Clinical and Experimental Immunology, 2026, **220**, uxaf084
https://doi.org/10.1093/cei/uxaf084
Advance access publication 22 December 2025
Research Article

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Development of a score derived from full blood count parameters to differentiate individuals with tuberculosis disease from those with tuberculosis infection

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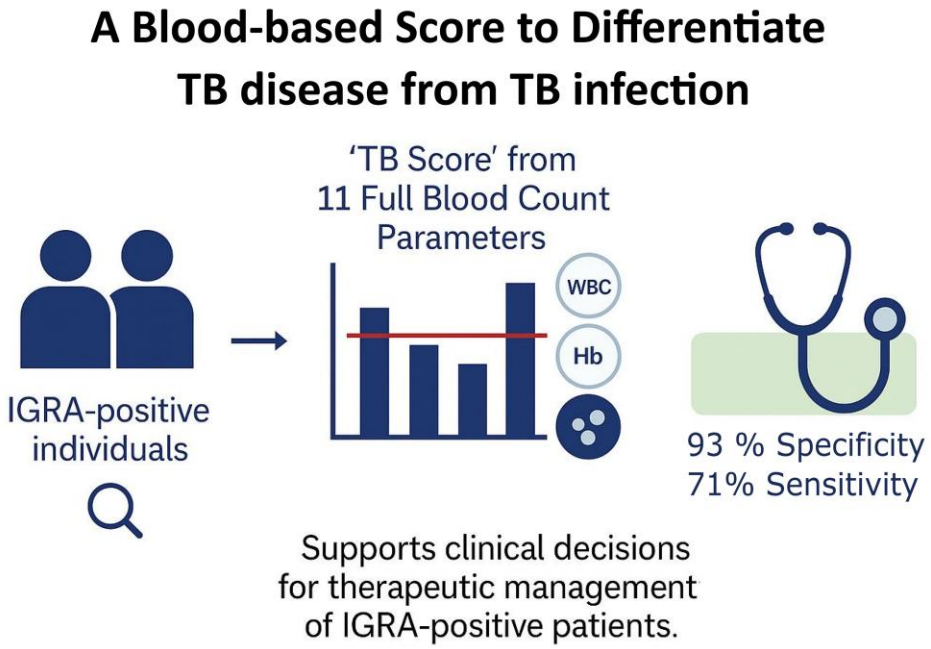
Abstract

In 2022, tuberculosis (TB) caused 1.3 million deaths worldwide, making it the second leading infectious cause of death. Diagnosing TB remains challenging because current immunological tests cannot distinguish between TB disease and TB infection (TBI). Research suggests that ratios such as monocyte-to-lymphocyte, neutrophil-to-lymphocyte, and platelet-to-lymphocyte, along with absolute counts of various blood cells, could help develop a low-cost and easy-to-use diagnostic tool to distinguish TB disease from TBI among IFN-γ release assay (IGRA)-positive subjects without relying on microbiological tests. We enrolled 112 TB-infected subjects and used blood cell count parameters and ratios to develop a TB score that can indicate TB status. We then validated the score in another cohort of IGRA-positive hospitalized patients. We developed a TB score based on 11 blood parameters to identify TB disease among IGRA-positive subjects, with 93% specificity and 71% sensitivity. This score can support physicians in making therapeutic decisions for IGRA-positive subjects, offering a practical approach to differentiate TB disease from TBI.

Received 7 August 2025; Revised 12 November 2025; Accepted for publication 21 November 2025

Graphical Abstract



Keywords: tuberculosis, myeloid cells, platelets, lymphocytes, TB score

Abbreviations: BCG, Bacillus Calmette-Guérin; EM, erythrocyte-to-monocyte; FBC, full blood count; HIV, human immunodeficiency virus; IGRA, IFN-γ release assay; ML, monocyte-to-lymphocyte; Mtb, Mycobacterium tuberculosis; NL, neutrophil-to-lymphocyte; NPV, negative predictive value; PE, platelet-to-erythrocyte; PL, platelet-to-lymphocyte; PPV, positive predictive value; PTB, pulmonary tuberculosis; QFT-PLUS, QuantiFERON®-TB Gold Plus; ROC, receiver operating characteristic; TB, tuberculosis; TBI, tuberculosis infection; WHO, World Health Organization.

Introduction

In 2023, the World Health Organization (WHO) reported 1.25 million tuberculosis (TB) estimated deaths, including 161 000 with human immunodeficiency virus (HIV) and 10.8 million people diagnosed with TB worldwide. Despite being curable and preventable, TB remains widespread. Multidrug-resistant TB is a major public health issue, with only 40% receiving treatment in 2022. Eliminating the TB epidemic by 2030 is a crucial target within the United Nations Sustainable Development Goals [1].

Diagnosing TB involves multiple complex steps and is often challenging because finding *Mycobacterium tuberculosis* (Mtb) or its DNA in biological samples represents the gold standard [2, 3]. The IGRA test, like QuantiFERON®-TB Gold Plus (QFT-PLUS), is a modern alternative to the tuberculin skin test and is not affected by Bacillus Calmette-Guérin (BCG) vaccination [4]. However, these tests cannot differentiate between TB disease and tuberculosis infection (TBI), leading to potential misdiagnosis or delays in treatment [5]. As both TB disease and TBI are prevalent in low-/middle-income countries, cost-effective and accessible tools to distinguish them are urgently needed.

TB affects hematopoiesis [6, 7], causing changes in blood cell counts. Typically, patients exhibit increased monocytes, neutrophils, and platelets, alongside reduced lymphocytes and erythrocytes [8], but values often remain within normal ranges and are nonspecific [9]. Hence, blood cell counts are not currently used to differentiate TB disease from TBI. However, studies consistently show elevated monocyte counts in TB disease patients compared to healthy individuals [10–12]. As circulating monocytes are macrophage precursors that, in turn, are central to Mtb immunity, their rise suggests an ongoing immune response and correlates with TB disease progression in exposed individuals [10]. Combining monocyte counts with other biomarkers, especially the monocyte-to-lymphocyte (ML) ratio, has shown promise in distinguishing TB disease from TBI [10, 12]. Additionally, monocyte-related gene signatures and the M/L ratio have been shown to be helpful in distinguishing TB disease from TBI [13].

Neutrophil counts also vary between TB disease and TBI. In TB disease, neutrophils are the predominant infected phagocytes in sputum and correlate with disease severity, particularly pulmonary damage and bacterial load [14, 15]. Conversely, in TBI subjects, neutrophil counts do not significantly correlate with TB severity [16], suggesting their different role in the pathogenesis of TB disease compared to TBI.

Lymphocyte counts are significantly lower in TB disease than in TBI or healthy controls [10, 16, 17], often accompanied by elevated monocytes and neutrophils, resulting in higher ML and neutrophil-to-lymphocyte (NL) ratios [10, 15]. In contrast, TBI subjects have normal lymphocyte counts like healthy controls [10, 16, 17]. The decreased lymphocyte count in TB disease patients is believed to impair adaptive immune responses, contributing to the disease's pathogenesis [16].

Platelet counts are markedly higher in TB disease and, when paired with reduced lymphocyte levels, result in elevated platelet-to-lymphocyte (PL) ratios. This increase in platelet count is a distinguishing feature of TB disease, helping to differentiate it from TBI [17–19]. In contrast, TBI subjects have a normal platelet count, which is not significantly different from healthy donors [18, 19]. The elevated platelet count in TB disease patients is thought to inhibit T-cell responses and contribute to the disease's pathogenesis [19, 20].

Table 1 Characteristics of the studied groups.

	TBI		TB disease		Total	
Enrolled subjects (%)	57	(50.9%)	55	(49.1%)	112	(100%)
Median age	46		38		42	
Range	21–76		22–76		21–76	
Male gender (%)	33	(58%)	42	(76%)	75	(67%)
Origin (%)						
Western Europe	43	(75%)	12	(21.8%)	55	(49.1%)
Eastern Europe	6	(10.9%)	22	(40%)	28	(25.0%)
Asia	1	(1.7%)	5	(9.2%)	6	(5.3%)
Africa[a]	5	(9%)	15	(27.2%)	20	(17.9%)
South America	2	(3.4%)	1	(1.8%)	3	(2.7%)
Comorbidities						
Cardiac disease	4	(7%)	5	(9.1%)	9	(8%)
Type II diabetes	2	(3.5%)	2	(3.6%)	4	(3.6%)
COPD	8	(14)	7	(12.7%)	15	(13.4%)
Anemia[b]	3	(5.3%)	9	(16.4%)	12	(10.7%)
Risk factors						
Alcohol abuse	4	(7%)	5	(9,1%)	9	(8%)
Smoking	18	(31,6%)	20	(36,4%)	38	(33.9%)
Household contact	5	(9%)	5	(9.1%)	10	(8.9%)
IGRA test (%)						
Positive	57	(100%)	52	(100%)	109	(97.3%)
Negative	0	(0%)	1	(1.8%)	1	(0.9%)
Indeterminate	0	(0%)	2	(3.6%)	2	(1.8%)
TB diagnosis (%)						
Microbiological diagnosis[c]			32	(58%)		
Smear positive			24	(44%)		
Molecular			31	(36%)		
Clinical			4	(7%)		
Pulmonary			50	(91%)		
Extra pulmonary			5	(9%)		

[a]Three TBI and 5 TB disease subjects of African origin had arrived in Italy less than one month.
[b]All TBI and TB disease subjects had mild anemia (Hb > 10 g/dl), except two TB disease patients who experienced moderate anemia (10 g/dl > Hb > 8 g/dl).
[c]Time to assess microbiological diagnosis varies from 9 to 59 days.

Red blood cell counts are lower in patients with pulmonary tuberculosis (PTB) than those with TBI [8]. Anemia is observed in 45.8–60% of TB cases and likely stems from malnutrition, malabsorption, or disease severity at diagnosis [21]. Macrophage-derived pro-inflammatory cytokines may also inhibit erythropoiesis and disrupt iron metabolism [22], while antituberculosis drugs can exacerbate anemia [22]. In contrast, TBI subjects do not exhibit these hematological changes [23]. In summary, PTB is commonly associated with anemia and reduced erythrocyte counts, likely due to the inflammatory effects of the disease on erythropoiesis and iron metabolism.

Several studies have already assessed the possible use of different parameters derived from complete blood cell count or cytokine production to categorize subjects TBI or TB disease. Of interest is the use of ratios between lymphocytes and myeloid cells such as monocytes or neutrophils [12, 19, 24–27].

While promising, these findings require further validation for routine clinical. use. According to this background, we assume that in TB disease, there is an appreciable decrease in lymphocytes and an increase in myeloid cells compared to TBI. This article aims to develop a scoring system (TB score) on routine CBC-derived immunological parameters to distinguish between TB disease and TBI in IGRA-positive subjects, even independently from microbiological and risk factors support. The score incorporates absolute counts of lymphocytes, monocytes, neutrophils, platelets, and erythrocytes, along with calculated ratios including ML, NL, PL, myeloid-to-lymphocyte, erythrocyte-to-monocyte (EM), and platelet-to-erythrocyte (PE). We aim to develop a tool with ≥80% sensitivity and specificity, enabling cost-effective, rapid TB assessment, particularly in resource-limited settings, thereby supporting timely diagnosis, improved patient outcomes, and enhanced public health interventions.

Materials and methods

Characteristics of enrolled individuals

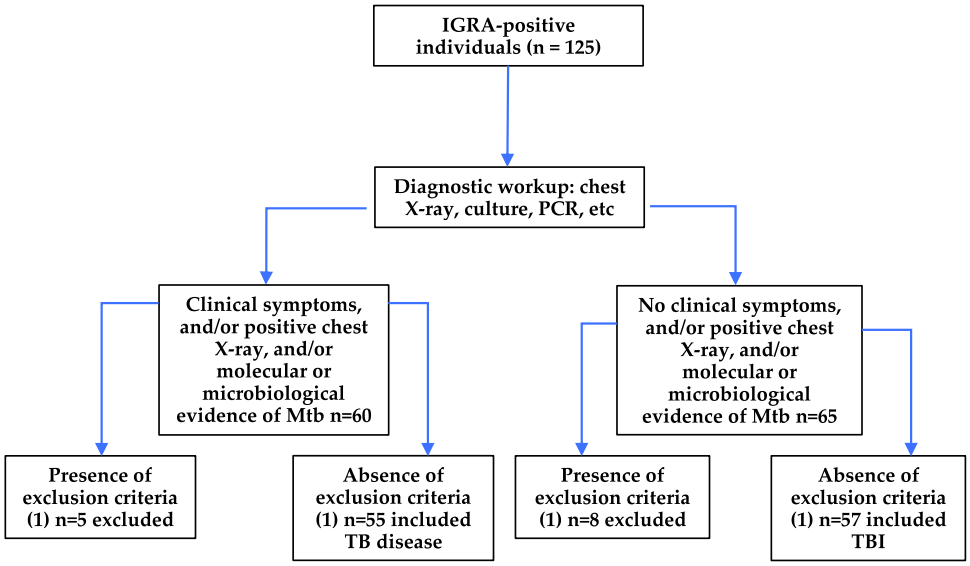
To develop the TB score, we retrospectively enrolled 112 individuals from a dataset of 125 IGRA-positive subjects comprising 55 patients with TB disease and 57 TBI; all the enrolled subjects were IGRA-positive. We collected demographic and clinical parameters relevant to TB diagnosis/infection, including age, sex, and risk factors such as origin from a TB-endemic country, immunodeficiency, history of TB, residence in high-risk communities, and alcoholism. Demographic characteristics of the enrolled participants are detailed in Table 1.

In accordance with WHO guidelines, TB disease diagnosis was based on a combination of clinical evaluation, microscopy, microbiological culture, molecular testing (GeneXpert, Sunnyvale, CA, USA), and X-ray findings.

In four cases, diagnosis was based on clinical presentation alone, including symptoms such as hemoptysis, recurrent night sweats (more than two episodes per month), persistent cough (≥2 weeks), pleuritic chest pain, or recurrent fever.

We excluded from the study patients with HIV infection or undergoing treatment with corticosteroids, immunosuppressants, or antitubercular drugs at the time of sampling, as indicated in the flow chart (Table 2).

Table 2 Flow chart of patients' selection.



(1) Exclusion criteria: HIV-positivity, treatment with corticosteroids, immunosuppressants, or anti-tubercular drugs at the time of sampling.

Individuals classified as having TBI were IGRA-positive without clinical symptoms and without radiological and microbiological evidence of TB disease.

Additionally, to validate the TB score, we enrolled 34 IGRA-positive individuals prior to a definitive clinical diagnosis of either TB disease or TBI. The characteristics of this validation cohort are presented in the corresponding results section.

The study was approved by the Ethics Committee of the University Hospital of Palermo (approval number 13/2013).

QFT-PLUS test

The QFT-PLUS test was performed according to the manufacturer's protocol. Briefly, blood samples were collected in the four tubes: Nil, TB1, TB2, and positive control, and gently inverted at least 10 times. The tubes were then incubated at 37°C for 18–20 hours. After incubation, the plasma was collected by centrifugation and stored at −20°C for up to 1 week or immediately used for the test. All plasma samples were tested using the QFT-PLUS test, and the results were analyzed and validated with QuantiFERON software provided by the manufacturer, available at www.quantiFERON.com. The spectrophotometer used to measure absorbance was set to a wavelength of 450 nm with a reference at 630 nm, following the manufacturer's instructions.

Quantitative analysis

Full blood counts (FBCs) of peripheral blood collected in ethylene-diamine tetra-acetic acid containing tubes were performed by one clinical diagnostic laboratory using a five-part differential hematology analyzer (Beckman Coulter 4.500, Brea, CA, United States). FBC measurement followed strict quality procedures, including twice-daily high and low internal quality control, fortnightly quality controls done by the clinical laboratory QC scheme, and annual quality assurance as part of the clinical laboratory QC scheme. The Italian National Accreditation System accredits the laboratory under international standards ISO 17025/2005 and ISO 15189/2007.

Statistical analysis

We studied our data starting with the Student *t* test and receiver operating characteristic (ROC) curve analysis. The Spearman rank test assessed the correlation between absolute values and ratio data. Based on the ROC analysis and Spearman Rank correlation, we built up our TB score by assigning a score of 1 for each parameter above the ROC analysis cutoff and 0 for the values below the cutoff. We assessed the specificity and sensitivity of our TB score using a contingency table and the following formulas: Sensitivity = true positive/total TB disease; specificity = true negative/total TBI; positive predictive value = true positive/total positive; negative predictive value (NPV) = true negative/total negative; accuracy = (true positive + true negative)/total tested. All the statistical analyses were performed using GraphPad Prism and Microsoft Excel.

Results

To build up our TB score, we first compared the absolute values of lymphocytes, monocytes, neutrophils, eosinophils, platelets, erythrocytes, and the sum of myeloid cells (Table 3). Most of these parameters showed significantly different values between TBI and TB disease; specifically, monocytes, neutrophils, and platelets numbers and myeloid cells sum were higher in TB disease than TBI (Fig. 1a-d), while lymphocyte numbers were lower (Fig. 1e); erythrocyte and eosinophil numbers did not display any significant difference between the two groups (Fig. 1f, g).

According to these results, we produced new parameters derived from the ratios between lymphocytes and myeloid cells or platelets. Moreover, we tested the EM and PE ratios, considering that other studies [12, 19, 24–27] reported an increase in platelets and a decrease in erythrocytes in TB disease. All the tested ratios displayed higher values in TB disease than in TBI (Fig. 2a-e), except the EM ratio, which was lower in TB disease than in TBI (Fig. 2f).

We performed a Spearman rank correlation between each ratio and its individual parameters to evaluate the specific contribution of single parameters in the ratios used. The results

Table 3 Absolute counts of blood cells/μl and descriptive statistics.

Cells		TBI	TB disease
Monocytes 1×10^3	Median	0.4800	0.7100
	25% percentile	0.4000	0.5400
	75% percentile	0.6000	0.9300
Neutrophils 1×10^3	Median	3.940	5.200
	25% percentile	3.400	4.075
	75% percentile	4.800	7.000
Myeloids 1×10^3	Median	2.350	4.395
	25% percentile	1.840	2.793
	75% percentile	2.990	6.373
Platelets 1×10^3	Median	230.0	328.0
	25% percentile	197.0	273.0
	75% percentile	274.0	391.0
Lymphocytes 1×10^3	Median	1880	1.500
	25% percentile	1.620	1.160
	75% percentile	2.450	2.015
Erythrocyte 1×10^6	Median	4.690	4.590
	25% percentile	4.340	4.110
	75% percentile	4.985	4.960
Eosinophils 1×10^3	Median	0.1800	0.1300
	25% percentile	0.1000	0.0750
	75% percentile	0.2200	0.2250

are displayed in Fig. 3. We observed a positive correlation between the ratio values and the values of neutrophils, monocytes, whole myeloid cells, and platelets (Fig. 3a-e).

At the same time, lymphocytes were always negatively correlated to the ratio values in which they are included, while erythrocyte values did not show any significant correlation (Fig. 3d, f).

The correlation data (Table 4) confirmed the pivotal impact of lymphocytes, myeloid cells, and platelets in discriminating TB disease from TBI. Erythrocyte values (r 0.170 P 0.2134) did not correlate with the EM ratio or display a low correlation (r −0.315 P 0.0193) with the PE ratio, meaning that monocytes and platelets are the main parameters correlated to the values of the respective ratios; in fact, correlations with the EM and PE ratios are very high (r −0.929 and r 0.879 for monocytes and platelets, respectively).

Then, for each absolute count and ratio, we performed a ROC curve analysis to assess a cutoff and evaluate sensitivity and specificity to decide whether they can be included in the TB score. The results of the ROC curve analysis are displayed in Table 4 and Fig. 4; the higher specificity was observed in the MY/L ratio, while the best sensitivity was observed in the PE ratio. The best area of ROC curve (AUC) was that of the PL ratio. In general, the ratios performed better than the absolute counts as predictive values, and among these, the platelet count was the best in our cohort.

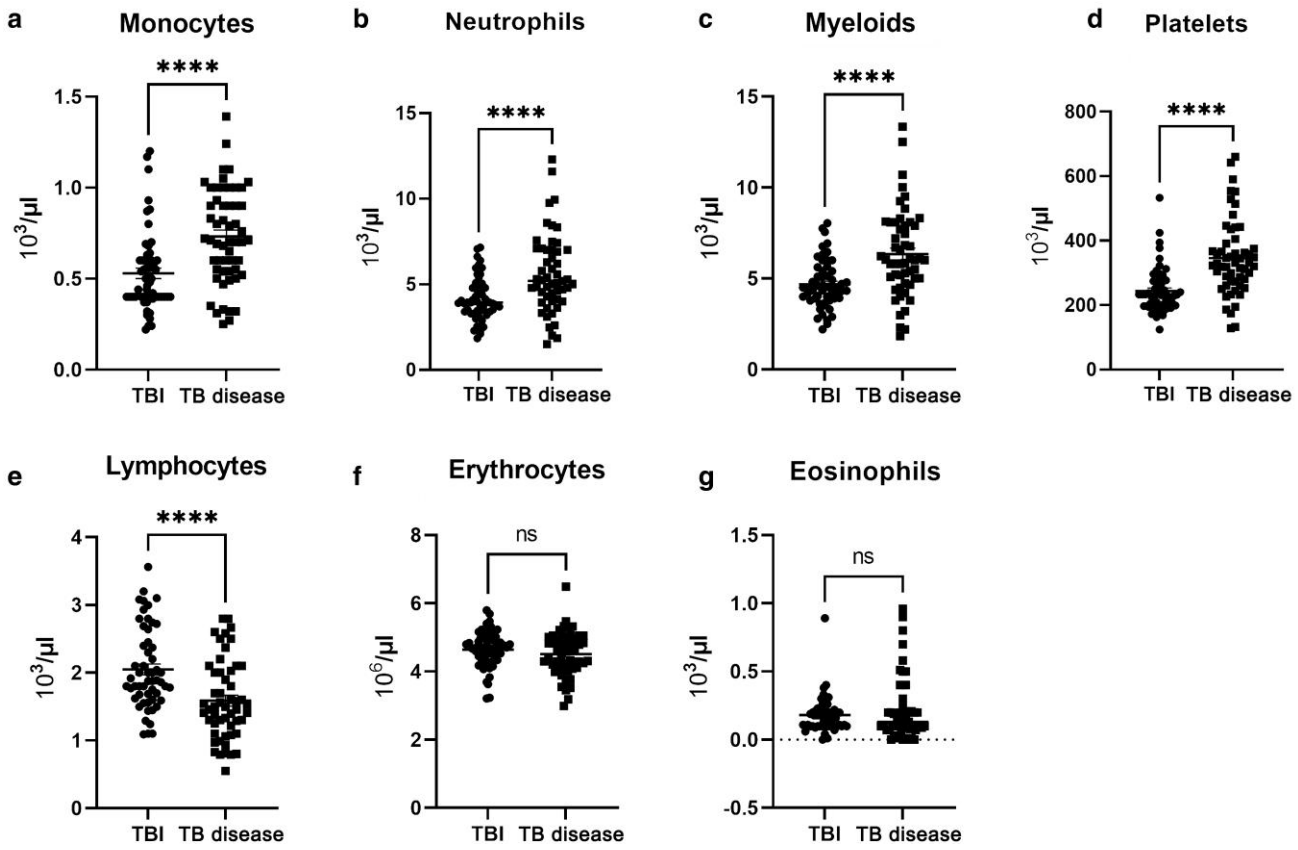


Figure 1 Comparison between TBI and TB disease of different absolute values from full blood cell count. (a) monocytes, (b) neutrophils, (c) total myeloid cells, (d) platelets, (e) lymphocytes, (f) erythrocytes, and (g) eosinophils. The graphs represent each parameter's mean and value distribution for TBI ($n = 57$) and TB disease ($n = 55$). The Student t test was used to compare the values of the two groups. **** = $P < 0.0001$.

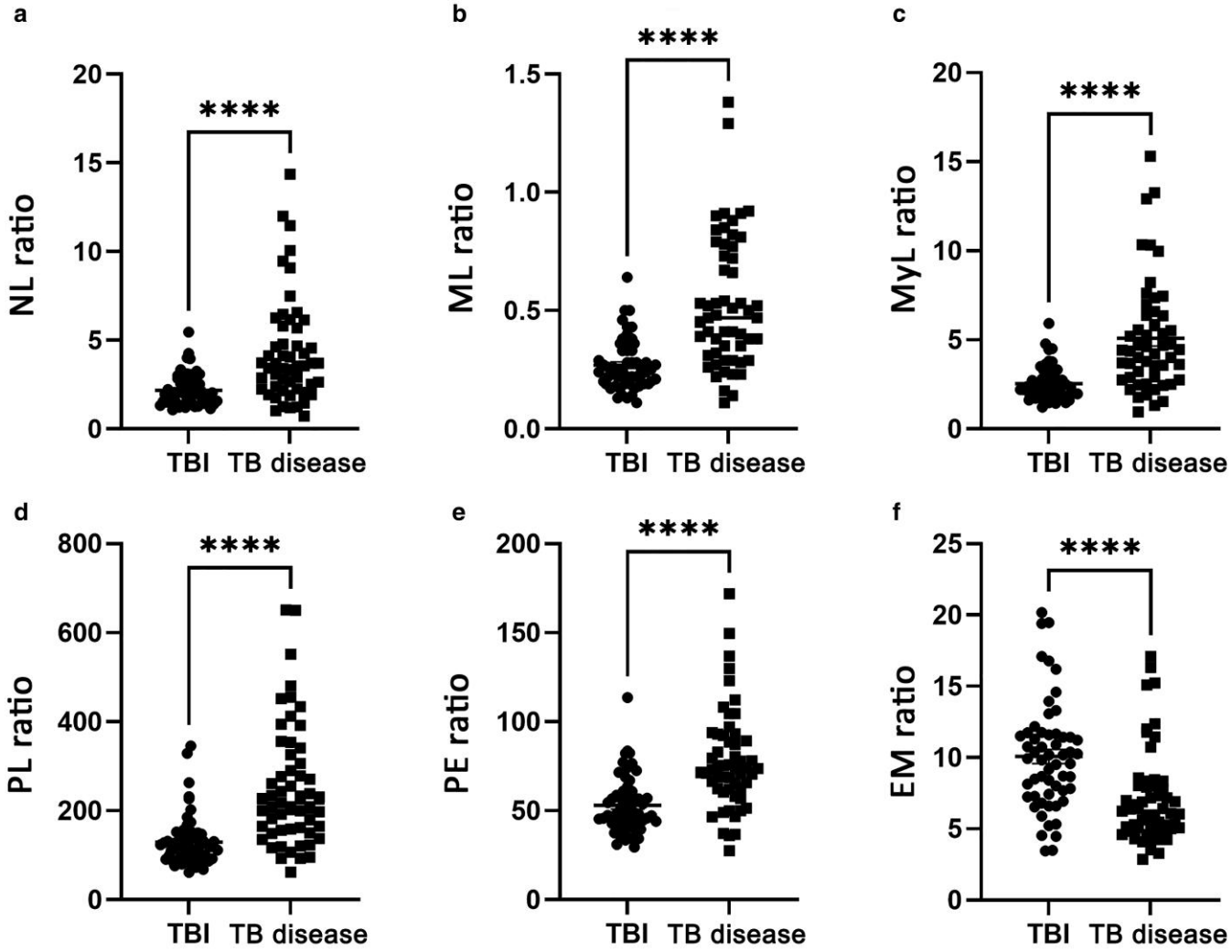


Figure 2 Evaluation of different ratios between TBI and TB disease. (a) NL, (b) ML, (c) MyL, (d) PL, (e) PE, (f) EM. The graphs represent each parameter's mean and value distribution for TBI ($n = 57$) and TB disease ($n = 55$). The Student t test was used to compare the values of the two groups. **** = $P < 0.0001$.

Based on the results of the parameter analysis, we decided to use the parameters reported in Table 5 to build our TB score. We used the cutoff obtained from the ROC analysis for each parameter and applied those cutoffs to our datasets for TB disease and TBI.

For each subject tested, we assigned a score of 1 to the parameters above the cutoff and 0 to those below the cutoff (Table 5). The sum of the scores represents the TB status of the subject and can range from 0 to 11. To adopt the best cutoff to distinguish between TBI and TB disease, we analyzed the TB score, along with the other parameters. First, we assessed the significant difference between TBI and TB disease (Fig. 5a), and then we used ROC analysis to establish the best cutoff for distinguishing the two groups (Fig. 5b).

Considering that the TB score should give a dichotomic result (positive or negative for the TB disease condition), we calculated the TB score sensitivity and specificity, using a 2×2 table, as reported in the statistical analysis paragraph of material and methods section. From our point of view, the TB score could provide a solid hypothesis of TB disease condition after a positive IGRA test, so we privilege the specificity rather than sensibility; for this reason, we settled a high value for the cutoff. A cutoff settled on 8 allows us to correctly classify 39 TB disease out of 55 and 53 TBI out of 57 (Table 1), with 71% sensitivity and 93% specificity. We also calculated the positive predictive value (PPV) at 91%, the NPV at 77%, and the accuracy of this test at 84%. The results shown in Table 6 confirmed the good performance of our TB score.

Compared to the other parameters analyzed, this score performed better, allowing a good result in distinguishing TBI from TB disease among the IGRA test-positive results. Of note, four out of the 16 TB disease patients negative for the TB score had a leukocyte count below the normal range ($< 4 \times 10^3$/µl); this parameter can be discriminant to decide whether to apply or not to apply the TB score. Importantly, all the components of the TB score are derived from a simple full blood cell count, ensuring no additional costs for its use.

Validation cohort results

To validate our clinical score, we enrolled an additional 34 IGRA-positive subjects. Twenty-eight patients displayed respiratory symptoms or fever and were hospitalized in the Infectious Diseases Unit of the University Hospital, but they

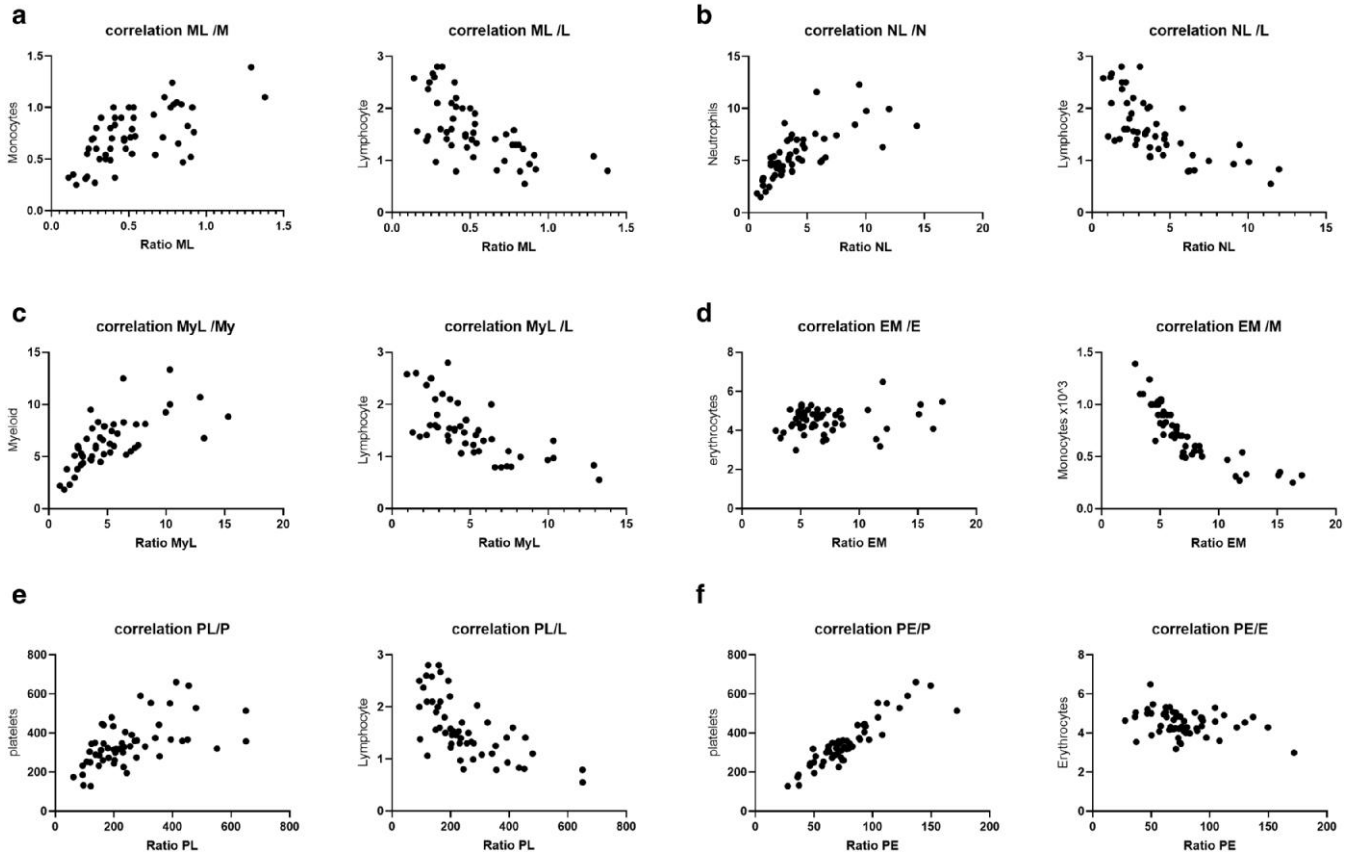


Figure 3 Correlation between each ratio and its components. (a) ML ratio and its correlation with monocyte (left) and lymphocyte (right) values; (b) NL ratio and its correlation with neutrophils (left) and lymphocyte (right) values; (c) MyL ratio and its correlation with the whole myeloid cells (left) and lymphocyte (right) values; (d) EM ratio and its correlation with erythrocyte (left) and monocyte (right) values; (e) PL ratio and its correlation with platelets (left) and lymphocyte (right) values; (f) PE ratio and its correlation with platelets (left) and erythrocyte (right) values. The Spearman rank correlation test analyzed the correlation between the ratios and absolute counts of their components.

Table 4 Correlation values between each ratio and its components.

	NL		ML		MyL		EM		PL		PE	
	N	L	M	L	My	L	E	M	P	L	P	E
Sp. r	0.754	−0.745	0.629	−0.659	0.7328	−0.746	0.170	−0.929	0.624	−0.693	0.879	−0.315
***P* val.**	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.2134	<0.0001	<0.0001	<0.0001	<0.0001	0.0193

All the myeloid cells positively correlate with the ratio values, except for monocytes in EM, which display an important negative correlation. On the contrary, lymphocytes negatively correlate in their ratios. Spearman r (Sp. r), *P* values (*P* val.) N = neutrophils; L = lymphocytes; M = monocytes; My = sum of all myeloid cells; E = erythrocytes; P = platelets.

had not yet been diagnosed with TB; four had TB disease and were at different weeks of antitubercular therapy, and two were household contacts with no symptoms. These subjects were used in a blind test to check if the TB score applied to their FBC could differentiate between TB disease and TBI or assess the efficacy of ongoing therapy. Clinical and microbiological data regarding the final diagnoses of these patients were later retrieved to confirm the TB score results. Table 7 displays the demographic data of the enrolled subjects, the Hb concentration, the leucocyte and platelet count, the results obtained with our score, the match with microbiological or molecular diagnosis, and, eventually, the time delay in weeks between microbiological results and TB score.

Among the 28 patients without a diagnosis, 16 were diagnosed with TB disease, while the other subjects were considered TBI. The TB score correctly indicated 14 out of 16 TB disease. Regarding the two TB disease patients missing, IDv 8 displayed a low number of platelets; this finding highlights the importance of platelet count as an inflammatory biomarker. Conversely, IDv 13 displayed a low leucocyte count, even if inside the normal range for each parameter, and a high platelet count. These two cases highlight the limitations of our TB score, which performs optimally only when both myeloid cells and platelets respond to the inflammatory signal characteristics of TB disease. It would be of high interest to analyze the causes that lead to the missing patient IDv8 to a low number of platelets, some kind of concomitant infectious disease or metabolic comorbidity is probably the main cause.

In three cases (IDv4, IDv9, and IDv14), TB score has anticipated microbiological diagnosis. The relatively low sensitivity did not allow TB score to identify all the TB disease, but it did not misclassify any TBI cases.

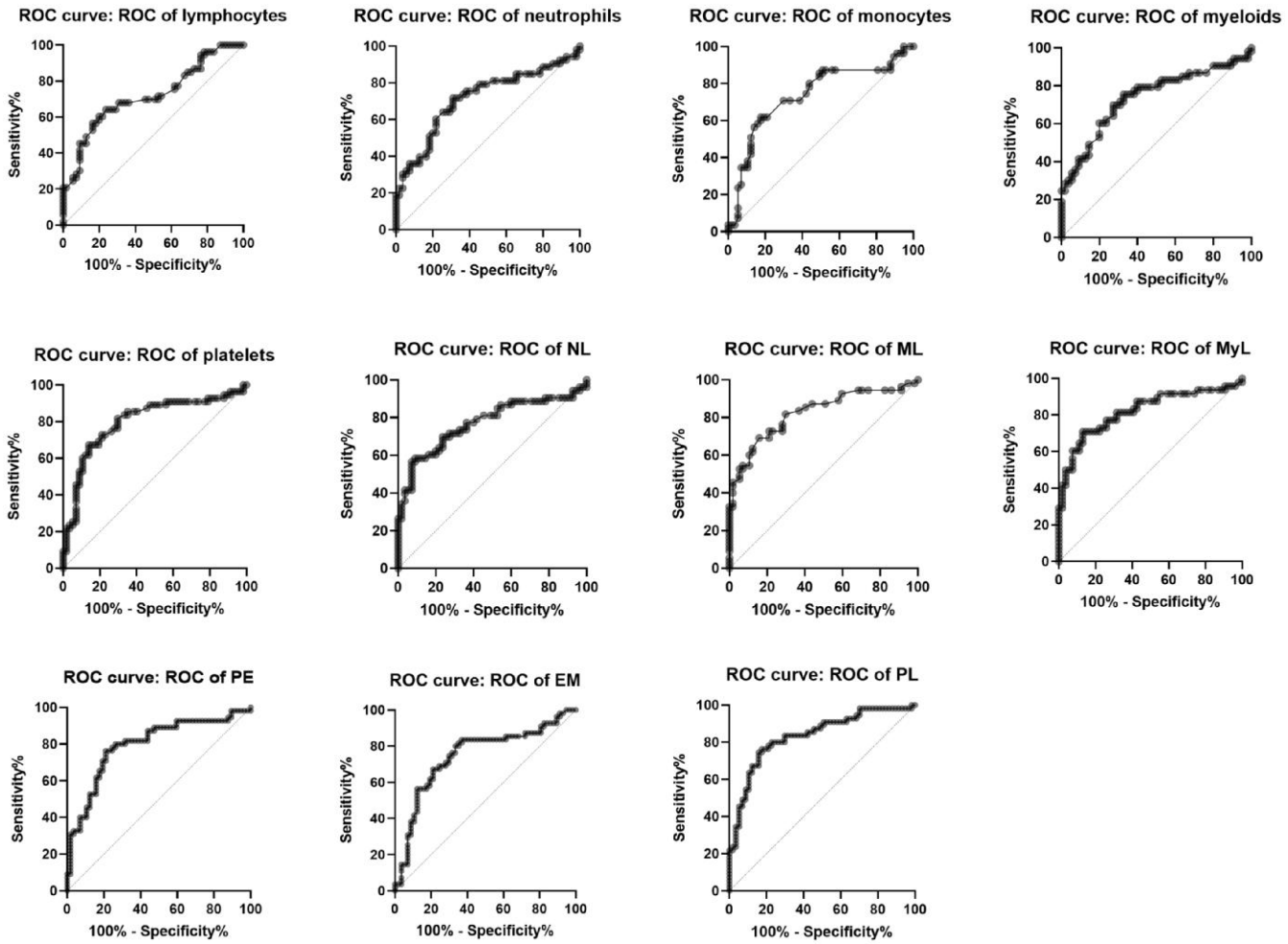


Figure 4 ROC curve analysis of the chosen parameters. The best performances were obtained by using the platelet and PL ratios. The *P* values of the ROC were all <0.0001 except for neutrophils, which had a $P = 0.0001$. The analysis was performed by GraphPad Prism software.

Of the four patients already in antitubercular therapy, three of them were in therapy for more than 2 months, so the inflammatory status that characterizes the TB disease was downregulated, and this affected the TB score, which was negative. These results suggest that TB score could potentially reflect and correlate with the efficacy of antitubercular therapy. This point is confirmed by the result of IDv18, a TB disease patient with less than 2 weeks of therapy, which displays a positive TB score.

Discussion

Understanding the immune responses that underlie protection from infection or progression to disease is essential to allow the development of diagnostic tools for the efficient prevention and management of TB [28].

Different studies have shown that mycobacterial infection can disrupt hematopoiesis [7], and the pathogen may also infect bone marrow mesenchymal stem cells [29, 30]. Research on humans and mice has demonstrated that subsets of hematopoietic stem cells exhibit distinct biases in the ratio of myeloid to lymphoid cells they produce [31, 32].

Significant differences were observed in the counts of monocytes, neutrophils, myeloid cells, and platelets, which were higher in TB disease patients compared to TBI patients. Conversely, lymphocytes and erythrocytes were lower in TB disease patients. These findings agree with several other papers [11, 12, 16, 18]. Particularly, monocyte absolute count, among the different parameters based on absolute count, distinguishes TBI from TB disease. The increase of myeloid cells can be explained by the ability of Mtb to affect the hematopoietic compartment, favoring myeloid cell production coupled with a reduction of lymphocyte populations [8]. Different reasons could explain this alteration. A paper based on the analysis of the whole-transcriptome microarrays in 10-week-old BCG-vaccinated and HIV-negative newborns displayed significant differences in the activation of the myeloid hematopoietic stem cell compartment, which was more prevalent among infants who developed TB, compared to those who did not develop the disease [33]. These findings are complementary to the study carried out on a cohort of 6.363 adolescents followed for over 2 years with periodic blood sample collections. In these subjects, it was observed that the progression from TBI to TB disease was preceded by several months of specific molecular changes. Before clinical signs of the disease appeared, there was a decrease in the levels of CD28 and CD79a mRNAs, which are associated with differentiation toward the T and B lymphocyte lineages, respectively. Additionally, there was an upregulation of mRNAs related to differentiation toward the myeloid lineage, coupled with an upregulation of the gene signature associated with a TB

Table 5 ROC curve analysis data.

	AUC	Cutoff	Sensitivity%	95% CI	Specificity%	95% CI	Likelihood ratio	
Neutrophils	0.7153	>	4.45	71.7	58.43% to 82.03%	69.09	55.97% to 79.72%	2.32
Monocytes	0.7356	>	0.595	70.91	57.86% to 81.23%	70.18	57.34% to 80.47%	2.378
Lymphocytes	0.7178	<	1.725	67.92	54.52% to 78.91%	69.09	55.97% to 79.72%	2.198
Myeloid cells	0.7369	>	5.095	71.7	58.43% to 82.03%	69.09	55.97% to 79.72%	2.32
Plt	0.8005	>	279	72.73	59.77% to 82.72%	78.95	66.71% to 87.53%	3.455
NL	0.771	>	2.525	71.7	58.43% to 82.03%	72.73	59.77% to 82.72%	2.629
ML	0.8265	>	0.345	72.73	59.77% to 82.72%	78.95	66.71% to 87.53%	3.455
MyL	0.8093	>	3.535	69.23	55.73% to 80.09%	87.27	75.98% to 93.70%	5.44
EM	0.749	<	7.37	69.09	55.97% to 79.72%	75.44	62.90% to 84.77%	2.813
PL	0.8338	>	158.3	74.55	61.70% to 84.19%	84.21	72.64% to 91.46%	4.721
PE	0.7971	>	61.62	76.36	63.65% to 85.63%	78.95	66.71% to 87.53%	3.627

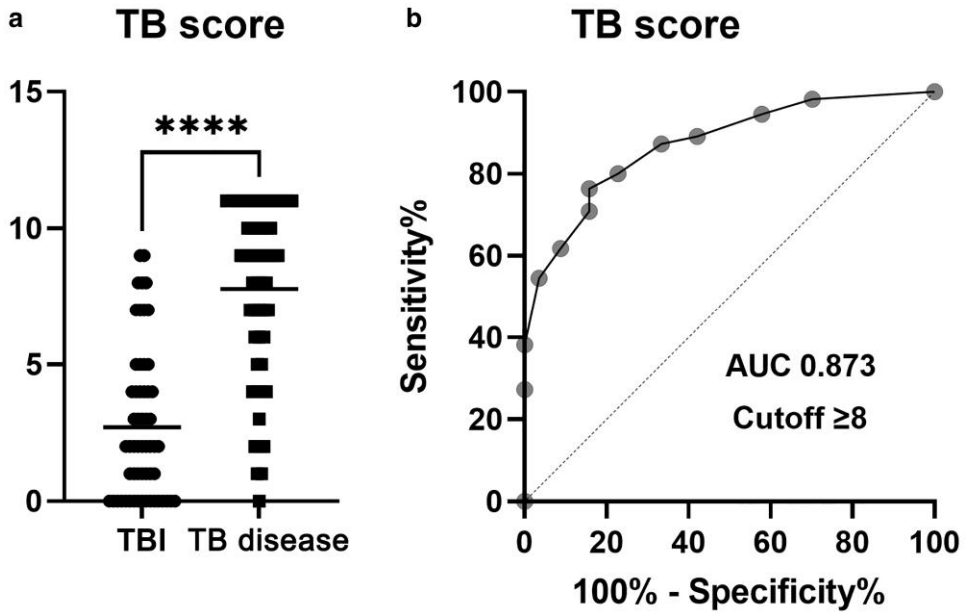


Figure 5 TB score evaluation between TBI and TB disease. (a) The Student *t* test of the TB score shows a significant difference between TBI and TB disease; (b) The TB score ROC curve analysis displays the best AUC compared to previously studied parameters.

risk that probably drove the unbalance between myeloid and lymphoid cells [13].

Infections can impact platelet production by megakaryocytes in the bone marrow, often leading to thrombocytosis due to inflammatory responses [34–36]. Cytokines such as IL-6 and TNF-α enhance platelet production by promoting megakaryocytopoiesis [37, 38]. In TB disease, elevated platelet counts and decreased lymphocyte counts are observed, resulting in a high PL ratio, similar to the ML and NL ratios [12, 39].

Several studies have demonstrated that the frequencies and absolute values of certain innate and adaptive immune cells can vary significantly between TB disease and TBI, either quantitatively or phenotypically, leading to the development of various methods and scoring systems to differentiate TB disease from TBI. Some of these approaches employ flow cytometry; in this context, we and others have shown that modulation of CD64 expression on monocytes can significantly distinguish between TBI and TB disease [12, 27].

Other cellular subsets, such as myeloid-derived suppressor cells and memory-like natural killer cells analyzed in peripheral blood, have also emerged as promising biomarkers for differentiating TBI from TB disease [25, 26].

However, assessing these markers requires laboratories equipped with specialized instruments and involves a more complex data analysis compared to methods based solely on complete blood counts.

Other studies, including ours, align with a line of research that leverages hematological data. In nearly all such studies, a noticeable increase in the myeloid compartment, relative to the lymphoid one, is observed in TB disease patients, reflecting the influence of Mtb on myelopoiesis [14–16]. In addition, the inflammatory state induced by TB is a distinguishing feature of TB disease compared to TBI. For this purpose, we examined levels of various cytokines [40], as well as platelet counts and their functional status [19].

In this study, we assume that the alteration of hemopoiesis can be mirrored, even if in a mild way, in the full blood cell count. In fact, starting from this point of view, we studied the possibility of exploiting the leukocyte, platelet, and erythrocyte absolute counts to assess a score for the orientation of physicians toward TB disease or TBI condition among IGRA-positive subjects.

The study introduced new parameters from the ratios between lymphocytes and myeloid cells or platelets. Most of these ratios

showed higher TB disease values than TBI, except for the EM ratio, which was lower in TB disease. A TB score was created using these parameters, where each parameter was assigned a score of 1 if above the cutoff and 0 if below. This score demonstrated a sensitivity of 71% and a specificity of 93% in distinguishing TB disease from TBI. This method offers a promising, low-cost, and easy-to-use tool for TB diagnosis using routine blood tests. In fact, the ratio can be easily calculated with software applications on the PC or the mobile phone. Thanks to its characteristics, this methodology can be useful in low-resource countries.

Obviously, this study has several limitations, starting from the number of subjects enrolled and the arbitrariness of the score criteria for assignment. Moreover, at 3% prevalence (typical of IGRA-positive subjects in contact investigation or other high-risk population screening), this score could display a very high negative NPV, that will be the strength for this tool, but a low PPV, suggesting that in most clinical settings where IGRA is used for TBI screening, a positive TB score could result in a high false-positive rate, potentially leading to unnecessary investigations or treatment. Nevertheless, this is an empiric-based method to help the physician better understand the underlying condition of the IGRA-positive subjects, especially hospitalized IGRA-positive patients. As other different scores, only the application can demonstrate this tool's usefulness or uselessness. Overall, this study highlights the potential of using specific blood

Table 6 Calculation of sensitivity, specificity, PPV, NPV, and accuracy of TB score.

	TB disease		
Test	TB disease = 55	TBI = 57	Total = 112
Positive	T.P. = 39	F.P. = 4	Total Pos. = 43
Negative	F.N. = 16	T.N. = 53	Total Neg. = 69
Total	Total TB disease = 55	Total TBI = 57	Total Tested = 112
Sensitivity	T.P./Total TB disease = 39/55 = 0.71		
Specificity	T.N./Total TBI = 53/57 = 0.93		
PPV	T.P./Total Pos. = 45/53 = 0.91		
NPV	T.N./Total Neg. = 53/69 = 0.77		
Accuracy	(T.P.+T.N.)/Total tested = (39 + 53)/ 112 = 0.82		

T.P.= true positive; F.N. = false negative; F.P. = false positive; T.N. = true negative

Table 7 Analysis of the subjects belonging to the validation cohort.

	Origin	Sex	Age years	Hb g/dl	Leuk. $\times 10^3$/µl	Plat. x 10^3/µl	ZN	Molecular assay	Mtb Culture	Weeks of delay between FBC and CP	TB score	Diagnosis
IDv1	Somalia	M	38	12.1	13.38	175	N	N	nd		N	TBI
IDv2	Guinea	F	20	9.10	5.50	84	N	N	nd		N	TBI
IDv3	Italy	M	78	12.0	8.76	212	N	N	nd		P	TB disease[a]
IDv4	Italy	F	57	10.5	13.20	670	N	N	P	11	P	TB disease
IDv5	Italy	M	35	13.8	6.67	245	P	P	P		P	TB disease
IDv6	Indonesia	F	47	14.5	4.82	229	N	N	N		N	TBI
IDv7	Bangladesh	M	21	15.7	8.14	308	N	N	N		N	TBI
IDv8	Italy	M	60	10.5	1.93	58	nd	N	N		N	TB disease[a]
IDv9	Gambia	M	37	10.7	9.52	330	P	P	P	21	P	TB disease
IDv10	Italy	F	64	13.0	4.00	106	P	P	P		N	TB in therapy[b]
IDv11	Italy	M	74	11.4	13.81	133	N	N	N		N	TBI
IDv12	Italy	F	79	10.4	8.57	281	N	P low	N		P	TB disease
IDv13	Italy	M	32	14.2	5.74	459	N	P low	P	26	N	TB disease
IDv14	Romania	F	40	8.80	11.90	565	N	P low	P	16	P	TB disease
IDv15	Italy	M	53	12.1	7.81	189	N	N	N		N	TB in therapy[b]
IDv16	Italy	M	67	13.8	7.18	201	N	N	N		N	TB in therapy[b]
IDv17	Syria	M	31	10.7	13.22	476	P	P	P		P	TB disease
IDv18	Italy	F	83	9.20	8.55	228	P	P	N		P	TB in therapy[c]
IDv19	Bangladesh[d]	M	20	15.7	8.28	366	N	P low	p		p	TB disease
IDv20	Gambia	M	17	13.2	6.97	340	N	N	N		N	TBI
IDv21	Italy	F	66	8.50	6.91	563	nd	nd	nd		N	TBI
IDv22	Bangladesh	M	45	11.6	11.66	235	P	P	P		P	TB disease
IDv23	Romania	F	40	14.0	9.22	277	N	N	N		N	TBI
IDv24	Senegal[d]	M	17	15.0	5.81	477	N	N	N		N	TBI
IDv25	Italy	F	36	9.3	5.96	472	N	N	nd		N	TBI
IDv26	Italy	M	71	11.6	6.42	197	nd	nd	nd		N	TBI
IDv27	Nigeria	M	41	8.4	10.31	502	N	N	N		N	TBI
IDv28	Italy	F	54	12.4	13.30	342	P	nd	nd		P	TB disease
IDv29	Nigeria	M	25	15.0	9.77	346	N	P	P		P	TB disease
IDv30	Ghana	M	44	8.9	10.61	310	nd	nd	P		P	TB disease
IDv31	Italy	M	47	16.3	9.92	264	N	N	N		N	TBI[e]
IDv32	Nigeria[d]	M	24	14.0	4.24	229	N	N	N		N	TBI
IDv33	Italy	M	56	13.1	12.39	564	P	P	P		P	TB disease
IDv34	Italy	M	61	14.3	10.06	173	nd	nd	nd		N	TBI[e]

ZN = Ziehl-Neelsen; FBC = full blood count; Leuk. = leukocytes, Plat. = platelets, CP = culture positivity; N = negative result; P = positive result; nd = not done; the bold letters represent the missing positivity of TB score in TB disease patients.
[a]Clinical diagnosis.
[b]More than 6 weeks of antitubercular therapy.
[c]Less than 2 weeks of antitubercular therapy
[d]Less than one month in Italy.
[e]Not hospitalized.

cell counts and derived ratios as biomarkers for distinguishing between TB disease and TBI, providing feasible support to more complex and expensive diagnostic methods.

Acknowledgements

Not applicable.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University Hospital in Palermo, approval number 13/2013. Patient consent was waived due to the use of their demographic laboratory data in an anonymous form.

Conflicts of interest

The authors declare no conflicts of interest.

Funding

This research was funded by the European Union-Next Generation EU (MUR PRIN PNRR grant number P202295SHW).

Author contributions

Giusto Davide Badami, Formal analysis, Investigation, Data curation, Writing—original draft; Bartolo Tamburini, Methodology, Formal analysis, Data curation, Writing original draft; Miriana Fallo, Data curation, Methodology; Mojtaba Shekarkar Azgomi, Formal analysis, Investigation; Methodology; Marco Pio La Manna, Methodology, Formal analysis, Data curation, Writing—original draft, Francesco Dieli, Conceptualization, Writing—review & editing, Supervision; Nadia Caccamo, Conceptualization, Resources, Writing—original draft, Supervision.

Data availability

Data presented in this study are available upon request from the corresponding author.

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Not applicable.

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