Detection of tuberculosis in HIV-infected children using an enzyme-linked immunospot assay

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\textbf{Objective:} To evaluate an enzyme-linked immunospot assay (ELISPOT) for the diagnosis of tuberculosis (TB) in HIV-infected children with suspected TB and to compare the performance of ELISPOT with the tuberculin skin test (TST).

\textbf{Methods:} Interferon-\(\gamma\) responses to Mycobacterium tuberculosis-specific antigens were measured by ELISPOT in HIV-infected children with suspected TB. HIV-infected and HIV-uninfected children without TB were taken for comparison.

\textbf{Results:} Results were available for 188 children, of whom 139 (74\%) were HIV-infected. Of these, 22 were classified as having definite TB; 24 probable TB, 14 possible TB and 128 not having TB. The median (range) age of patients was 20 (10–54.1) months. Median interferon-\(\gamma\) responses to early-secreted antigenic target-6 and culture filtrate protein-10 were higher in children with definite or probable TB compared with children without TB (\(P < 0.002\)). In HIV-infected children with an interpretable ELISPOT result, the ELISPOT was positive in 14/21 (66\%) with definite TB. A significantly higher proportion of HIV-infected children with definite or probable TB had a positive ELISPOT compared with a positive TST [25/39 (64\%) vs. 10/34 (29\%), \(P = 0.005\)]. In contrast to TST, results from ELISPOT were not affected by young age or severe immunosuppression. In HIV-infected children without active TB disease, 27\% had a positive ELISPOT, suggesting latent TB infection.

\textbf{Conclusion:} ELISPOT is more sensitive than TST for the detection of active TB in HIV-infected children. However, the sensitivity of current ELISPOT assays is not sufficiently high to be used as a rule out test for TB.

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\textbf{Keywords:} culture filtrate protein-10, diagnosis, early secreted antigenic target-6, interferon, tuberculosis

\textbf{Introduction}

The Western Cape region of South Africa has one of the highest recorded incidence rates of tuberculosis (TB). Fuellmed by the HIV pandemic, rates in excess of 1600/100,000 have been reported [1]. Historically, it has been thought that children contribute little to the TB burden but recent evidence challenges this dogma [2–4]. It was...
estimated that 11% of the eight million cases of TB worldwide in 2000 occurred in children [5]. This is likely to be an underestimate because of difficulties with TB diagnosis in children coupled with poor recording and reporting of child TB cases in endemic areas. Childhood TB accounts for 39% of all cases in the Western Cape [6] and the importance of TB as a cause of childhood death in TB endemic areas has also been highlighted in other studies [7,8].

HIV infection is the major risk factor for the development of TB in sub-Saharan Africa [9]. The risk of TB has recently been reported to be more than 20-fold greater in HIV-infected children compared with uninfected children [10]. The detection of TB in HIV-infected children remains problematic for a number of reasons [11]. There is epidemiological and clinical overlap with the presentation of HIV and TB in children, and investigations such as chest radiography and tuberculin skin testing (TST) have reduced specificity and sensitivity [12,13]. Mycobacterial culture confirms the diagnosis of TB in children and provides important drug susceptibility data [14]. However, culture is not available in most TB/HIV endemic settings. Further, a decision to commence TB treatment usually needs to be made before confirmation of diagnosis by culture, especially in HIV-infected children, as disease progression can be rapid [8,11,14].

Interferon gamma (IFN-\(\gamma\)) release assays (IGRA) have emerged as potential tools for the rapid and reliable detection of TB [15–17]. Few studies [16–19] have assessed their performance in children. Data on the use of IGRA for the diagnosis of TB in HIV-infected children are even more limited [20]. The aims of this study were to evaluate an enzyme-linked immunospot assay (ELISPOT) in HIV-infected children with suspected TB in a high TB incidence area; to compare the performance of ELISPOT against the TST and to investigate the effect of age, nutritional status and degree of HIV-associated immune suppression on the performance of both tests.

Methods

Patients

The study was approved by the University of Cape Town Research Ethics Committee. Children known to be HIV-infected and with symptoms suggestive of TB were prospectively recruited from the short-stay unit or general paediatric wards at the Red Cross Children’s Hospital, Cape Town, South Africa. The presence of one or more of the following symptoms was considered suggestive of TB: cough for longer than 2 weeks, persistent fever, night sweats, weight loss and fatigue. Children already receiving TB treatment for more than 7 days were excluded. A second group of known HIV-infected children presenting with an illness other than TB or in whom TB had been actively excluded were recruited from the infectious diseases clinic, short-stay unit as well as from an ongoing efficacy study of isoniazid preventive treatment (IPT) in HIV-infected children. A major entry criterion for participants in the IPT study was exclusion of active TB disease. A group of HIV-uninfected children with an illness not consistent with TB were also prospectively recruited (Fig. 1). These children had a range of alternative diagnoses, including asthma, malaria, pneumonia, croup and urinary tract infection.

Written witnessed informed consent was obtained from parents in their preferred language. Demographic and clinical details were obtained using a questionnaire and included age, weight, presence of bacillus Calmette–Guerin (BCG) scar, TB contact history, previous history of TB, symptoms suggestive of TB, WHO HIV clinical stage and details of antiretroviral medications.

Diagnostic tests

Children with suspected TB were clinically assessed, had a TST, chest radiograph and at least one induced sputum or two gastric washings or other site-specific clinical specimen sent for TB microscopy and culture. TST was performed by intradermal installation of 2 TU (PPD RT-23) and read after 48–72 h. A positive TST was defined as greater than or equal to 5 mm in HIV-infected children and greater than or equal to 10 mm in HIV-uninfected children. HIV enzyme-linked immunosorbent assay (ELISA) testing was performed in all children, with positive ELISA results confirmed by HIV RNA PCR. A blood sample was obtained from each child for ELISPOT and, in HIV-infected children, a concomitant CD4 cell count. HIV-infected children were classified as having mild, advanced or severe immunodeficiency according to WHO criteria based on age-related CD4% values. All children in the study who qualified for antiretroviral treatment based on the WHO eligibility guidelines that were in use at the time of the study were referred to the appropriate clinic for assessment. HIV-infected and HIV-uninfected children with illnesses not consistent with TB had specific investigations at the discretion of the child’s physician and their classification subsequently confirmed at a follow-up appointment 14–28 days after initial assessment. Patients failing to attend follow-up visits were contacted.

All blood samples were processed within 4 h of phlebotomy and ELISPOT was performed as previously described [21]. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation, washed, resuspended and counted. PBMCs (2 × 10^6/ml) were added to duplicate wells (96-well polyvinylidene fluoride plates precoated with anti-IFN-\(\gamma\) monoclonal antibody (mAb) 1-DIK antibody (Mabtech, Stockholm, Sweden) containing antigen. Stimulatory antigens were added to PBMC and consisted of early secretory antigen target-6 (ESAT-6; a pool of 15-mer
peptides overlapping by 10 amino acids, Peptide Protein Research UK; final concentration 5 μg/ml/peptide) and culture filtrate protein-10 (CFP-10, a pool of 15-mer peptides overlapping by 10 amino acids, Peptide Protein Research, UK; final concentration 5 μg/ml/peptide). All stimulatory antigens were placed in separate wells and samples were processed in duplicate. No antigen was added to the negative control well. Anti-CD3 mAb CD3-2 (Mabtech) at a final concentration of 100 ng/ml was included as a positive control. During the study, samples from a subset of 22 HIV-infected children were tested with both the ELISPOT assay and the T.SPOT.TB (Oxford Immunotec, Oxford, UK), a commercially available IGRA.

ELISPOTs were scored by two independent observers using a stereo microscope (Carl Zeiss MicroImaging, Inc, Germany). Laboratory scientists were blinded to the final categorization of patients. Study investigators also confirmed spot counts in a blinded manner. The mean IFN-γ response to each of the antigens (ESAT-6 and CFP-10) and positive control was calculated after subtraction of background IFN-γ responses obtained from the negative control wells. IFN-γ responses were expressed as spot forming cells (SFCs)/million (10^6) PBMC. The ELISPOT was considered positive if the number of SFCs in the antigen-stimulated wells was greater than or equal to 5 SFCs/2 × 10^5 (25 SFCs/10^6) above the background response, and, in cases in which the background response was greater than or equal to 5 SFCs/10^6, more than twice the background response. Responses were classified as indeterminate when the number of SFCs in the antigen-stimulated wells, corrected for background, was less than 5 (25 SFCs/10^6) in the presence of a failed positive control response; the number of SFCs in the antigen-stimulated wells was less than twice the background response when the background response was greater than or equal to 10 SFCs (50 SFCs/10^6) and there was an insufficient number of PBMCs to undertake the assay (technical). The overall ELISPOT result was considered positive if the response to ESAT-6 or CFP-10 or both was positive.

Children were classified as having definite, probable, possible or no TB using the clinical and microbiological criteria detailed in Table 1. TST was not included as part of the diagnostic algorithm in assigning children to clinical categories.

**Statistical analysis**

Data were analysed using Prism Graphpad (Graphpad Software, Inc, San Diego, California, USA). The Mann–Whitney U-test was used to compare nonparametric unpaired data. Fisher’s exact test or χ² was used to
compare proportions when test results were analysed as dichotomous outcomes. McNemar’s-paired test was used to compare the results of TST and ELISPOT. Correlations were assessed using the Spearman’s correlation coefficient. Weight-for-age Z-scores (WAZ) were calculated using Epi-info 2000, version 1.0 (Division of Surveillance and Epidemiology, CDC, Atlanta, Georgia, USA).

Results

A total of 188 children were enrolled between April 2006 and May 2007, of whom 139 (74%) were HIV-infected (Fig. 1). Demographic details are shown in Table 2. The median (interquartile range; IQR) age of patients included in the study was 20 (10.0–54.1) months. Phlebotomy failed in two (1%) children who were subsequently excluded from analysis. Of 104 HIV-infected children presenting with suspected TB, 22 (21%) had definite TB as defined by study criteria. There was no significant difference in age, presence of BCG scar or absolute CD4 cell count between HIV-infected children in each diagnostic group (Table 2). HIV-infected children with possible TB had a lower CD4% compared with HIV-infected children with definite TB (14.0 vs. 24.2%, \( P = 0.050 \)). HIV-infected children with definite or probable TB had significantly lower WAZ scores than HIV-infected or HIV-uninfected children without TB.

The median (IQR) IFN-\( \gamma \) responses to ESAT-6 and CFP-10 were higher in children with definite TB compared with children without TB [ESAT-6 30 (2.5–162.5) vs. 5 (0–15) SFCs/10\(^6\) PBMC, \( P = 0.002 \); CFP-10 45 (12.5–407.5) vs. 5 (0–20), \( P < 0.0001 \)]. Similarly, median (IQR) IFN-\( \gamma \) responses to ESAT-6 and CFP-10 were higher in children with definite or probable TB compared with children without TB [ESAT-6 30 (5–110) vs. 5 (0–15) SFCs/10\(^6\) PBMC, \( P < 0.0001 \); CFP-10 45 (10–190) vs. 5 (0–20), \( P < 0.0001 \)]. Furthermore, children with definite or probable TB had higher median IFN-\( \gamma \) responses compared with children with possible TB [ESAT-6 30 (5–110) vs. 5 (0.0–37.5) SFCs/10\(^6\) PBMC, \( P = 0.03 \); CFP-10 45 (10–190) vs. 2.5 (0.0–37.5), \( P = 0.006 \)]. The response rates to the individual antigens alone and in combination are shown in Table 3. Of the 22 children with definite TB, 21 (95%) had an interpretable ELISPOT result. Of these 21 children, 14 (66%) had a positive response. Of the 22 children with definite TB, 12 (55%) were sputum smear negative and eight (67%) of these had a positive ELISPOT. ELISPOT was positive in six (67%) of nine sputum smear positive children and was indeterminate in one child with sputum smear positive disease. Of 19 children with definite TB who had a TST undertaken, 15 (79%) returned for their TST reading. Of these 15 children, five (33%) had induration greater than or equal to 5 mm.

Combining the results of TST and ELISPOT resulted in a marginal increase in sensitivity over the use of ELISPOT alone. Of the 22 children with definite TB, 16 (73%) had a positive result in at least one of the two tests. Similarly, of the 46 HIV-infected children with definite or probable TB, 27 (69%) had a positive result in at least one of the two tests. Overall, a significantly higher number of children with definite or probable TB had a positive ELISPOT compared with a positive TST [ELISPOT 25/39 (64%) vs. 10/34 (29%) TST-positive, \( P = 0.005 \)]. Compared with children without TB, children with definite or probable TB were more likely to have a positive ELISPOT than a positive TST [ELISPOT: odds ratio (OR) = 6.0 (95% confidence interval (CI) = 2.7–13.1, \( P < 0.0001 \) vs. TST: OR = 2.6 (95% CI = 0.9–7.2, \( P = 0.12 \)). In HIV-infected children with definite or probable TB, four children were TST-positive.

Table 1. Clinical and microbiological criteria used for assigning children to diagnostic groups.

<table>
<thead>
<tr>
<th>Diagnostic classification</th>
<th>Criteria</th>
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<tbody>
<tr>
<td>Definite TB (n = 22)</td>
<td>Isolation of Mycobacterium tuberculosis in culture or detection of acid-fast bacilli on microscopy of appropriate site-specific specimen</td>
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<td></td>
<td>Symptoms and signs suggestive of TB(^a) and two or more of the following</td>
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<td></td>
<td>Chest radiograph findings consistent with TB(^b)</td>
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<td></td>
<td>Response to TB treatment(^c)</td>
</tr>
<tr>
<td>Probable TB (n = 24)</td>
<td>Symptoms suggestive of TB and only one of the following</td>
</tr>
<tr>
<td></td>
<td>Chest radiograph findings consistent with TB(^b)</td>
</tr>
<tr>
<td></td>
<td>No alternative definitive diagnosis established</td>
</tr>
<tr>
<td>Possible TB (n = 14)</td>
<td>Confirmed HIV infection and no symptoms or signs of TB (or symptoms potentially suggestive of TB but full resolution on clinical follow-up) and/or alternative definitive diagnosis established</td>
</tr>
<tr>
<td>Not TB, HIV-positive (n = 79)</td>
<td>As above but confirmed HIV uninfected</td>
</tr>
<tr>
<td>Not TB, HIV-negative (n = 49)</td>
<td>As above but confirmed HIV uninfected</td>
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TB, tuberculosis.

\(^a\)Symptoms suggestive of TB included cough for longer than 2 weeks, persistent fever, night sweats, weight loss and fatigue.

\(^b\)A chest radiograph with hilar lymphadenopathy, right upper lobe infiltrates, pleural effusion or a miliary pattern.

\(^c\)Resolution of clinical symptoms, radiological improvement and weight gain.
Table 2. Demographic details for patients included in the study.

<table>
<thead>
<tr>
<th></th>
<th>All patients (n = 188)</th>
<th>Definite (n = 22)</th>
<th>Probable (n = 24)</th>
<th>Possible (n = 14)</th>
<th>HIV-negative (n = 49)</th>
<th>HIV-positive (n = 79)</th>
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<tr>
<td><strong>Demographic details</strong></td>
<td></td>
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<tr>
<td><strong>TB</strong></td>
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<tr>
<td><strong>Not TB</strong></td>
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<tr>
<td><strong>Median age in months (IQR)</strong></td>
<td></td>
<td>20 (10.0–54.1)</td>
<td>34.7 (14.6–75.6)</td>
<td>17.9 (11.5–34.9)</td>
<td>14.3 (7.1–34.9)</td>
<td>14.3 (7.1–34.9)</td>
</tr>
<tr>
<td><strong>Male; n (%)</strong></td>
<td></td>
<td>100 (53)</td>
<td>14 (64)</td>
<td>16 (67)</td>
<td>6 (43)</td>
<td>36 (46)</td>
</tr>
<tr>
<td><strong>Median weight for age score (IQR)</strong></td>
<td></td>
<td>0.47 (0.4–0.88)</td>
<td>2.31 (3.3–13.1)</td>
<td>6.4 (4.3–9.5)</td>
<td>2.3 (3.2–13.1)</td>
<td>2.3 (3.2–13.1)</td>
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<tr>
<td><strong>WHO HIV stage</strong></td>
<td></td>
<td>4 (19)</td>
<td>5 (36)</td>
<td>5 (36)</td>
<td>4 (28)</td>
<td>4 (28)</td>
</tr>
<tr>
<td><strong>BCG scar present; n (%)</strong></td>
<td></td>
<td>144 (76)</td>
<td>16 (73)</td>
<td>17 (71)</td>
<td>10 (71)</td>
<td>64 (81)</td>
</tr>
<tr>
<td><strong>Median CD4 (IQR) (%)</strong></td>
<td></td>
<td>20 (14.6–280)</td>
<td>107 (7.6–30.3)</td>
<td>19.9 (14.6–29.5)</td>
<td>24.2 (14.1–29.9)</td>
<td>22.3 (16.0–31.1)</td>
</tr>
<tr>
<td><strong>Median absolute CD4 cell count (IQR) (cells/ml)</strong></td>
<td></td>
<td>788 (450–1258)</td>
<td>107 (7.6–30.3)</td>
<td>19.9 (14.6–29.5)</td>
<td>24.2 (14.1–29.9)</td>
<td>22.3 (16.0–31.1)</td>
</tr>
<tr>
<td><strong>On antiretrovirals at diagnosis; n (%)</strong></td>
<td></td>
<td>3/19 (16%)</td>
<td>2/5 (25)</td>
<td>5 (36)</td>
<td>40 (51)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Median TST induration (range) (mm)</strong></td>
<td></td>
<td>0 (0–27)</td>
<td>0 (0–32)</td>
<td>0 (0–27)</td>
<td>0 (0–27)</td>
<td>0 (0–27)</td>
</tr>
</tbody>
</table>

**ELISPOT and tuberculosis and child**

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ELISPOT-negative or indeterminate and 11 were ELISPOT-positive/TST-negative.

Of the 128 children without TB, 118 (93%) had an interpretable ELISPOT result and 27 (23%) were positive. The median (IQR) IFN-γ responses to ESAT-6 and CFP-10 in children with a positive ELISPOT in the no TB group were significantly lower compared with the responses in children with definite or probable TB and a positive ELISPOT [ESAT-6 45 (32–65) SFCs/10⁶ PBMC vs. 110 (70–280), P = 0.003; CFP-10 40 (27–52) vs. 177 (48–508), P = 0.0005]. There was poor agreement between the TST and ELISPOT in HIV-infected children without TB [κ = 0.12 (95% CI = −0.1–0.3)]. A higher proportion of these children had a positive ELISPOT than a positive TST (McNemar-paired analysis, P = 0.06).

We investigated the effect of age, nutritional status and degree of immunosuppression on test performance by comparing the results of TST and ELISPOT in children less than or greater than 24 months, in children with CD4% greater than or less than 15 and in children with moderate-to-severe malnutrition.

In those with definite or probable TB, children less than 24 months of age were less likely to have a positive TST compared with children 24 months of age or older [3/19 (16%) vs. 7/15 (47%), P = 0.06] (Fig. 2a). The median (IQR) age of children with definite or probable TB and a positive TST was significantly higher than those with a negative TST [63.1 (17.8–86.1) vs. 16.3 (7.6–30.3) months, P = 0.01]. The magnitude of TST induration correlated with age (Spearman’s correlation 0.46, P = 0.006). In contrast, age did not affect the proportion of children with definite or probable TB having a positive ELISPOT [11/19 (58%) children <24 months vs. 14/21 (67%) children ≥24 months, P = 0.75] (Fig. 2c).

None of 12 (0%) children with a CD4% less than 15% had a positive TST compared with nine of 19 (47%) children with a CD4% greater than 15% [P = 0.005; Fig. 2b]. However, there was no difference in the proportion of children with a positive ELISPOT in those with CD4% less than or greater than 15% [8/12 (67%) vs. 11/19 (58%), P = 0.71] (Fig. 2d).

WHO guidelines classify HIV-associated immunodeficiency as mild, advanced or severe on the basis of age-related CD4% values. Therefore, we investigated the influence of the degree of immunosuppression on the results of TST and ELISPOT in HIV-infected children with definite or probable TB. Of 36 HIV-infected children with definite or probable TB with interpretable results of TST and ELISPOT, 15 (42%) were classified as severely immunodeficient. Of these 15 children, none had a positive TST. In contrast, 10 (67%) of these 15 children had a positive ELISPOT (P = 0.0002).
Only four (9%) HIV-infected children with definite or probable TB with moderate-to-severe malnutrition (WAZ ≤ −2) had a positive TST. In contrast, 13 (68%) HIV-infected children with definite and probable TB with moderate-to-severe malnutrition had a positive ELISPOT [TST 4/21 (19%) vs. 13/19 (68%), P = 0.003].

For the whole cohort, the sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of ELISPOT for the diagnosis of TB in children with definite or probable TB were 64 (95% CI = 47–79), 77 (95% CI = 68–84), 48 (95% CI = 34–62) and 87% (95% CI = 78–92), respectively. For TST, the corresponding values in children with definite or probable TB were 29 (95% CI = 15–47), 86 (95% CI = 75–93), 55 (95% CI = 31–78) and 67 (95% CI = 55–78), respectively. In HIV-infected children with definite TB, the sensitivity, specificity, PPV and NPV of ELISPOT were 67 (95% CI = 43–85), 77 (68–84), 34 (95% CI = 21–50) and 93 (95% CI = 86–97), respectively. For the TST, the corresponding values in HIV-infected children with definite TB were 33 (95% CI = 11–62), 86 (95% CI = 74–93), 38 (95% CI = 13–68) and 83% (95% CI = 71–91), respectively.

The interobserver agreement in assigning results for the ELISPOT assays was high (96%). There was a strong correlation between individual spot counts recorded by both observers (Spearman’s correlation 0.98, P < 0.0001). In the 22 patients who had both ELISPOT and T-SPOT.TB performed, IFN-γ responses to ESAT-6 and CFP-10 were significantly correlated between the two assays (ESAT-6: Spearman’s correlation 0.71, P = 0.0002; CFP-10: Spearman’s correlation 0.72, P = 0.0002). Of 188 baseline ELISPOT assays, 17 (9.0%) yielded results that were considered indeterminate by study criteria (two inadequate PBMC, one technical, one failed positive control, 13 high-negative control). Therefore, overall, 171 (91%) ELISPOT assays yielded interpretable results. There was no significant difference in the proportion of indeterminate ELISPOT results between HIV-infected and HIV-uninfected children [12/139 (8.6%) vs. 5/49 (10.2%), P = 0.78].

### Discussion

The potential for immunodiagnosis of TB has improved with the discovery of the TB-specific antigens [22]. IGRAs are increasingly being proposed as replacement tests for the TST [23–25]. The majority of studies have evaluated IGRA for the detection of TB in HIV-uninfected people and there are several recent reports of their use in HIV-infected adults [26–36]. There are limited data from children and only one study reporting their use in a small number of HIV-infected children [18–20]. This is the first study to describe the effect of immunosuppression on the performance of IGRA in children.

Our study confirms the poor sensitivity of TST in HIV-infected children with TB [13] and shows that sensitivity of ELISPOT is significantly higher than TST in children with definite or probable TB, including those with malnutrition. These data are consistent with the findings of the previous study on South African children [20]. In addition, we found that compared with TST, ELISPOT responses were well maintained in young children with severe immunosuppression. These data are of practical relevance because the diagnosis of TB in children is challenging and important in those at greatest risk of disease: young, HIV-infected children with advanced immunosuppression and malnutrition [11].

The sensitivity of ELISPOT in HIV-infected children in this study is inferior to that reported in HIV-infected adults. Chapman et al. [27] reported a sensitivity of 90% for ELISPOT for the diagnosis of active TB in HIV-infected Zambian patients. In South Africa, Rangaka et al. [1] compared T-SPOT.TB and Quantiferon-TB Gold for the detection of latent TB infection in both HIV-infected and HIV-uninfected adults and found that...
Proportion scored positive by either IGRA was fairly well maintained with advanced immunosuppression. Concerns, however, have been raised about potential false negative IGRA responses in this setting. For example, Brock et al. [26] reported a higher rate of indeterminate assay results in individuals with CD4 cell counts less than 100 cells/\(\mu\)l in a low-incidence setting. Similarly, Jones et al. [32] found indeterminate results overrepresented in HIV-infected individuals with a CD4 cell count less than 200 cells/\(\mu\)l screened for latent TB infection. In contrast, the majority of indeterminate assay results in our study resulted from a high background nil control response in HIV-infected children, a finding that has been reported in other cohorts of HIV-uninfected African children [18,37].

The sensitivity of ELISPOT in our study was consistent with the 73% sensitivity reported by Liebeschuetz et al. [20] in a smaller group of 30 HIV-infected children (median age 50 months) with confirmed or highly probable TB. A case report detailed the use of an ELISPOT for the early detection of TB in an 11-year-old HIV-infected child with advanced immunosuppression (CD4 2%) in whom the TST was negative [38]. The detection of an immune response to TB prompted the early introduction of antituberculous treatment prior to...
culture confirmation and illustrates the potential benefit of IGRA to influence management decisions [39].

A sensitivity of 64% and NPV of 87% is an improvement over TST but indicates that ELISPOT cannot be used to definitively exclude a diagnosis of TB in HIV-infected children. The potential for ELISPOT, however, to provide an early indication of TB is important in clinical practice and may allow for earlier initiation of antituberculous treatment in this highly vulnerable cohort. In our study, over two-thirds of children with smear negative TB had a positive ELISPOT.

Currently available IGRA are unable to discriminate between active TB disease and latent TB infection (LTBI). A relationship between bacillary load and IFN-γ responses may exist, with active TB disease associated with higher IFN-γ responses compared with latent TB infection [40,41]. An unanticipated finding of our study was that the magnitude of IFN-γ responses to ESAT-6 and CFP-10 was significantly higher in HIV-infected children with definite or probable TB compared with IFN-γ responses in HIV-infected children without TB who had a positive ELISPOT. Our data, therefore, are consistent with the possibility that the magnitude of the IGRA response may help to differentiate active TB disease from latent TB infection in children, as has been suggested in adults [28,36].

There were several limitations to our study. First, the ELISPOT assay used in the study was not the commercially available T-SPOT.TB assay. However, the assay, incorporating pools of peptides of ESAT-6 and CFP-10 as stimulatory antigens, is similar to the commercial T-SPOT.TB assay. In addition, there was a significant correlation in the magnitude of IFN-γ responses to ESAT-6 and CFP-10 between ELISPOT and T-SPOT.TB assays. An in-house version of the ELISPOT assay has been used in many previous published comparison studies [21,42–46]. A further limitation of our study was that not every child deemed uninfected had a thorough work-up to exclude TB. Follow-up of this cohort was for a relatively short period of time and therefore we cannot be certain that some of these children did not develop TB.

In conclusion, we found the ELISPOT assay to be superior to TST in the evaluation of HIV-infected children with suspected TB. The performance of ELISPOT was relatively unaffected by young age or HIV-associated immunosuppression. However, the sensitivity of current ELISPOT assays is not sufficiently high enough to be used as rule out test for TB in children.

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