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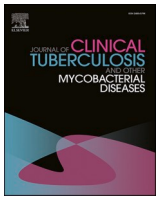

Is analysis of inflammatory biomarkers and lymphocyte subpopulations useful in prediction of tuberculosis treatment outcomes?

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ABSTRACT

Analysis of inflammatory biomarkers and lymphocytes during the treatment of tuberculosis (TB) could yield findings that influence the routine clinical practice and use of new anti-TB drugs. This study aimed to evaluate whether the selected biomarkers—soluble intercellular adhesion molecule type 1, soluble urokinase-type plasminogen activator receptor (suPAR), and C-reactive protein (CRP)—and T-cell subpopulations are useful for predicting culture conversion, treatment outcomes, and the extent of radiological lesions (calculated using X-ray score) in patients with drug-sensitive pulmonary TB. This study included 62 patients with drug-sensitive pulmonary TB. CRP and suPAR levels significantly decreased after 1 month of treatment. Before treatment initiation, CRP and suPAR levels were significantly higher in patients without culture conversion; however, none of the selected host biomarkers appeared to significantly influence the conversion status or treatment outcomes. Some lymphocyte subpopulations were correlated with X-ray scores before TB treatment initiation, but lung destruction, as determined using X-ray scores, showed the highest correlation with the baseline CRP value. We conclude that selected host biomarkers have a very limited role in predicting TB treatment outcomes and culture conversion and do not appear to be superior to CRP in monitoring TB treatment.

1. Introduction

The discovery of tuberculosis (TB) biomarkers could influence routine clinical practice by helping clinicians to assess patients' response to anti-TB treatment and confirm the sterilizing activity of the drugs, and could thus help shorten clinical trials or even indicate new clinical endpoints [1–3]. Current TB treatment monitoring relies heavily on culture conversion. However, this approach is time consuming because of the slow growth of *Mycobacterium tuberculosis*. New biomarkers, if found, could help in the prognosis of TB treatment and the decision to modify treatment. Results of our previous study suggested that several factors could be used to predict sputum culture conversion in pulmonary TB after 1 month of treatment [4]. In this study, we investigated whether biomarkers of the host's immune response are useful in predicting pulmonary TB treatment outcomes and sputum culture conversion.

Studies on the use of single host biomarkers for assessing TB therapy have repeatedly demonstrated poor sensitivity and specificity [3]. We hoped that the use of a combination of biomarkers could improve the prediction of TB outcomes. After analyzing the available literature, we selected several immune biomarkers for this study [1,2,5,6]. The first selected biomarker was soluble intercellular adhesion molecule type 1 (sICAM-1), which is reportedly a sensitive biomarker for evaluating the action of anti-TB drugs. Levels of sICAM-1 were higher in patients with TB than in those with certain other pulmonary diseases [6].

The second selected biomarker was C-reactive protein (CRP), a widely studied acute-phase protein that is produced in the liver and can opsonize pathogens and facilitate phagocytosis [3]. Studies have shown that CRP levels decrease within the first few days of anti-TB therapy [7]. High baseline CRP levels are also associated with worse TB treatment outcomes [8].

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The third selected biomarker was urokinase-type plasminogen activator receptor (uPAR), which is mostly expressed by monocytes and macrophages and is involved in cell motility and adhesion [3]. Levels of a soluble form of uPAR, suPAR, were shown to be elevated in active TB and were correlated with the number of acid-fast bacilli in sputum [9]. Compared with CRP, suPAR has not been widely studied in pulmonary TB. Several studies have shown that suPAR may be useful in the prognosis and differentiation of TB [10–14].

We also measured T-cell subpopulations. A better understanding of T-cell mechanisms is vital for developing vaccines and new therapies [15]. The cellular immune response plays a crucial role in controlling M. tuberculosis replication [16]: CD4 T cells help control primary *M. tuberculosis* infection and granuloma formation [17], whereas CD8 T cells help inhibit TB reactivation [18]. CD45RA is a marker of naive T-cell subsets that is also expressed on the CD4 and CD8 effector cells. After antigen exposure, memory T cells stop expressing CD45RA [19]. CD4 T cells with low expression of CD27 have been described as a biomarker of active TB [20]. Decreased CD27 expression may indicate that differentiated effector T cells are appearing as a result of antigen encounter [20]. CD38, one of the activation markers on M. tuberculosis-specific CD4 T cells [21], is believed to modulate inflammatory gene expression in helper T cells. This is because CD38 ligation results in the secretion of interferons, secretion of interleukins 6 and 10, and overexpression of CD38 on lymphocytes, which is a predictor of $CD4^+$ T-cell depletion [22].

We studied whether changes in these selected biomarkers are useful in predicting culture conversion, treatment outcomes, and the extent of radiological lesions in drug-sensitive pulmonary TB.

2. Methods

2.1. Study population and design

This prospective study was conducted in Romainiai Tuberculosis Hospital, a branch of Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Kaunas, Lithuania. Approximately 95% of the patients (adults only) treated in this hospital are from the Kaunas region. Directly observed treatment (DOT) is fully implemented; the staff ensure that patients with TB take their medications 7 days a week.

The clinical research protocol was approved by Kaunas Regional Biomedical Research Ethics Committee (BE-10–9 2015–10-09) and informed written consent was obtained from all participants. All patients received the standardized TB treatment regimen in line with national and World Health Organization TB treatment guidelines (isoniazid, rifampicin, ethambutol, pyrazinamide for two months, then isoniazid and rifampicin for four months, doses adjusted according to the body weight). TB drugs were distributed every day by hospital nurses, who ensured that all patients, including ambulatory patients, swallowed the drugs. There were no shortages of the TB drugs during the study.

Based on the standard finite sample size calculation formula, we determined that a sample size of 55 was needed for this study. From November 2015 to November 2017, we assessed 110 patients with new pulmonary TB diagnosed for the first time in their lives. Exclusion criteria were significant morbidity due to other illnesses (e.g., cancer, autoimmune diseases, diabetes mellitus, and renal insufficiency) and human immunodeficiency virus positivity. Pregnant or breastfeeding women were excluded, as were patients in whom drug-resistant TB was diagnosed. The final study population included 62 patients, in whom drug-susceptible pulmonary TB was diagnosed. Inclusion required acid-fast bacilli to be observed in sputum using Ziehl–Nielsen staining or sputum culture positivity for *M. tuberculosis.* All TB cases were confirmed bacteriologically (BACTEC system, Middlebrook media; BD, Franklin Lakes, NJ, USA). All included patients were treated in the hospital for at least 1 month. Treatment was continued until either treatment success or patient's death. Length of treatment was 6–7 months (7-month treatment was administered if sputum showed no microscopic conversion after 2 months of treatment, as per national guidelines).

After enrollment in the study and before TB treatment initiation, standard posteroanterior chest X-rays were obtained and blood laboratory tests (CRP, flow cytometry, suPAR, and sICAM-1) were performed. Chest X-rays were evaluated by an experienced radiologist, and the score of disease extension was calculated according to the method described by Ralph et al. (percentage of lung affected plus 40 if cavitation was present) [23].

After 1 month of DOT in the hospital setting, sputum microscopy and culture and CRP, sICAM-1, and suPAR tests were repeated. Sputum culture conversion after 1 month of treatment was confirmed via a second negative culture 30 days later (after 2 months of treatment). Patients were assigned to one of two groups (conversion or non-conversion) after 1 month of TB treatment.

Five months after treatment initiation, sputum microscopy and culture were repeated and CRP, sICAM-1, and suPAR levels were remeasured. Flow cytometry and posteroanterior chest X-rays were repeated as well. Outcomes were recorded 1 year after treatment initiation: successful (treatment completion and cure) and unsuccessful (death). No patients experienced treatment failure (defined as a patient who is sputum smear or sputum culture positive at 5 months or later after the initiation of anti TB treatment). One patient was lost to follow-up; the data for that patient were not included in the analysis of treatment outcomes.

2.2. Laboratory testing

Laboratory testing was performed at the hospital of Lithuanian University of Health Sciences Kauno klinikos. AFB smear positive results were confirmed as per WHO/International Union Against Tuberculosis and Lung Disease grading: "scanty" with of 1–9 AFB per 100 oil immersion fields; "1+" with 10–99 AFB per 100 oil immersion fields; "2+" with 1–10 AFB per 1 oil immersion field and "3+" with > 10 AFB per oil immersion field.

Peripheral blood for ELISA assays was collected into plain BD Vacutainer tubes (BD, USA), centrifuged at 1600x*g* for 15 min. Sera were separated from the blood, aliquoted and stored at − 70 °C temperature until analysis. Serum suPAR was assessed using commercial kits Human suPAR ELISA (BioVendor – Laboratorní medicína, Czech Republic) according to manufacturer's instructions. Lower detection limit was 5.1 pg/mL for suPAR. RayBio Human sICAM-1 ELISA commercial kits (RayBiotech, USA) with a lower detection limit of 150 pg/ml were used for assessment of sICAM-1 in serum. CRP level was detected using clinical chemistry system Becman Coulter UniCel DxC Synchron 800 (USA). Peripheral blood for flow cytometry and hematology analysis was collected into BD Vacutainer K3EDTA tubes (BD, USA).

For flow cytometry cell aliquots were directly stained following a standard procedure with monoclonal antibodies (BD Biosciences Pharmingen, USA): anti-human CD3 (FITC, clone UCHT1), anti-human CD4 (PE-Cy™7, clone SK3), anti-human CD8 (APC-Cy™7, clone SK1), anti-human CD45RA (PerCP-Cy™5.5, clone HI100), anti-human CD27 (APC, clone M−T271), anti-human CD38 (PE, clone HIT2). Subsequently samples were analysed using FACS Canto flow cytometer (BD Immunocytometry Systems, USA). Up to 50 000 total events were collected per sample. Data were analysed using FACSDiva software (Becton Dickinson, USA). The T lymphocyte population was identified based on SSC and the level of CD3 expression. The number of positive cells with CD expression was evaluated as a percentage of cells in the T lymphocyte gate. These T-cell subsets were assessed during analysis: CD4 + CD45RA + CD27+ (naive); CD4 + CD45RA-CD27+ (memory); CD4 + CD45RA + CD27-; CD4 + CD45RA-CD27- (memory/effector); CD8 + CD45RA + CD27+ (naive); CD8 + CD45RA-CD27+ (memory); CD8 + CD45RA + CD27- (cytotoxic effector); CD8 + CD45RA-CD27- (memory/effector); CD8 + CD38 + CD3+ (activated suppressor/cytotoxic); CD8 + CD38-CD3 + . Absolute counts of T-cell subsets were calculated from total cell counts, enumerated by automated hematology

system Sysmex XE-5000 (Sysmex Corporation, Japan).

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 23.0 for Windows (Statistical Package for the Social Sciences, USA). The following descriptive statistics were reported: proportions with their 95% confidence intervals for dichotomous variables and medians with their interquartile ranges (IQR) for continuous variables. Comparisons of continuous variables between groups were made using Mann-Whitney *U* test for independent samples, as there were relatively few observations and no normal distribution. For correlation Spearman's coefficient was used. To estimate the risks of non-conversion and death by biomarker levels, we used logistic regression analysis. We also estimated the association between treatment outcomes, culture conversion and biomarkers using general linear model for repeated measures. $P \leq 0.05$ was considered statistically significant.

3. Results

Of the 62 patients, 48 (77.4%) were male (age range: 22–65 years, median: 46.5 years) and 14 (22.6%) were female (age range: 21–89 years, median: 48 years). Baseline levels of host biomarkers and T-cell subpopulations are presented in Table 1.

3.1. Prediction of culture conversion

After 1 month of TB treatment, sputum culture conversion was observed in 24 patients (38.7%). In other patients, sputum culture conversion was not observed after 1 month of treatment or adequate sputum samples could not be produced. Some patients died during the first month of treatment. Culture conversion was observed in every patient still alive after 5 months of TB treatment.

Table 1
Baseline levels of biomarkers and T-cell subpopulations in 62 patients with drug-sensitive pulmonary tuberculosis.

Baseline Characteristics	Median [Interquartile range]
CRP, mg/l	39.22 [8.25–98.56]
sICAM-1, ng/ml	59.94 [57.01–65.79]
suPAR, pg/ml	2621.05 [1898.36–4372.91]
Lymphocytes (N) x10^9/l	1.53 [1.14–2.15]
Lymphocytes (%)	19.85 [12.95–26.6]
CD3+ (x 10^9/l)	0.99 [0.59–1.50]
CD3+ (%)	66.55 [52.4–71.52]
CD4+ (x 10^9/l)	0.64 [0.33–0.85]
CD4+ (%)	63.0 [53.27–70.6]
CD8+ (x 10^9/l)	0.34 [0.21–0.55]
CD8+(%)	35.25 [29.03–43.15]
CD4/CD8	1.79 [1.21–2.45]
CD4+/CD45RA+/CD27- (x 10^9/l)	0.0041 [0.0002–0.0179]
CD4+/CD45RA+/CD27- (%)	0.7 [0.07–3.4]
CD4+/CD45RA+/CD27+ (x 10^9/l)	0.20 [0.11–0.37]
CD4+/CD45RA+/CD27+ (%)	40.65 [30.55–54.9]
CD4+/CD45RA-/CD27- (x 10^9/l)	0.06 [0.03–0.1]
CD4+/CD45RA-/CD27- (%)	11.2 [7.07–16.9]
CD4+/CDRA-/CD27+ (x 10^9/l)	0.29 [0.14–0.4]
CD4+/CD45RA-/CD27+(%)	41.6 [35.73–51.05]
CD8+/CD45RA+/CD27-(× 10^9/l)	0.07 [0.02–0.15]
CD8+/CD45RA+/CD27- (%)	23.0 [13.25–44.35]
CD8+/CD45RA+/CD27+ (x 10^9/l)	0.09 [0.04–0.15]
CD8+/CD45RA+/CD27+ (%)	23.70 [14.7–38.95]
CD8+/CD45RA-/CD27- (x 10^9/l)	0.06 [0.03–0.11]
CD8+/CD45RA-/CD27- (%)	15.3 [10.18–26.15]
CD8+/CD45RA-/CD27+ (x 10^9/l)	0.08 [0.04–0.12]
CD8+/CD45RA-/CD27+ (%)	22.15 [13.48–30.75]
CD8+/CD38- (x 10^9/l)	0.27 [0.17–0.5]
CD8+/CD38- (%)	83.35 [70.45–90.57]
CD8+/CD38+ (x 10^9/l)	0.05 [0.02–0.1]
CD8+/CD38+ (%)	16.65 [9.42–29.55]

Before treatment initiation, CRP ($p = 0.003$) and suPAR ($p = 0.01$) levels were significantly higher in the nonconversion group than in the conversion group, but sICAM-1 levels did not differ between the two groups. CRP, suPAR, and sICAM-1 levels were also measured at different time points (baseline and after 1 and 5 months of treatment) according to a general linear model (Table 2).

During general linear modeling, we found that CRP values varied significantly depending on the treatment period (Wilks' lambda; $F = 0.61$; $p < 0.001$) and interception between the time period and conversion (Wilks' lambda; $F = 0.85$; $p = 0.05$). Bonferroni post hoc test revealed that CRP values differed significantly among all tested time points ($p < 0.05$ for all cases). Levels of sICAM-1 and suPAR in general linear modeling were low as a result of laboratory errors after 5 months of treatment. Because of these limitations, we did not find statistically significant differences between the conversion and nonconversion groups.

While analyzing T-cell subpopulations, we observed statistically significant differences in the absolute numbers of total lymphocytes, and the levels of two T-cell subpopulations, $CD4^+/CD45RA^-/CD27^+$ ($p = 0.007$) and $CD8^+/CD45RA^+/CD27^+$ ($p = 0.001$), were higher in the conversion group. There were also statistically significant differences in the percentage of total lymphocytes ($p = 0.005$) and percentages of these T-cell subpopulations: CD4+/CD45RA-/CD27- ($p = 0.002$); CD4+/CD45RA-/CD27+ ($p = 0.007$); CD8+/CD45RA+/CD27+ ($p = 0.008$); CD8+/CD45RA-/CD27- ($p = 0.001$). Higher levels of CD4+/CD45RA-/CD27-; CD8+/CD45RA-/CD27 were found in non-conversion group.

When analyzing T-cell subpopulations using general linear models for repeated measures, we observed significant variations, depending on the treatment period, in the absolute numbers of total lymphocytes (Wilks' lambda; $F = 0.87$; $p = 0.012$) and the total number of lymphocytes in the following T-cell subpopulations: $CD3^+$ ($F = 0.85$; $p = 0.007$); $CD4^+$ ($F = 0.33$; $p < 0.001$); $CD4^+/CD45RA^+/CD27^-$ ($F = 0.817$; $p = 0.012$); $CD4^+/CD45RA^+/CD27^+$ ($F = 0.9$; $p = 0.031$); $CD4^+/CD45RA^-/CD27^+$ ($F = 0.858$; $p = 0.009$); and $CD8^+/CD38^+$ ($F = 0.769$; $p = 0.001$). Also, depending on treatment period, the percentages of total lymphocytes varied significantly ($F = 0.44$; $p < 0.001$), as did the percentages of the following T-cell subpopulations: $CD3^+$ ($F = 0.88$; $p = 0.017$); $CD8^+$ ($F = 0.85$; $p = 0.007$); $CD4^+/CD45RA^+/CD27^-$ ($F = 0.866$; $p = 0.034$); $CD4^+/CD45RA^-/CD27^-$ ($F = 0.576$; $p < 0.001$); and $CD8^+/CD38^-$ ($F = 0.652$; $p < 0.001$). The general linear model analysis included 27 patients without sputum culture conversion and 20 patients with sputum culture conversion. In regression analysis, none of the host biomarkers or T-cell subpopulations showed a statistically significant effect on the

Table 2
Biomarker levels in different conversion groups at different time points during the treatment of drug-sensitive pulmonary tuberculosis. General linear model for repeated measures. Only data from patients without missing variables were included.

Biomarker	Treatment period	Conversion group (N = 17)		Non conversion group (N = 24)	
		Mean	St. deviation	Mean	St. deviation
CRP	Baseline	30,67	41,14	74,71	67,27
	After 1 month	17,41	29,84	30,58	29,23
	After 5 months	9,8	15,26	8,22	9,67
sICAM-1	Baseline	Conversion group (N = 1)		Non conversion group (N = 4)	
		57,52	–	65,84	10,6
	After 1 month	55,87	–	63,19	7,12
	After 5 months	48,38	–	58,44	2,35
suPAR	Baseline	Conversion group (N = 4)		Non conversion group (N = 10)	
		2038,23	746,56	4047,7	2403,24
	After 1 month	1678,6	190,9	2543,51	930,16
	After 5 months	1936,77	219,27	2374,89	602,96

conversion status.

3.2. Prediction of treatment outcomes

Of the patients with TB, 56 eventually recovered, 5 died during treatment, and 1 was lost to follow-up. TB treatment failure and acquired drug resistance was not observed in any patients during the study.

Baseline CRP and suPAR levels were higher in patients who died ($p < 0.001$); no differences were noted in baseline sICAM-1 levels. The general linear model for repeated measures was used to analyze CRP, sICAM-1, and suPAR levels at different time points. Values at baseline and 1 month were analyzed. All deaths occurred before the fifth month of treatment; hence, values at the fifth month could not be determined in these cases. We found that CRP values varied significantly between patients who died and those who survived, depending on the treatment period (Wilks' lambda; $F = 0.685$; $p < 0.001$), but did not vary significantly with regard to the interval between the time period and conversion (Wilks lambda; $F = 0.892$; $p = 0.052$). The suPAR and sICAM-1 levels could not be measured in the patients who died.

The baseline levels of total lymphocytes ($p = 0.001$) and the absolute number of the following T-cell subpopulations were lower in patients with worse prognosis: $CD3^+$ ($p < 0.001$); $CD4^+$ ($p = 0.001$); $CD8^+$ ($p < 0.001$); $CD4^+/CD45RA^+/CD27^+$ ($p = 0.021$); $CD4^+/CD45RA^-/CD27^+$ ($p < 0.001$); $CD8^+/CD45RA^+/CD27^-$ ($p < 0.001$); $CD8^+/CD45RA^+/CD27^+$ ($p < 0.001$); $CD8^+/CD45RA^-/CD27^+$ ($p = 0.032$); and $CD8^+/CD38^-$ ($p < 0.001$). Comparisons of percentages of lymphocyte subpopulations revealed that the percentages of all lymphocytes and of $CD4^+/CD45RA^-/CD27^+$ ($p < 0.001$), $CD8^+/CD45RA^-/CD27^+$ ($p = 0.032$), and $CD8^+/CD38^-$ ($p < 0.001$) T-cell subpopulations were lower in patients with worse prognoses.

We were unable to use the general linear model for repeated-measures analysis of T-cell subpopulations because no measurements were made at the fifth month of treatment. In regression analysis, none of the host biomarkers or T-cell subpopulations showed a statistically significant effect on the conversion status.

3.3. Host biomarkers, T-cell subpopulations and extent of lung destruction

We found significant correlations between some T-cell subpopulations and X-ray scores before TB treatment initiation: when these T-cell subpopulations were smaller, lung destruction was more pronounced (Table 3). Correlations of total lymphocyte percentages and X-ray scores before TB treatment initiation are depicted in Fig. 1.

Baseline CRP and suPAR levels were correlated with baseline lung destruction. X-ray scores were correlated with CRP (Spearman's rho, 0.762; $p < 0.001$) and suPAR (Spearman's rho, 0.468; $p = 0.002$) levels. sICAM-1 levels were not significantly correlated with lung destruction. The correlation between CRP levels and X-ray scores is depicted in Fig. 2.

Table 3
Statistically significant correlations of baseline T-cell subpopulations measured using flow cytometry with baseline X-ray scores in 62 patients with drug-sensitive pulmonary tuberculosis.

Baseline characteristics	Correlation coefficient (Spearman's rho)	P value
CD3+ (x 10^9/l)	−0.523	<0.001
CD3+ (%)	−0.556	<0.001
CD4+ (x 10^9/l)	−0.523	<0.001
CD8+ (x 10^9/l)	−0.456	0.001
CD4+/CD45RA-/CD27- (x 10^9/l)	−0.36	0.008
CD4+/CD45RA-/CD27+ (x 10^9/l)	−0.504	<0.001
CD8+/CD45RA+/CD27+ (x 10^9/l)	−0.488	<0.001
CD8+/CD45RA-/CD27+ (x 10^9/l)	−0.439	0.001
CD8+/CD38- (x 10^9/l)	−0.531	<0.001
CD8+/CD38- (%)	−0.687	<0.001
CD8+/CD38+ (%)	−0.687	<0.001

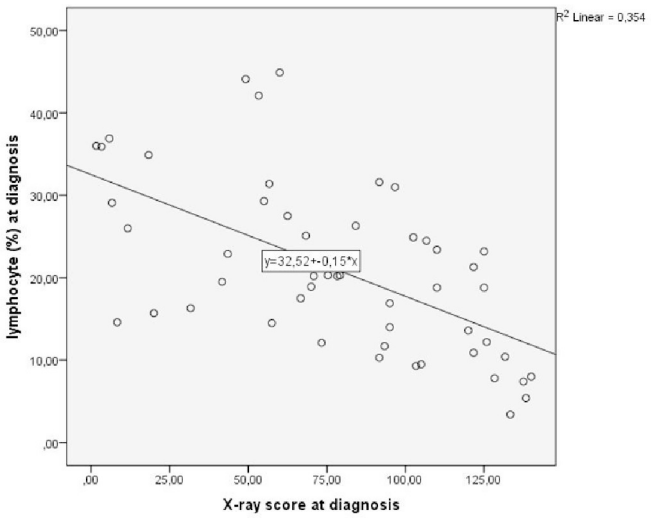

Fig. 1. Correlations of baseline total lymphocyte percentages and baseline X-ray scores in 62 patients with drug-sensitive pulmonary tuberculosis.

4. Discussion

Our exclusion criteria were chosen to avoid data reflecting laboratory test results and radiological changes resulting from or influenced by diseases other than TB.

CRP is possibly one of the most studied host biomarkers in pulmonary TB. CRP tends to increase most significantly from baseline as disease severity increases, is associated with week-8 culture status, and is overall a good indicator of the patient's clinical condition at the time of diagnosis [24]. We found similar results: CRP significantly decreased after 1 month of treatment, and its baseline levels were higher in patients who died and in the nonconversion group than in the conversion group. CRP was also strongly correlated with X-ray scores.

suPAR is not as well studied as CRP in pulmonary TB. However, some

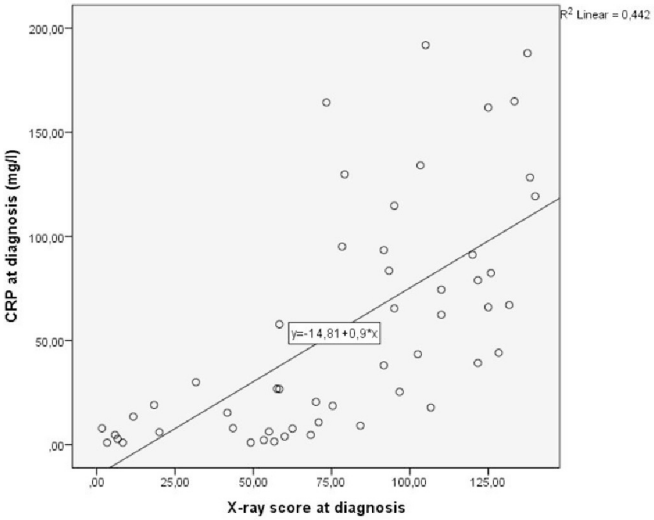

Fig. 2. Correlation of baseline C reactive protein and baseline x-ray score before in drug sensitive pulmonary tuberculosis patients who were included in the study in Romainiai tuberculosis hospital (n = 62).

published data have shown that suPAR levels increase in patients with bacterial infections [10], sepsis [11], lung cancer [12], exacerbated chronic obstructive pulmonary disease [24], and acute respiratory distress syndrome [25]. Our findings are similar to those of previously published studies [13,14]: suPAR levels tended to decrease after initiating appropriate TB treatment and were higher in patients with an unsuccessful treatment outcome.

Studies on sICAM-1 and suPAR with regard to TB diagnosis and TB treatment monitoring are lacking. We were also unable to find much published data concerning the relationship of T-cell subpopulations with the severity of TB or radiological evidence of the extent of lung damage. These were the main reasons that we wanted to conduct this study.

In our study, sICAM-1 baseline values were not correlated with baseline X-ray scores. We were unable to find any studies using sICAM-1 levels and X-ray scoring method proposed by Ralph et al. In some studies, a simpler radiological scoring method was used [1,6,26] to show the correlation of sICAM-1 levels with the radiological extent of lung damage in TB. When sICAM-1 was used in TB treatment monitoring, the results were contradictory [1,6]. Some of these conflicting results are probably attributable to the small patient populations in these studies.

Host T-cell responses are essential for an effective immune response to *M. tuberculosis*. To gage this response, T-cell phenotypes in the peripheral blood can be measured [15]. To evaluate response to TB treatment, some researchers have analyzed *in vitro* stimulated peripheral blood mononuclear cells to evaluate a specific response [27]. However, we decided to evaluate the nonspecific T-cell response to *M. tuberculosis* infection because testing this would be cheaper and easier to perform in clinical practice. The number of studies in which researchers evaluated nonspecific T-cell response to TB treatment is very limited [15,28,29], and we did not find any other studies in which flow cytometry results were analyzed as a factor in determining culture conversion. We did not find that T-cell subpopulation differences had a statistically significant effect on sputum conversion or treatment outcomes.

We were only able to find one validated chest X-ray score in the current literature on pulmonary TB, which could be used in monitoring radiological changes in the lungs during the treatment course [23]. In some studies, chest X-rays of patients with TB are evaluated using simpler methods, such as the presence of cavities [30]. When we used the scoring method of Ralph et al., we detected a significant correlation of X-ray scores with T-cell subpopulations, as previously mentioned. We could not find any other studies comparing T-cell subpopulations with radiological changes of active pulmonary TB. It would have been interesting to compare our results with those of other authors.

We believe that one of the strengths of our study is that it was a prospective study of in-hospital patients on DOT. Hence, we have no doubt that our patients were taking their medication and were not missing doses. All the patients were in the same environment, at least for the first month of the treatment, and were treated using the same methodology. All patients admitted to our hospital during the 2-year study were asked to participate, and the study population may represent the population of patients with TB in the Kaunas district. One of the limitations of this study was the relatively low number of participants. However, further study will be performed and will yield more data.

5. Conclusions

Selected host biomarkers play a very limited role in predicting TB treatment outcomes and culture conversion. suPAR and sICAM-1 levels did not appear to be superior to CRP levels for monitoring TB treatment. T-cell subpopulations and X-ray scores were correlated, but baseline lung destruction showed the highest correlation with baseline CRP levels.

Ethical statement

All patients were examined as part of a clinical research protocol approved by Kaunas Regional Biomedical Research Ethics Committee (BE-10–9 2015–10-09) and informed written consent was obtained from all participants.

CRediT authorship contribution statement

Greta Musteikienė: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Funding acquisition. **Skaidrius Miliauskas:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing - review & editing, Project administration, Funding acquisition. **Jurgita Zaveckienė:** Methodology, Investigation, Resources, Writing – review & editing. **Daiva Urbonienė:** Conceptualization, Methodology, Investigation, Resources, Writing - review & editing. **Astra Vitkauskienė:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition. **Marius Žemaitis:** Resources, Writing – review & editing, Project administration. **Albinas Naudžiūnas:** Resources, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, and revision of the manuscript. Furthermore, each author certifies that this material has not been and will not be submitted to or published in any other publication before its appearance in your journal.

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