Mycobacterium tuberculosis-specific cytokine biomarkers for the diagnosis of childhood TB in a TB-endemic setting

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ABSTRACT

The tuberculin skin test and interferon-gamma release assays have limitations in diagnosing tuberculosis (TB), particularly in children. This study investigated the performance of candidate *M. tuberculosis*-specific cytokine biomarkers for TB in children in a TB-endemic setting. A total of 237 children with a household contact with smear-positive pulmonary TB were recruited. Importantly, a group of children with illnesses other than TB (sick controls) was included to assess specificity.

Median IFN-ɣ, IL-1ra, IL-2, IL-13, IP-10, MIP-1β and TNF-α responses were significantly higher in children with active TB and latent TB infection (LTBI) than in both healthy and sick control children. Three of these cytokines – IL-2, IL-13 and IP-10 – showed better performance characteristics than IFN-ɣ, with IL-2 achieving positive and negative predictive values of 97.7% and 90.7%, respectively. Furthermore, IL-1ra and TNF-α responses differed significantly between active TB and LTBI cases, suggesting that they may be stage-specific biomarkers.

Our data indicate that incorporating these biomarkers into future blood-based TB assays could result in substantial performance gains.

1. Introduction

Tuberculosis (TB) is responsible for the greatest number of deaths from a single infectious disease worldwide [1,2]. Of the 10 million new cases of active TB each year, approximately 10% occur in children less than 15 years of age, resulting in an estimated 80,000 deaths [1,3]. For most children, the source of infection is a close contact, usually a household member [1]. The proportion of children infected with latent TB infection (LTBI) who subsequently progress to active disease is significantly greater than in adults [4]. In its 'End TB Strategy', the World Health Organization emphasises the need to target the most vulnerable groups, such as children with LTBI, to successfully halt the global TB pandemic [5,6].

Diagnosis of LTBI remains a significant challenge, especially in children [7]. The diagnosis of LTBI currently relies on a positive immuno-diagnostic test result, namely a tuberculin skin test (TST) and/or interferon (IFN)-ɣ release assay (IGRA). The TST has been in use for close to a century and is still the mainstay for the diagnosis of LTBI in most parts of the world [8,9]. In addition to significant inter-operator variability in measuring and interpretation of the test result, the test has poor specificity due to the test reagent, purified protein derivative (PPD), comprising over 200 peptides, many of which are shared between mycobacterial species, including Bacille Calmette-Guérin (BCG) vaccine strains [10,11]. In contrast, commercial IGRAs in current use

rely on the use of two relatively *M. tuberculosis*-specific antigens, ESAT-6 and CFP-10, which are absent in BCG, and are therefore thought to be more specific than the TST, particularly in BCG-vaccinated individuals [12–17]. However, substantial data show that IGRAs have suboptimal sensitivity in the setting of active TB [18,19], and there is considerable evidence that IGRAs perform worse in children compared to adults [20–23]. A recent longitudinal study in a high TB prevalence setting suggests that IGRAs are less sensitive than TSTs for the detection of LTBI [24]. The updated version of the QFT assay, QuantiFERON-TB Gold Plus, may perform better, but the available data in children currently remain limited [25–27].

The World Health Organization has called for the development of new diagnostic tests to address these shortcomings and allow more accurate diagnosis of TB in children. Biomarkers other than IFN-γ have shown promise for the diagnosis of both LTBI and active TB [16,28–31], but the vast majority of previous studies have not included sick control participants (i.e. patients with infections other than TB) with symptoms that resemble TB. Therefore, the specificity of these biomarkers is currently uncertain.

In this study, we aimed to detemine the performance, in particular the specificity, and positive and negative predictive values, of previously identified candidate *M. tuberculosis*-specific cytokine biomarkers for the diagnosis of LTBI and active TB in children in a TB-endemic setting.

2. Methods

2.1. Participants

Infants and children less than 15 years of age who were household contacts of a TB index case were recruited from 27 community health centres in the district of San Juan de Miraflores in Lima, Peru, between January and December 2014. An index case was defined as an adult diagnosed with smear-positive pulmonary TB. Exclusion criteria comprised: immunocompromise (including known HIV infection); index case with multi-drug resistant TB; previous anti-tuberculous therapy; current antibiotic treatment; clinically unstable or requiring immediate medical management; and inability to obtain informed consent from parents or guardians. Demographic data, clinical history and investigation findings were recorded by trained study nurses using a standardised data collection form.

2.2. Management of participants

Participants were managed according to the Peruvian Ministry of Health guidelines, which recommend a TST for all household contacts, and a chest radiograph and sputum sample at the discretion of the physician when clinically indicated. In line with these guidelines, participants diagnosed with LTBI and all participants under five years of age were started on a 6-month course of isoniazid preventive therapy. Participants diagnosed with active TB were treated with anti-tuberculous therapy guided by drug susceptibility testing.

2.3. Tuberculin skin tests

All participants had a TST administered by intradermal injection of 0.1 ml of Tubersol (5 Tuberculin Units PPD-S) into the volar surface of the forearm with any induration measured after 48 to 72 h. Nurses working in the community health centres were specifically trained to standardise TST placement and interpretation.

2.4. Blood sample collection

For cytokine assays, 3 ml of blood were collected into QuantiFERON-TB Gold In-Tube (QFT-GIT) tubes (Cellestis/Qiagen, Carnegie, Australia). The QFT-GIT samples, were incubated overnight at 37°C in the Immunology Laboratory of the Instituto de Medicina Tropical Alexander von Humboldt at the Universidad Peruana Cayetano Heredia (UPCH) laboratory and, after centrifugation and harvesting according to the manufacturer's instructions, plasma supernatants were cryopreserved. Samples were stored at $-70\,°C$ and shipped frozen to Melbourne for cytokine measurement. QFT-GIT IFN-γ assays were measured by ELISA at the Victorian Infectious Diseases Reference Laboratories in accordance with the manufacturer's instructions.

2.5. Cytokine analyses

Cytokine concentrations in supernatants were measured using Bioplex Pro Human Cytokine Group 1 assay (Bio-Rad Laboratories, Hercules, CA, U.S.) according to the manufacturer's instructions, with a Luminex 200 analyser (Luminex Corp., Austin, TX, U.S.). Based on previous optimisation experiments (data not shown), IFN-γ, interleukin (IL)-10, IL-13, IL-1ra, IL-2 and tumour necrosis factor (TNF)-α were analysed in 1/4 diluted samples with a customised 6-plex assay, and IFN-inducible protein-10 (IP-10) and macrophage inflammatory protein-1β (MIP-1β) were analysed in 1/100 diluted samples with a customised 2-plex assay. The laboratory scientists analysing the samples were blind to the participant category.

2.6. Categorisation of participants and study definitions

Participants were categorised into four groups based on their symptoms, TST result, IGRA result and chest radiograph results as detailed in Fig. 1. In brief, i) participants with a positive TST (defined as an induration diameter ≥10 mm) or positive IGRA result, no symptoms or signs and a normal chest radiograph (if done) were categorised as LTBI; ii) those with negative TST and IGRA results, symptoms consistent with active TB (fever, cough, weight loss or night sweats) and a physician-designated alternative diagnosis and not started on TB treatment were categorised as sick controls; iii) those with a negative TST and IGRA and no symptoms were categorised as healthy controls; iv) those with a positive TST or IGRA and radiological findings consistent with active TB were categorised as probable active TB. Excluded from the final analyses were the remaining participants: a) in whom no IGRA was performed or the IGRA produced an indeterminate result, b) in whom insufficient data regarding symptoms were recorded, and c) who had a positive TST or IGRA result, symptoms consistent with active TB and a normal chest X-ray (as those participants could have either had LTBI but be presenting with an alternative illness or early active TB) to avoid data contamination introduced by participants with uncertain disease category.

2.7. Statistical analysis

Comparisons of continuous variables between multiple groups were done using Kruskal-Wallis tests. In instances where the Kruskal-Wallis *p*-value was less than 0.1, indicating a potential difference between the groups, additional two-group comparisons were done using Mann-Whitney *U* tests.

Cytokine concentrations were background-corrected for analysis (by subtracting the concentration measured in the negative (nil) control sample from the concentration measured in the MTB antigen-stimulated sample). Following analysis of receiver operating characteristics, optimal cut-offs were determined for each cytokine by using the Youden index. Analyses were done using Stata V14 (StataCorp, College Station, TX) and Prism V7 (GraphPad Software Inc., La Jolla, CA); figures were constructed with Prism.

2.8. Ethical approval

The study was approved by the Royal Children's Hospital Human Research Ethics Committee (34251) and by the Comité Institucional de
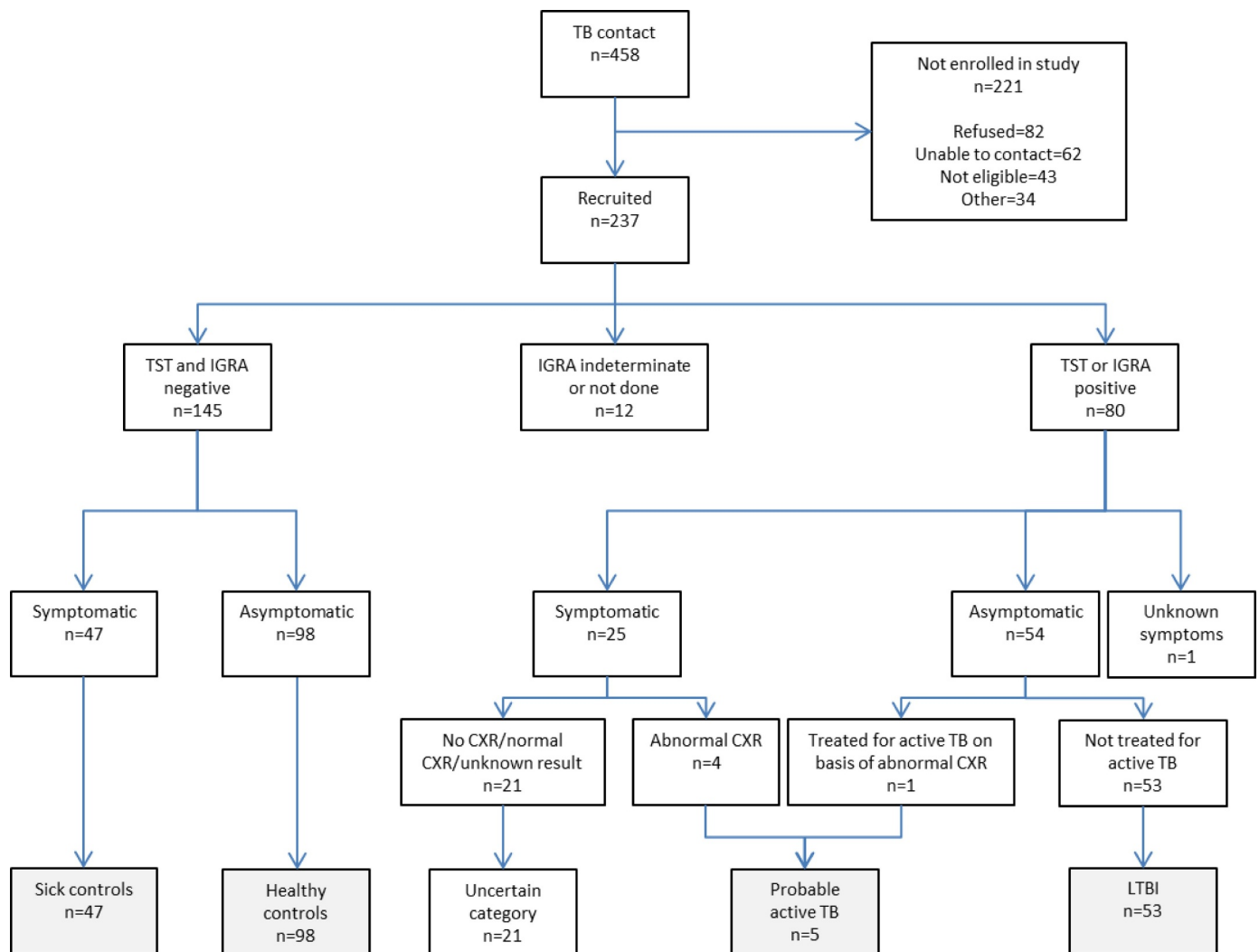
Fig. 1. Flow diagram outlining the categorisation of study participants.

3. Results

3.1. Participants

Of 458 children who were household contacts of an index case with pulmonary TB, 237 were recruited (Fig. 1). A total of 203 participants could be unambiguously categorised (53 LTBI, 98 healthy controls, 47 sick controls and 5 probable active TB) and were included in the final analysis. The 34 participants who could not be categorised unambiguously (12 IGRAs indeterminate or not done; 1 symptoms unrecorded; 21 symptomatic with positive TST or IGRA but normal chest radiograph) were excluded. Table 1 shows the demographic and other details of the 203 included participants and their TST and IGRA results are shown in Fig. 2. Table 2 shows the demographic and other details of the adult index cases with whom the participants had household contact. In comparison with participants in the healthy or sick control group, a higher proportion of those with LTBI had their mother as the index case and shared a bedroom with the index case.

3.2. Mycobacteria-specific cytokine responses in supernatants

There were significant differences between the four groups of participants in the median cytokine concentrations in the MTB antigen (ESAT-6, CFP-10 and TB7.7)-stimulated sample for seven of the eight cytokines: IFN-γ, IL-1ra, IL-2, IL-13, IP-10, MIP-1β and TNF-α (Table 3, Fig. 3). IL-10 responses did not differ significantly between the groups and were therefore not analysed further. Overall, median concentrations of these cytokines were highest in the probable active TB group, followed by the LTBI group. The lowest median concentrations were observed in the sick control group for all cytokines except IP-10. These results were not appreciably affected by: i) participants who had a failed negative control (high cytokine concentration in the nil sample) or failed positive control (low cytokine concentration in the mitogen-stimulated sample), ii) cytokine results that were potentially outliers, or iii) analysis without background correction.

Compared with healthy controls, sick controls had lower median IL-1ra levels but there were no other significant differences in MTB antigen-induced cytokine responses between these two groups (Table 3, Fig. 3). Median concentrations of IFN-γ, IL-1ra, IL-2, IL-13, IP-10, MIP-1β and TNF-α were higher in the LTBI group compared with the healthy control group. These comparisons were statistically significant with p-values below 0.0005 for all cytokines except TNF-α. Median

Table 1
Demographic and other features of participants (child contacts).

Participant at enrolment	Healthy controls (n = 98)	Sick controls (n = 47)	LTBI (n = 53)	Active TB (n = 5)	Total (n = 203)
Age (years)					
Mean	7.1	7.0	8.4	7.4	7.4
Range	0.2-14.3	0.4-14.5	0.4-14.9	3-14.3	0.2-14.9
Under 5 years	35 (36%)	20 (43%)	14 (26%)	3 (60%)	72 (35%)
Sex					
Male	44 (45%)	25 (53%)	25 (47%)	3 (60%)	97 (48%)
Other household members with TB in the last 5 years	42 (43%)	11 (23%)	32 (60%)	3 (60%)	88% (43%)
Symptoms in the last two weeks					
Fever	0	17 (36%)	0	2 (40%)	19 (9%)
Cough	0	34 (72%)	0	4 (80%)	38 (19%)
Night sweats	0	7 (15%)	0	2 (40%)	9 (4%)
Weight loss	0	4 (9%)	0	1 (20%)	5 (2%)
BCG immunisation					
Immunisation card	24 (25%)	22 (47%)	11 (21%)	2 (40%)	59 (29%)
Scar	95 (97%)	43 (91%)	49 (92%)	5 (100%)	192 (95%)
TST induration (mm)					
Median	0	0	17	13	0
0-4	90 (92%)	43 (91%)	1 (2%)	0	134 (66%)
5-9	8 (8%)	4 (9%)	2 (4%)	0	14 (7%)
10-14	0	0	10 (19%)	3 (60%)	13 (6%)
≥15	0	0	40 (75%)	2 (40%)	42 (21%)
Chest X-ray					
Done	13 (13%)	8 (17%)	19 (36%)	5 (100%)	45 (22%)
Abnormal	0	0	0	5 (100%)	5 (11%)
Sputum sample					
Done	5 (5%)	4 (9%)	5 (9%)	5 (100%)	19 (9%)
Smear positive	0	0	0	0	0

concentrations of these cytokines, except for TNF-α, were also significantly higher in the LTBI group compared with the sick control group.

For all cytokines, median cytokine concentrations were also significantly higher in the probable active TB group compared with both the healthy control group and the sick control group. Compared with the median cytokine concentrations in the LTBI group, median cytokine concentrations were higher in the probable active TB group, although this was statistically significant only for IL-1ra and IL-2.

3.3. Receiver operating characteristic analyses

Receiver operating characteristic analyses were done on the seven cytokines that showed significant differences between the diagnostic groups in the previous analyses. Comparisons were made between patients with TB infection (i.e. LTBI and probable active TB cases combined) and those without TB infection (i.e. sick and healthy controls combined). Three cytokines - IL-2, IL-13 and IP-10 - had higher test accuracy than IFN-γ for identifying TB infection (Table 4). In addition, IL-2 had high specificity (99.3%, 95% CI 96–100%) and positive predictive value (97.7%, 95% CI 85.9–99.7%), compared with the lower
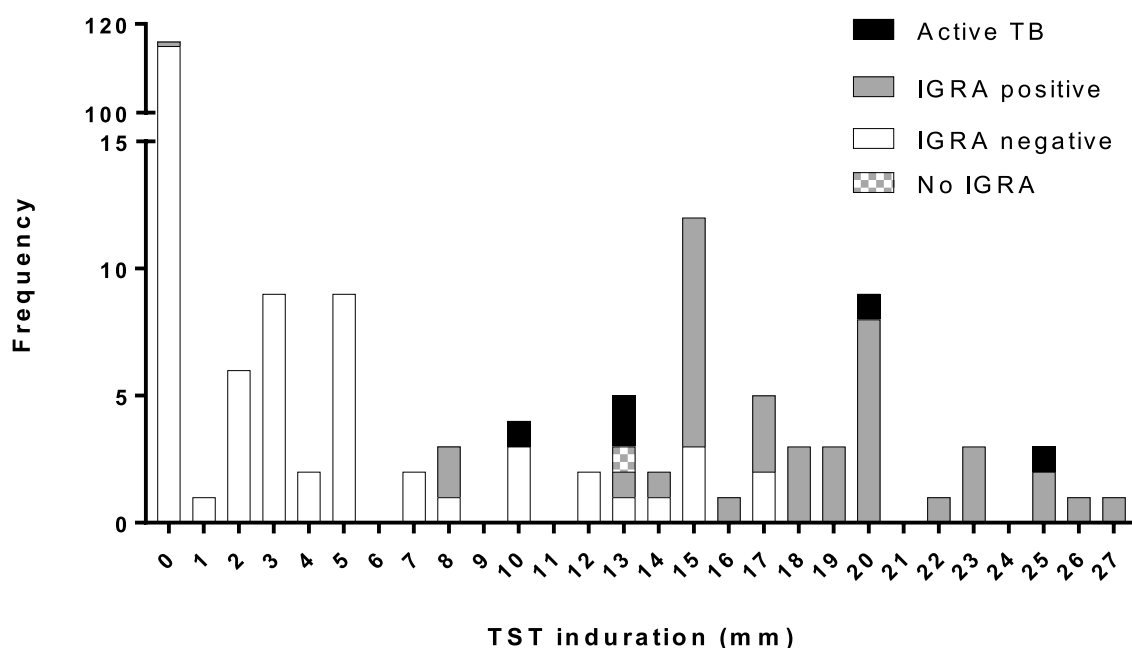

Fig. 2. IGRA results in relation to TST induration size in study participants.

Table 2
Demographic and other features of index cases.

Index case	Healthy controls ($n = 98$)	Sick controls ($n = 47$)	LTBI ($n = 53$)	Active TB ($n = 5$)	Total ($n = 203$)
Age (years)					
Mean	33.5	36.1	30.7	21.9	33.1
Range	15.4-72.9	15.5-72.9	16.8-62.2	16.9-27.1	15.4-72.9
Sex					
Male	63 (64%)	29 (62%)	27 (51%)	3 (60%)	122 (60%)
Relation to participant					
Mother	10 (10%)	6 (12%)	17 (32%)	0	33 (16%)
Father	13 (13%)	5 (11%)	6 (11%)	1 (20%)	25 (12%)
Sibling	18 (18%)	5 (11%)	10 (19%)	4 (80%)	37 (18%)
Grandparent	10 (10%)	10 (21%)	4 (8%)	0	24 (12%)
Uncle/aunt	30 (31%)	14 (30%)	6 (11%)	0	50 (25%)
Cousin	11 (11%)	5 (11%)	9 (17%)	0	25 (12%)
Other	5 (5%)	2 (4%)	0	0	7 (3%)
Unknown	1 (1%)	0	1 (2%)	0	2 (1%)
Shared bedroom with participant	24 (24%)	15 (32%)	22 (42%)	2 (40%)	63 (31%)
Duration shared bedroom (months)					
<1	-	13%	-	50%	5%
1-6	17%	7%	14%	-	13%
>6	83%	80%	86%	50%	82%
Cough	98 (100%)	45 (96%)	53 (100%)	5 (100%)	201 (99%)
Duration of symptoms prior to diagnosis (months)					
<1	19%	11%	11%	0%	15%
1-6	63%	72%	68%	80%	67%
>6	17%	13%	15%	20%	16%
Chest X-ray					
Done	97 (99%)	47 (100%)	53 (100%)	5 (100%)	202 (99%)
Pulmonary cavitation	65 (66%)	21 (45%)	35 (66%)	3 (60%)	124 (61%)
Sputum microscopy					
+	46 (47%)	25 (53%)	26 (49%)	1 (20%)	98 (48%)
++	16 (16%)	10 (21%)	6 (11%)	2 (40%)	34 (17%)
+++	36 (36%)	12 (26%)	19 (36%)	2 (40%)	69 (34%)

values for IFN-γ (specificity: 83.8%, 95% CI 76.5–89.6%; positive predictive value: 67.2%, 95% CI 57.7–75.4%). Receiver operating characteristic curves for the seven cytokines showed higher area under the curve (AUC) values for IL-2, IL-13 and IP-10 than for IFN-γ (Fig. 4). The combination of IL-2, IL-13 and IP-10 had a high negative predictive value (92.6%, 95% CI 87.2–95.8%).

Receiver operating characteristic analyses were also done to compare cases with probable active TB to participants with LTBI for the two cytokines where differences between those groups were greatest, IL-1ra and TNF-α (Fig. 5). Using a cut-off of 410.3 pg/ml for IL-1ra, all participants with probable active TB had 'positive' results (sensitivity 100%, 95% CI 47.8–100%), and the AUC value was 0.91.

Comparing the probable active TB group to the 'non-active TB' (LTBI, sick control and healthy control) groups, provides further evidence for the favourable performance characteristics of IL-1ra responses. Using a cut-off of 4600 pg/ml, all probable active TB cases were correctly classified (sensitivity 100%; 95% CI 54.1–100%), and only 9 'false-positive' results occurred in the remaining 3 groups (specificity: 95.2%, 95% CI 91.1–97.8%; negative predictive value: 100%; test accuracy: 95.4%, 95% CI 91.4–97.9%).

4. Discussion

To our knowledge, this study is the first to determine the specificity and positive and negative predictive values of a broad range of mycobacteria-specific cytokine responses, which have previously shown promise as diagnostic biomarkers for TB, in children in a TB endemic setting.

We found that seven cytokines distinguished between asymptomatic patients with and without TB infection (i.e. healthy controls and LTBI) and between symptomatic patients with and without TB (i.e. sick controls and probable active TB). In addition, two cytokines - IL-1ra and TNF-α - were significantly higher in the active TB group than in the LTBI group (i.e. potentially stage-specific marker), and significantly

higher in the active TB group and control groups (healthy controls and sick controls).

In this study, we found that, for child household contacts of adults with pulmonary TB, seven of eight previously identified cytokine biomarkers (IFN-γ, IL-1ra, IL-2, IL-13, IP-10, TNF-α, and MIP-1β) were able to distinguish between participants with LTBI and TB-uninfected healthy controls [28].

Importantly, in contrast to the vast majority of previous studies focusing on mycobacteria-specific cytokine responses, we included a 'sick' control group of patients that clinically resembled patients with active TB, therefore allowing us to demonstrate the specificity of these biomarkers in the context of suspected active TB.

The ability of IFN-γ, IL-1ra, IL-2, IL-13, IP-10, TNF-α, and MIP-1β to distinguish individuals with TB infection (i.e. LTBI or active TB) from those who are uninfected in a population with endemic TB is consistent with the findings of our previous studies in children and adults in a low TB prevalence setting [28,32,33]. This is despite a critical difference in methodology. While our previous study used *ex vivo* whole blood assays stimulated separately with ESAT-6 and CFP-10 peptide pools in combination with co-stimulatory antibodies, in this study we used supernatants from commercial QFT-GIT assays that had been stimulated with three MTB antigens simultaneously (ESAT-6, CFP-10 and TB7.7). Moreover, the median response for six cytokines was higher in the active TB group than in the LTBI group, which is again consistent with our previous observations. The finding that participants with active TB had higher cytokine responses than sick controls, indicates that these responses are truly mycobacteria-specific and are not influenced by a non-specific inflammatory response.

The performance characteristics of three cytokines (IL-2, IL-13 and IP-10) exceeded those of IFN-γ for the comparison of TB infection versus absence of TB infection, validating our previous findings [28]. The high negative predictive value (97.7%) of IL-2 alone highlights its potential as a rule-out test for TB infection. Notably, IFN-γ had a specificity of only 83.8%, suggesting that as many as one in five patients

Table 3
Median cytokine concentrations (background-corrected) in MTB antigen-stimulated samples by diagnostic category, with significance testing for differences between groups.

Median [IQR] (pg/mL)	Active TB (n = 5)	LTBI (n = 53)	Healthy controls (n = 98)	Sick controls (n = 47)	4-group test	Healthy controls vs Sick controls	LTBI Vs Healthy controls	LTBI vs Sick controls	Active TB vs Healthy controls	Active TB vs Sick controls	Active TB vs LTBI
					Kruskal-Wallis				Mann Whitney U		
IFN-γ	735.0 [291.8, 1275.2]	233.2 [20.8, 733.9]	0 [−20.8, 3.8]	0 [−17.2, 4.7]	0.0001	0.7920	<0.0001	<0.0001	0.0002	0.0002	0.0902
IL-1ra	5674.1 [5044.3, 7796.0]	1594.9 [417.4, 3532.2]	184.5 [−70.2, 641.0]	−30.3 [−732.1, 496.0]	0.0001	0.0195	<0.0001	<0.0001	0.0002	0.0004	0.0021
IL-2	586.4 [413.9, 681.2]	238.3 [7.9, 524.5]	0 [−0.7, 8.0]	0 [−0.5, 0]	0.0001	0.9614	<0.0001	<0.0001	0.0001	0.0001	0.0481
IL-10	3.3 [0, 8.0]	−0.9 [−4.0, 2.1]	0 [−2.3, 1.7]	−0.6 [−4.6, −0.8]	0.1119	-	-	-	-	-	-
IL-13	90.1 [51.5, 290.0]	27.6 [27.6, 156.3]	0 [−0.1, 1.1]	0 [−0.1, 0]	0.0001	0.2991	<0.0001	<0.0001	0.0001	<0.0001	0.2355
IP-10	34,336.2 [28,743.3, 51,625.5]	15,533.8 [2429.1, 47,348.1]	0 [−331.5, 720.8]	85.5 [−193.5, 438.4]	0.0001	0.8632	<0.0001	<0.0001	0.0002	0.0005	0.1849
MIP-1β	23,340.9 [5067.6, 43,574.5]	3337.1 [337.4, 12,848.4]	−103.7 [−1434.4, 1970.6]	−745.9 [−4273.8, 2061.7]	0.0001	0.1118	0.0004	0.0001	0.0215	0.0073	0.1079
TNF-α	168.3 [96.4, 204.5]	−135.2 [−451.4, 228.9]	−87.3 [−311.4, 4.2]	−201.8 [−458.9, 16.8]	0.0803	0.1527	0.9557	0.2391	0.0245	0.0176	0.1349

Mann-Whitney U p-values were done when Kruskal-Wallis p-value was <0.10.
Abbreviations: IFN-γ = interferon γ; IL-1ra = interleukin 1 receptor antagonist; IL-2 = interleukin 2; IL-10 = interleukin 10; IL-13 = interleukin 13; IP-10 = interferon-inducible protein 10; MIP-1β = macrophage inflammatory protein 1β; TNF-α = tumour necrosis factor α.
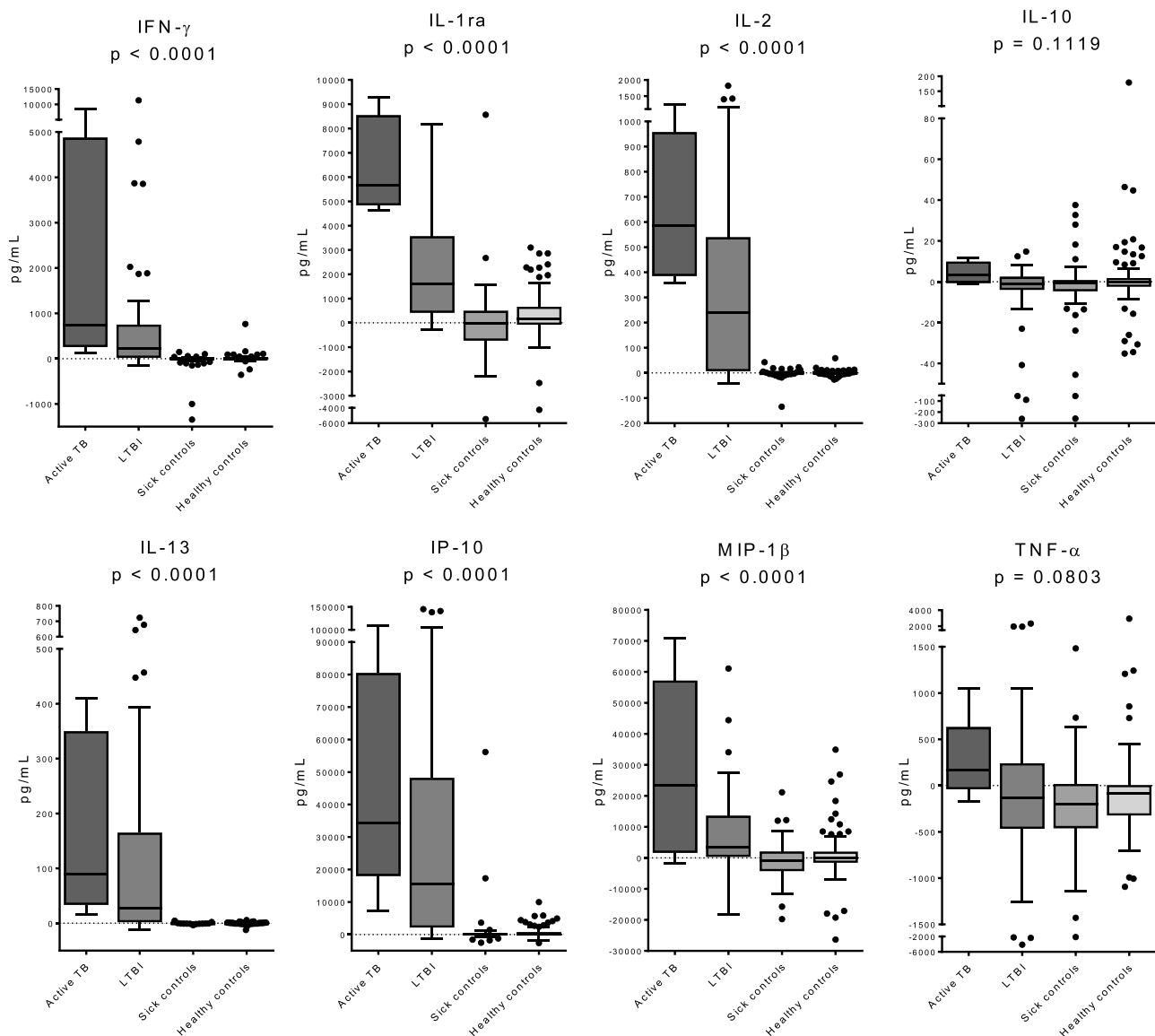
Fig. 3. Mycobacterial-specific cytokine responses (background-corrected) in participants with probable active TB and LTBI, and sick controls and healthy controls. Tukey whiskers denote the highest and lowest values, excluding outliers.

would have a false-positive result. The high sensitivity (84.2%) and negative predictive value (92.6%) for the combination of IL-2, IL-13 and IP-10 indicates these biomarkers have the potential to form the basis of a combined rule-in/rule-out test for TB infection.

Several previous studies have attempted to identify biomarkers in blood that can robustly distinguish between LTBI and active TB [34–40], but only few identified candidate biomarkers potentially allowing this distinction have been validated in subsequent, prospective studies [15,31,33]. Considering that currently used immune-based tests (i.e. TST and IGRA) cannot distinguish between both infection states, a blood-based assay that has this ability would be a real advance for patient care, especially in TB endemic regions, where a large proportion of the population has LTBI and where consequently a positive TST or IGRA result in a symptomatic patient provides little help to clinicians having to decide whether the patient has active TB or alternatively a respiratory tract infection caused by an organism other than *M. tuberculosis* and coincidentally co-existing LTBI.

We have previously shown that a combination of TNF-α, IL-1ra and IL-10 responses can be used to distinguish between children with LTBI and children with active TB [28]. The current study confirms that IL-1ra responses are significantly higher in patients with probable active TB

than in those with LTBI, as well as healthy and sick controls. The latter comparison provides evidence that these responses are highly specific and not influenced by background inflammation. The remarkable performance characteristics of IL-1ra, including both a sensitivity and negative predictive value of 100%, indicate that this single cytokine could act as a stage-specific biomarker. IL-1ra is known to be involved in the immune response to TB infection, particularly the containment of infection through formation of granulomas [41]. The importance of IL-1ra in the pathogenesis of TB is further highlighted by data showing that therapy with monoclonal IL-1 receptor antagonists is associated with a significantly increased risk of developing active TB [42].

The strengths of our study include the use of well-defined participant groups and, critically, the inclusion of a sick control group enabling us to determine the specificity of the cytokine responses investigated. In addition, our study was based in a TB endemic setting in contrast to our previous studies that were based in low TB prevalence settings and largely comprised participants from African and Asian backgrounds [28,32]. The results of this study confirm that our diagnostic cytokine biomarkers perform well in a population with an entirely different genetic background, and that a greater background prevalence of LTBI on population level does not impact on their

Table 4
Results of the receiver operating characteristic analyses of the seven MTB-specific cytokine responses with the potential ability to discriminate between TB-uninfected and TB-infected participants.

|	Cut-off* (pg/mL)	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	Test accuracy [95% CI]
IFN-γ	20.3	79.0% [66.1–88.6%]	83.8% [76.5–89.6%]	67.2% [57.7–75.4%]	90.5% [85.1–94.0%]	82.4% [76.3–87.5%]
IL-1ra	410.3	80.7% [68.1–90.0%]	68.4% [59.9–76.1%]	51.7% [44.8–58.6%]	89.4% [83.1–93.6%]	72.0% [65.1–78.2%]
IL-2	50.1	75.4% [62.2–85.9%]	99.3% [96.0–100%]	97.7% [85.9–99.7%]	90.6% [86.0–93.9%]	92.2% [87.5–95.6%]
IL-13	1.1	80.7% [68.1–90.0%]	93.4% [87.8–97.0%]	83.6% [72.9–90.7%]	92.0% [87.1–95.2%]	89.6% [84.5–93.6%]
IP-10	1557	80.7% [68.1–90.0%]	86.0% [79.1–91.4%]	70.8% [61.0–78.9%]	91.4% [86.2–94.8%]	84.5% [78.6–89.3%]
MIP-1β	847.1	70.2% [56.7–81.6%]	67.7% [59.1–75.4%]	47.6% [40.3–55.0%]	84.4% [78.1–89.1%]	68.4% [61.3–74.9%]
TNF-α	120.9	30.9% [19.1–44.8%]	88.2% [81.6–93.1%]	51.5% [36.7–66.1%]	76.0% [72.4–79.2%]	71.7% [64.8–78.0%]
IL-2, IL-13, IP-10	as above	84.2% [72.1–92.5%]	82.4% [74.9–88.4%]	66.7% [57.8–74.5%]	92.6% [87.2–95.8%]	82.9% [76.8–87.9%]

Abbreviations: IFN-γ = interferon γ; IL-1ra = interleukin 1 receptor antagonist; IL-2 = interleukin 2; IL-10 = interleukin 10; IL-13 = interleukin 13; IP-10 = interferon-inducible protein 10; MIP-1β = macrophage inflammatory protein 1β; NPV = negative predictive value, PPV = positive predictive value, TNF-α = tumour necrosis factor α.

* Optimal cut-offs were determined for each cytokine using the Youden index.

diagnostic accuracy. The study limitations include the low number of participants with active TB, which resulted in wide confidence interval estimates regarding test sensitivities. However, despite this, we were able to detect highly significant differences in cytokine responses between the different diagnostic groups, consistent with our previous findings [28]. Although these differences may not be sufficient for clinical purposes, these results provide proof-of-principle that certain cytokines responses could be useful in diagnostic assays. Another limitation is the fact that, due to local resource limitations, only 36% of the

patients categorised as LTBI (based on a positive TST or IGRA without symptoms) had a chest x-ray. It is therefore possible that some of these patients had an abnormal chest x-ray and had early, subclinical active TB. However, none of these participants had any signs of disease on detailed physical examination or were treated for active TB subsequently. Also, one of our previous studies has shown that chest x-ray changes potentially indicating active TB are rare in asymptomatic children screened after TB contact [43].

In conclusion, we found that in children in a highly TB endemic
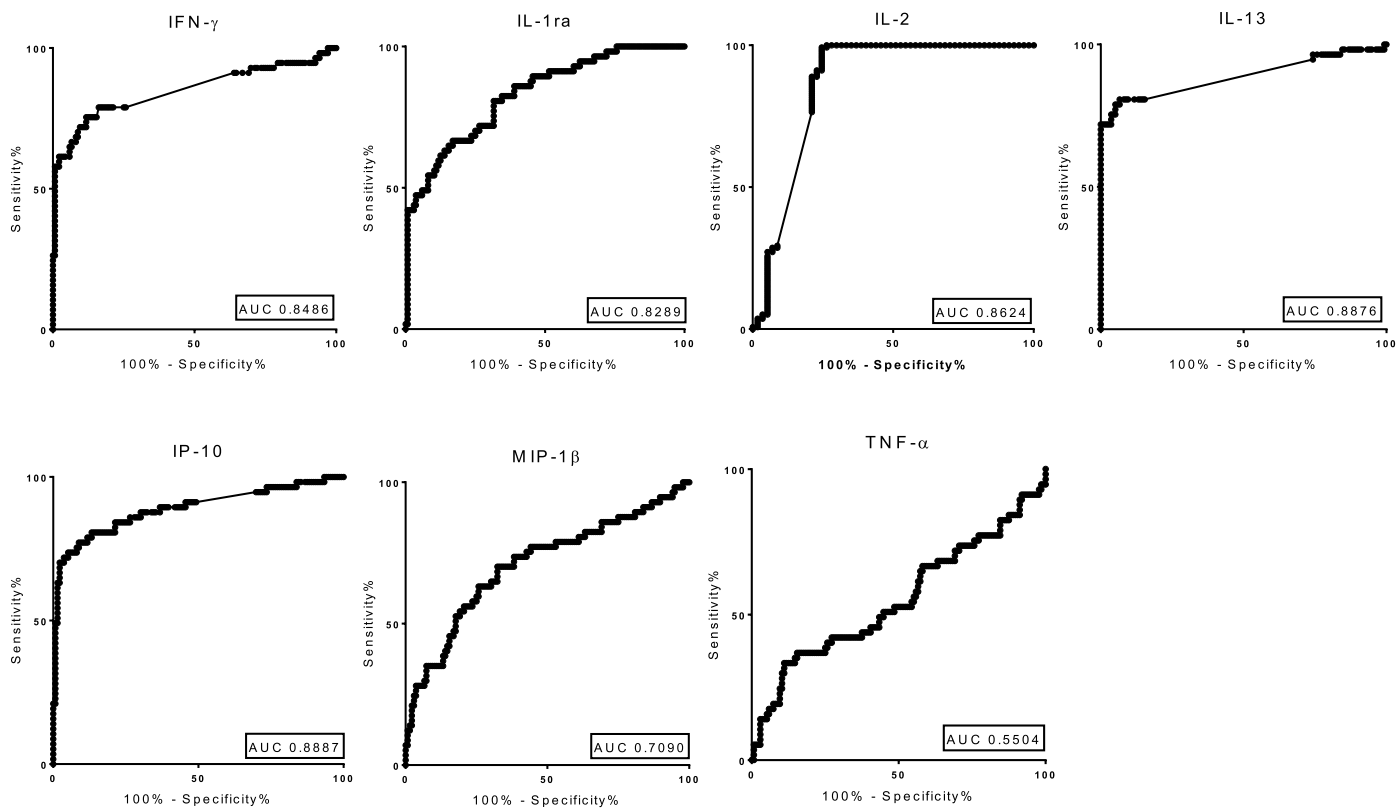
Fig. 4. Receiver operating characteristic curves for IFN-γ, IL-1ra, IL-2, IL-13, IP-10, MIP-1β and TNF-α responses for identifying TB infection. Case values comprised background-corrected cytokine concentrations in participants with LTBI or probable active TB (TB-infected group); control values comprised cytokine concentrations in uninfected participants (sick controls and healthy controls). AUC = area under the curve.
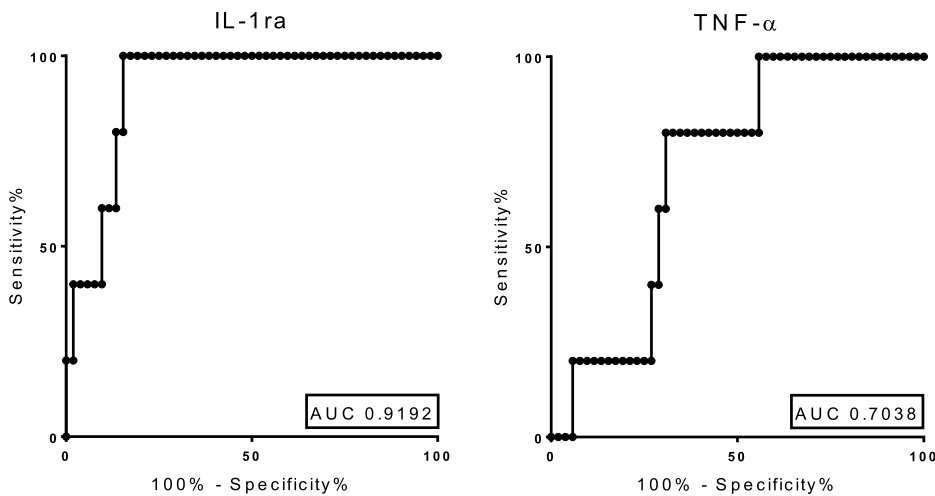
Fig. 5. Receiver operating characteristic curves for IL-1ra and TNF-α responses for the distinction between LTBI and probable active TB. Case values comprised background-corrected cytokine concentrations in participants with probable active TB; control values comprised cytokine concentrations in participants with LTBI. AUC = area under the curve.

setting, seven cytokine biomarkers (IFN-γ, IL-1ra, IL-2, IL-13, IP-10, TNF-α, and MIP-1β responses) had the ability to distinguish between TB-infected and TB-uninfected individuals with high levels of sensitivity and specificity. Combining three of these cytokines - IL-2, IL-13 and IP-10 -achieved high sensitivity and a high negative predictive value. Importantly, inclusion of both healthy and sick control groups also allowed us to arrive at robust specificity estimates. Furthermore, in accordance with our previous studies, our results show that TB-specific IL-1ra responses allow to discriminate between LTBI and probable active TB based on a blood test alone, a finding that has the potential to significantly improve patient care. The observed high negative predictive value of IL-1ra responses indicates that this cytokine biomarker could provide the basis for future rule-out tests for active TB. Further studies with larger numbers of patients with pulmonary and extrapulmonary TB are needed to validate these findings, and allow more precise estimation of test sensitivity in the context of active TB.

Competing interests

None declared.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jctube.2019.100102.

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