictability of each immune disturbance that achieved the statistical difference at the comparison between the groups of patients was assessed. This way, it was determined that high predictable value for treatment failure indicates: deficiency of T cell count OR=62.5 (CI 95%:14.231–274.49), deficiency of T helper count OR=12.5 (CI 95%: 3.42–45.04), high count of T suppressors OR=2.10 (CI 95%: 0.62–7.14). Humoral hyperactivity assessed using the serum titer of immunoglobulins were established as low risk factor: high level of IgE was estimated with OR=1.131 (CI 95%: 0.098–13.114), high level of IgA with OR=1.545 (CI 95%: 0.241–9.905) and high count of B lymphocytes with OR=1.158 (CI 95%: 0.348–3.847). High serum concentration of immune circulating complexes was established as high risk factor OR=9.801 (CI 95%: 2.895–33.175) and functional deficiency of innate immune was low risk factor OR=1.158 (CI: 95%: 0.341–3.847). Data are shown in the table IV.

Table I. Cellular immunity (M±m).

Indices	Sample Group	Control Group		Study Group	
		1	2	1	2
LBTR – PHA %	79.9±1.16	65.1±1.23•	68.9±1.18♦	56.3±1.33•	61.7±1.21♦
LBTR -Ag Mbt%	2.0±0.21	3.4±0.42•	5.6±0.54♦	3.3±0.28•	4.4±0.28♦
T-lymphocytes %	60.2±0.75	63.3±1.24•	68.5±1.52♦	52.2±0.94•	56.6±0.78♦
Th-lymphocytes %	43.7±0.85	42.3±1.20	44.1±1.39	34.1±0.81•	36.7±0.73♦
Ts- lymphocytes %	16.6±0.72	20.9±0.83•	24.4±1.23♦	18.1±0.64	19.8±0.55♦
B-lymphocytes %	24.9±0.70	26.8±0.34•	23.5±0.51♦	28.8±0.71•	26.1±0.58♦

Legend:
• - statistical difference in comparison with healthy group individuals;
♦- statistical difference in comparison with healthy group individuals; losis treatment was lower in
LBTR – PHA lymphocyte blast transformation reaction induced by phytohemagglutinin;
LBTR -Ag Mbt - lymphocyte blast transformation reaction induced by mycobacterial antigens;
Th-lymphocytes - helper T (CD4+) lymphocytes;
Ts-lymphocytes suppressor (CD8+) lymphocytes.

Table II. Humoral immunity (M±m).

Indices	Sample Group	Control Group		Study Group	
		1	2	1	2
IgG g/l	12.3±0.27	17.2±0.33•	15.7±0.40♦	18.2±0.31•	17.1±0.31♦
IgA g/l	2.6±0.10	3.2±0.11•	2.6±0.13♦	3.6±0.14•	3.2±0.11♦
IgM g/l	1.4±0.06	1.6±0.09	1.2±0.07♦	2.2±0.11•	1.9±0.10♦
Total IgE U/ml	17.4±1.28	98±11.0•	51±6.9♦	118±13.6•	74±9.6♦
Anti-MBT antibodies o.d.u	2.3±0.09	4.5±0.25•	3.6±0.27♦	5.4±0.33•	5.0±0.31

Legend:
• - statistical difference in comparison with healthy group individuals;
♦- statistical difference in comparison with healthy group individuals; losis treatment and an optimal
Anti-MBT antibodies o.d.u - anti-mycobacteria antibodies optical density units.

Table III. Innate immunity (M±m).

Indices	Sample Group	Control Group		Study Group	
		1	2	1	2
CIC %	49.3±2.38	52.2±4.53	33.8±3.10♦	97.3±5.55•	76.8±4.23♦
C3 g/l	1.20±0.06	0.73±0.030•	0.91±0.035♦	0.73±0.021•	0.82±0.23♦
C4 g/l	0.49±0.02	0.32±0.010•	0.39±0.017♦	0.36±0.008•	0.41±0.010♦
NBT CU	0.14±0.006	0.14±0.008	0.17±0.008♦	0.12±0.005•	0.17±0.023♦
PNr %	76.9±0.86	77.9±1.08	85.2±1.31♦	73.9±1.25•	81.6±1.17♦
PI CU	4.61±0.17	5.1±0.23	6.2±0.21♦	4.4±0.16	5.0±0.13♦

Legend:
• - statistical difference in comparison with healthy group individuals;
♦- statistical difference in comparison with healthy group individuals; y at t of intensive phase,
NBT CU nitro-blue-tetrasolium reduction test, conventional units;
PN-Phagocytating number,
PI - Phagocytating index, conventional units.

Table IV. Predictability of the immune disturbances in the development of treatment failure.

Risk factor	Odds Ratio CI 95%
Low count of T lymphocytes	62.5 (CI 95%: 14.231–274.49)
Low count of T helper lymphocytes	12.5 (CI 95%: 3.42–45.04)
High concentration of CIC	9.801 (CI 95%: 2.895–33.175)
Decreased phagocytating index	2.875 (CI 95%: 0.926–8.928)
Increased count of T suppressor cells	2.10 (CI 95%:0.62–7.14)

Legend: Confidence Interval (CI).

Discussion

Tuberculosis is an infectious disease, with an immunological substrate. Cell mediated immunity represents the basic immune response that locks mycobacterium infection in latent form. Due to the complexity of risk factors, to which is submitted the human host, the cellular immune deficiency causes the re-activation of latent mycobacterial infection and active tuberculosis development. Actual immune research established that the same immune disturbances are responsible for failing the anti-tuberculosis treatment. Thus, the qualitative and quantitative assessment of cellular immunity established more severe defficiency in the group of patients which failed the standard anti-tuberculosis treatment and a better evolution in the group of patients which successfully ended the treatment. The increased quantity of B lymphocytes and high titers of serum immune globulins demonstrated a higher activation of humoral immunity in the group of patients that failed the treatment and an optimal rehabilitation of the group of patients successfully ended the treatment. Innate resistance assessed by different biomarkers evidenced that serum concentration of circulating immune complexes was high in both groups of patients, but higher in the group of patients that failed the standard treatment. Serum concentration of the C3 and C4 fragments of complement were decreased at the same level in both groups of patients, despite this a more favorable evolution was identified only in the group of patients that successfully ended the anti- tuberculosis treatment. Functional activity of innate immunity assessed using the test of nitro-blue-tetrasolium reduction, phagocytating number and phagocytating index, identified a higher degree of activation under the influence of the standard anti- tuberculosis treatment in the group of patients that successfully ended the anti-tuberculosis treatment. Despite of a large number of investigated indices, only a small number showed predictability for standard antituberculosis treatment failure: quantitative deficiency of T cells and T helper cells, high concentration of immune circulating complexes, as well as qualitative deficiency of innate resistance.

Conclusion

Immune disturbances evidenced in patients with treatment failure were: important deficiencies of cellular immunity, hyperactivity of humoral immunity and deficiencies of cellular immunity.

High predictive value for treatment failure showed the indices: deficiency of T lymphocytes count (OR=62.5) and T helper count (OR=12.5), high level of circulating immune complexes (OR=9.801), deficiency of innate resistance (decreased phagocytating index (OR=2.875).

The study of immune disturbances, especially of cellular immunity, in tuberculosis patients will contribute to the early initiation of immune adaptive treatment, that will favor the treatment quality and will contribute to the successful outcome.

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