IMMUNE DISORDERS IN PULMONARY TUBERCULOSIS AND CONCOMITANT SARS-CoV-2 INFECTION

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ABSTRACT
The study aimed to analyze immune-metabolic disorders and biomarkers of endogenous intoxication in patients with pulmonary tuberculosis (TB) caused by different *Mycobacterium tuberculosis* strains, including multidrug-resistant TB (MDR-TB), and in cases with concomitant SARS-CoV-2 infection, to assess the impact of coinfection on immune–metabolic homeostasis and systemic inflammation. This prospective study included 191 newly diagnosed TB patients divided into four groups: drug-susceptible TB (n=80), primary MDR-TB (n=40), acquired MDR-TB (n=49), and TB with SARS-CoV-2 coinfection (n=22). A control group comprised 36 conventionally healthy individuals. A more severe disease course was observed in patients with acquired MDR-TB and SARS-CoV-2 coinfection, associated with decreased $CD3^+$, $CD4^+$, $CD16^+$, and $CD8^+$ T lymphocytes. Circulating immune complexes, ESR, acute-phase proteins, and derived hematological indices were elevated in all groups, with the highest levels in coinfected patients. Pro-inflammatory cytokines (TNF-α, IL-6, IL-8) were significantly increased; TNF-α peaked in acquired MDR-TB, while IL-6 and IL-8 were highest in coinfection. Early detection of MDR-TB and SARS-CoV-2 coinfection, combined with timely immunopathogenic interventions, is essential to modulate immune–metabolic disturbances and improve outcomes in severe TB.
Keywords: Tuberculosis, COVID-19, Immune factors.

1. INTRODUCTION

The Republic of Moldova (RM) is among the high tuberculosis (TB) burden countries in Eastern Europe, reporting some of the highest rates of multidrug-resistant tuberculosis (MDR-TB). It is estimated that approximately 33% (30-35%) of newly diagnosed TB cases annually in RM are caused by MDR/rifampicin-resistant (RR-TB) strains, while acquired resistance occurs in nearly 60% (56-64%) of cases [1]. The COVID-19 pandemic further worsened the global MDR-TB situation by disrupting TB detection in high-risk groups and weakening reporting systems, leading to a decrease in newly registered TB cases and an increase in TB-related mortality, especially among patients with MDR-TB and those with concomitant SARS-CoV-2 infection [2]. Early detection of drug resistance and SARS-CoV-2 coinfection, followed by prompt initiation of appropriate treatment, may prevent further resistance and improve treatment outcomes. In addition, early identification of immune-metabolic disturbances and timely initiation of pathogenetic therapy can enhance treatment effectiveness [3, 4].

The study aimed to analyze immune-metabolic disorders in patients with pulmonary tuberculosis (TB) caused by different strains of Mycobacterium tuberculosis (MTB) and concomitant SARS-CoV-2 infection, to assess the impact of coinfection on homeostasis and systemic inflammatory responses.

2. MATERIAL AND METHODS

This prospective study included 191 newly diagnosed patients with pulmonary TB, divided into four groups: the 1st study group (1st SG) comprised 80 patients with drug susceptible TB, the 2nd study group (2nd SG) included 40 patients with primary drug resistant TB (MDR TB), the 3rd study group (3rd SG) consisted of 49 patients with acquired MDR TB, and the 4th study group (4th SG) included 22 patients (11 with drug susceptible TB and 11 with primary MDR TB) co infected with SARS CoV 2. All indices were compared with those of a control group (CG) comprising 36 conventionally healthy individuals. Inclusion criteria for the study were: age> 18 years, signed informed consent, microbiologically confirmed diagnosis of pulmonary TB, and availability of Mycobacterium tuberculosis drug-susceptibility testing results. All patients underwent clinical evaluation, chest radiography, and microbiological investigations, including Ziehl-Neelsen acid-fast staining, culture of Löwenstein-Jensen solid medium and BACTEC liquid medium, and the GeneXpert MTB/RIF assay. Immunological assessments included quantitative flow cytometric immune phenotyping of lymphocyte subsets (CD3+, CD4+, CD8+, CD16+) and measurement of inflammatory and intoxication biomarkers.

3. RESULTS

Patients were similarly distributed across the four study groups by demographic characteristics, with no significant differences in sex distribution. The male to female ratios were 2.1:1 in the 1st SG (54 men [67%] vs. 26 women [32%]), 2.1:1 in the 2nd SG (27 men [67%] vs. 13 women [32%]), 1.5:1 in the 3rd SG (29 men [59%] vs. 20 women [41%]), and 2.0:1 in the 4th SG (14 men [64%] vs. 8 women [36%]). Age distribution revealed a statistically higher proportion of young patients (18–44 years) in the 2nd SG (25 cases [62%]) and the 4th SG (17 cases [77%]), compared with the 1st SG (42 cases [52%]) and the 3rd SG (19 cases [39%]). Patients older than 45 years predominated in the 1st SG (38 cases [48%]) and the 3rd SG (30 cases [61%]), compared with the 2nd SG (15 cases [38%]) and the 4th SG (4 cases [23%]). Evaluation of general symptoms established a significantly higher prevalence of prolonged asthenia in the 3rd SG (49 cases [100%]) and the 4th SG (22 cases [100%]), compared with the 1st SG (62 cases [77%]) and the 2nd SG (21 cases [52%]). Persistent weight loss was more frequent in the 3rd SG (49 cases [100%]) and the 4th SG (22 cases [100%]), compared with the 1st SG (71 cases [87%]) and the 2nd SG (28 cases [71%]). Low-grade fever was present in all patients of the 3rd SG (49 cases [100%]) and in all 22 patients of the 4th SG (100%), compared with 25 cases (31%) in the 1st SG and 8 cases (20%) in the 2nd SG, and was associated with night sweats. Radiological evaluation revealed a statistical higher proportion of patients who met all radiological severity criteria - extensive lung involvement (more than three segments), parenchymal destruction, and disseminated lesions in the 3rd SG (35 cases [71%]) and the 4th SG (16 cases [73%]), compared with half of the 1st SG (44 cases [55%]) and the 2nd SG (23 cases [57%]) ($p<0.05$ for both comparisons). Clinical-radiological severity of pulmonary TB was defined by a pronounced intoxication syndrome (altered general condition, persistent fever, and weight loss), persistent respiratory manifestations (episodes of hemoptysis, dyspnea of MRC grades 2–3), radiological severity criteria, and AFB-positive sputum. Those criteria statistically predominated in the 3rd SG (31 cases [63%]) and the 4th SG (17 cases [78%]) compared with the 1st SG (22 cases [25%]) and the 2nd SG (18 cases [45%]) ($p<0.01$ for both comparisons). To assess cell-mediated immunity, peripheral blood lymphocytes were isolated and their immune phenotypes analyzed, revealing that the proportion of $CD3^+$ T cells, including the T helper subset, was reduced in all SGs compared with the control group (CG) ($p<0.001$), with the lowest levels in the 3rd and 4th SGs compared with the 1st and 2nd SGs ($p<0.001$). The proportion of $CD4^+$ (T helper) cells was also significantly decreased in all SGs vs. the CG ($p<0.001$), statistically

more reduced in the 3rd and 4th SGs than in the 1st and 2nd SGs ($p<0.01$). These findings indicated more pronounced depletion of cell-mediated immunity in patients with acquired MDR TB and concomitant SARS-CoV-2 infection. In contrast, $CD8^+$ (T suppressor) cells were slightly elevated in the 1st and 2nd SGs, but significantly reduced in the 3rd and 4th SGs compared with the CG ($p<0.001$). The $CD4^+/CD8^+$ ratio was approximately 1 in the 1st and 2nd SGs and increased to 1.4 in the 3rd SG and 1.5 in the 4th SG, indicating cell-mediated immune hyperactivity in these groups. The proportion of $CD16^+$ (natural killer) cells was mildly reduced in the 1st and 2nd SGs and significantly decreased in the 3rd and 4th SGs compared with the CG ($p<0.001$), reflecting impaired innate immunity and diminished cytotoxic function in these groups.

Table 1. Cell-mediated immunity indices (%)

Indices	1st SG	2nd SG	3rd SG	4th SG	CG
CD3+ (%)	63.6±0.9□	66.4±2.5□◊	46.1±1.1□○	37.3±2.1□●◘	67.9±0.5
CD4+ (%)	32.3±0.6□	34.6±1.7□◊	27.5±1.2□○●	22.4±1.13□○◘	38.3±0.6
CD8+ (%)	31.4±0.7□	32.5±1.5□◊	18.6±1.5□○●	15.9±1.2□○◘	29.6±0.7
CD16+ (%)	10.9±1.1 *	11.2±1.6	7.3±2.2□○●	7.5±1.9□	12.1±0.5

Note: □ – statistically significant vs. CG; ◊ 1st vs. 2nd SGs; ○ 1st vs. 3rd SGs; ◌ 1st vs. 4th SGs; ● 2nd vs. 3rd SGs; ♦ 2nd vs. 4th SGs; ◘3rd vs 4th SGs.

Table 2 summarizes the changes in endogenous intoxication and pro-inflammatory biomarkers.

Table 2. Endogenous intoxication and pro-inflammatory biomarkers

Indices	1st SG	2nd SG	3rd SG	4th SG	CG
CIC-2.5%	22.2±4.64 □◊ 300%*	28.2±3.77 □ 400%	47,0±6,56 □● 670%	37.3±5.1□● ◘ 530%	7.2±0.35
CIC-4.2%	45.6±6.79 □◊ 180%*	62.6±5.55 □ 250%	78.3±9.38 □● 300%	52.5±5.73□ ○◘ 200%	25.2±0.84
CIC-8.0%	322±27.4 □◊ 130%	487±39.1 □○ 200%	523±83.7 □ 210%	412.9±1.2□ ○◘ 170%	245.5±7.2
LII_{Kk}	1.3±0.4 130%	1.8±0.2□ 180%	1.9±0.3□○ 190%	2.4±0.5□○◘ 240%	1.0±0.04
HII	1.4±0.2 120%*	1.5±0.3 130%	2.1±0.4□○ 170%	2.5±0.4□○◘ 210%	1.2±0.01
ESR mm/h	25±6.1□ 350%*	28±5.6□ 400%	32±6.8□○ 450%	65±5.9□○◘ 900%	7±2.3
CRP mg/L	36±3.5□ 970%*	39±4.2□ 1030%	64±3.9□○● 1730%	85±5.6□○◘ 2300%	3,7±1,2
CP mg/L	870±32□ 2310%	892±39□ 2350%	1027±35□○● 2730%	1254±41□○◘ 3330%	376±23
IL-6 pg/mL	6.3±0.42□ 170%	7.6±0.61□ 200%	8.2±0.81□○ 210%	20.8±0.62□○◘ 560%	3,7±0,32
IL-8 pg/mL	13.2±0.17□ 1280%	15.4±0.22□ 1320%	16.7±0.27□○● 1430%	19.6±0.24□○◘ 1670%	1,17±0,23
TNF-α pg/mL	21.1±0.67□ 1850%	27.8±0.64□◊ 4270%	39.8±0.67□○● 6120%	29.6±0.87□○◘ 4550%	6,5±1,2

Note: □ – statistically significant vs. CG; ◊ 1st vs. 2nd SGs; ○ 1st vs. 3rd SGs; ◌ 1st vs. 4th SGs; ● 2nd vs. 3rd SGs; ♦ 2nd vs. 4th SGs; ◘3rd vs 4th SGs.

Assessment of circulating immune complexes (CICs) revealed that the concentration of high-molecular-weight CICs (2.5%) was significantly higher in all SGs compared with the CG ($p<0.001$), with significantly higher levels observed in 3rd and 4th SGs compared with 1st

and 2nd SGs ($p<0.001$). The concentration of medium molecular weight CICs (4.2%) was significantly higher in all SGs compared to the CG ($p<0.001$), with significantly higher levels in the 3rd SG than in the 1st and 4th SGs ($p< 0.01$). Similarly, low molecular weight CICs (8.0%) were significantly higher in all SGs vs the CG ($p<0.001$), with higher levels in the 2nd and 3rd SGs compared to the 1st and 4th SGs ($p<0.001$). CIC concentrations across all molecular weight fractions were significantly increased in all study groups relative to the CG. Notably, CICs (2.5%) exceeded CICs (4.2%) and (8.0%) only in patients with acquired MDR TB and concomitant SARS-CoV-2 infection, indicating a higher antigenic load in those groups. This pattern may be explained by SARS-CoV-2 coinfection, which promotes the formation of larger CICs through sustained antibody production and complement activation, thereby contributing to persistent systemic inflammation and increased disease severity. Calculation of the hematological indices LIIkk and HII revealed significantly higher values in all SGs compared to the CG ($p < 0.01$ for all comparisons). The erythrocyte sedimentation rate (ESR) was significantly elevated in all SGs versus the CG, with the highest levels observed in the 4th SG compared to the other SGs ($p < 0.001$ for all comparisons). Serum C-reactive protein (CRP) levels were significantly higher in all SGs than in the CG, with significantly greater values in the 3rd and 4th SGs compared to the 1st and 2nd SGs ($p < 0.01$ for both comparisons). Similarly, serum ceruloplasmin (CP), a copper-binding glycoprotein with ferroxidase activity, was significantly elevated in all SGs versus the CG ($p < 0.001$ for all comparisons), with higher concentrations in the 3rd and 4th SGs compared to the 1st and 2nd SGs ($p < 0.001$). Serum concentrations of TNF-α, IL-6, and IL-8 were significantly increased in all SGs relative to the CG ($p < 0.001$ for all comparisons). Among the SGs, TNF-α levels were highest in the 3rd SG ($p < 0.001$), whereas IL-6 and IL-8 concentrations peaked in the 4th SG ($p < 0.001$).

4. DISCUSSION

Our study included TB patients comparably distributed across SGs, with a consistent predominance of males. A higher proportion of younger patients (18-44 years) was observed in the 2nd and 4th SGs, whereas patients aged ≥45 years predominated in the 1st and 3rd SGs. Clinical and radiological assessments indicated a more severe disease course in patients with acquired MDR-TB and concomitant SARS-CoV-2 infection. This was associated with marked depletion of $CD3^+$, $CD4^+$, and $CD16^+$ T cells, as well as reduced $CD8^+$ T-cell counts, findings that are consistent with previous reports [5]. CIC concentrations were elevated across all study groups, reflecting an increased antigenic load and coordinated immune complex formation across all molecular weight fractions. Hematological indices, ESR values, and acute-phase proteins (CRP and ceruloplasmin) were also increased in all SGs, with the highest levels observed in patients with concomitant SARS-CoV-2 infection. Serum concentrations of the pro-inflammatory cytokines TNF-α, IL-6, and IL-8 were significantly elevated in all SGs. Among these, TNF-α reached peak levels in patients with acquired MDR-TB, whereas IL-6 and IL-8 were highest in those with concomitant SARS-CoV-2 infection, in agreement with findings reported in other studies [6,7]. The main limitations of this study include the relatively small sample size, particularly in the 4th SG, which reduces statistical power and limits the generalizability of the findings, especially for comparisons involving concomitant SARS-CoV-2 infection and acquired MDR-TB. In addition, the lack of adjustment for comorbid conditions (such as HIV infection, diabetes, or other immunosuppressive states) may confound the interpretation of immune and inflammatory parameters. The absence of spatial or regional epidemiological data also limits insights into local transmission dynamics and environmental influences [8]. These limitations underscore the need for larger, multicenter studies to better characterize immune-metabolic responses and treatment outcomes in patients with MDR-TB and SARS-CoV-2 coinfection.

5. CONCLUSIONS

Clinical and radiological evaluations revealed a more severe disease course in patients with acquired MDR-TB and concomitant SARS-CoV-2 infection. These patients exhibited marked depletion of $CD3^+$, $CD4^+$, and $CD16^+$ T cells, along with reduced $CD8^+$ T-cell counts, indicating profound impairment of both cell-mediated and innate immunity. Elevated CIC levels across all SGs reflected an increased antigenic load and coordinated immune complex formation across molecular weight fractions. In parallel, hematological indices, ESR, acute-phase proteins, and the cytokines IL-6 and IL-8 were highest in patients with SARS-CoV-2 coinfection, indicating an intensified systemic inflammatory response. Serum TNF-α concentrations were significantly elevated in all groups, with peak levels observed in patients with acquired MDR-TB. Early identification of MDR-TB and concomitant SARS-CoV-2 infection, combined with timely immunopathogenetic interventions, is essential to modulate immune-metabolic disturbances, limit the progression of drug resistance, and improve treatment outcomes in severe forms of TB.

DECLARATIONS

Conflict of Interest Statement: The authors declare that they have no conflict of interest.

Author Contributions: E. L. designed the study; E. L. and S. G. analyzed data; E. L. drafted the manuscript; Both authors have reviewed and approved the final version of the manuscript.

Ethics Statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the State Pharmacy and Medicine University ethics committees on 13.11.2017. All participants provided informed consent, and data were anonymized to ensure confidentiality.

Originality Statement: The authors confirm that this manuscript is original, has not been published previously, and is not under consideration elsewhere.

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