Potential of interferon-γ-inducible protein 10 in improving tuberculosis diagnosis in HIV-infected patients

To the Editors:

In patients latently infected with *Mycobacterium tuberculosis*, immunosuppression significantly augments the risk of progression to active tuberculosis (TB) and TB is still one of the most frequent opportunistic infections worldwide. Prevention of TB in HIV-positive patients using the tuberculin skin testing (TST) followed by targeted preventive treatment is an effective strategy, but has thus far shown limited clinical success, in part due to the lack of a reliable test for latent TB infection (LTBI) [1]. Interferon (IFN)-γ release assays (IGRAs) have shown great potential in the improvement of LTBI diagnosis, but the tests perform suboptimally in immunocompromised populations. We and others have previously shown that HIV-positive patients have high rates of indeterminate QuantiFERON®-TB Gold In-Tube test (QFT-IT) results and that this, at least in part, is due to low CD4 T-cell count [2–4].

We and others have also shown that IFN-γ-inducible protein 10 (IP-10/CXCL-10) is an alternative biomarker may improve immunodiagnosis of *M. tuberculosis* infection. IP-10 is induced specifically and in large quantities upon *in vitro* stimulation of whole blood with *M. tuberculosis*-specific antigens. We have developed an IP-10 based test [5] and shown that the IP-10 test performs comparably to the QFT-IT in adult patients with intact immune function [6].

We compared the sensitivity of an IP-10 based test with the QFT-IT for diagnosing *M. tuberculosis* infection in HIV-negative and HIV-positive patients with culture-confirmed active pulmonary tuberculosis (PTB). 300 patients newly diagnosed with PTB were recruited prospectively through the National Tuberculosis and Leprosy Programme (Mwanza, Tanzania). Blood was drawn for IGRA and HIV testing, and CD4 cell counts. Sputum was collected for microscopy and *M. tuberculosis* culture. Patients >15 yrs of age with a positive sputum culture result were enrolled. All HIV-positive patients were newly diagnosed in the study context. A detailed description of recruitment, testing and sample handling has been published previously [3]. After QFT-IT testing, supernatants were frozen at -80°C. 1 yr later, samples were thawed and analysed for IP-10 using xMAP technology on the Luminex platform (Luminex Corp., Austin, TX, USA) as described previously [5]. Algorithms for interpretation of IP-10 test results have been developed previously using receiver operating characteristic analysis [5]; however, the mitogen cut-off was chosen arbitrarily (see online supplement 1). One IU corresponds to 50 pg IFN-γ. In the literature, there is a lack of consensus on how to present IGRA sensitivity and specificity: including or excluding indeterminate results. We suggest the designations ''sensitivity'' for calculation after exclusion of indeterminate results, and ''positivity rate'' for calculation amongst all possible test outcomes. High rates of indeterminate results (*i.e.* low positivity rate) mainly affect usability and cost-effectiveness, and may impair the feasibility of implementing the test in severely immunocompromised individuals. Positivity rate was calculated among all possible tests outcomes (*i.e.* including indeterminate results), whereas sensitivity was calculated after exclusion of indeterminate test results. All comparisons were done using nonparametric paired tests. A p-value $\leqslant 0.05$ was considered significant. Permission to conduct the study was granted by the ethics committee of the National Institute for Medical Research (Mwanza, Tanzania) and was approved by The Danish National Committee on Biomedical Research Ethics (Copenhagen, Denmark; reference 2005-7041-57).

HIV-negative (n=92) and HIV-positive (n=65) patients were included in the study. HIV-positive patients were older, had lower CD4 cell counts and were more likely to be female ($p<0.05$ for all, see online supplement 2). The IP-10 test had comparable positivity rate and sensitivity to the QFT-IT in both HIV-positive and HIV-negative patients. Positivity rate was lower and indeterminate rate higher in HIV-positive patients for both tests, while there was no significant difference in sensitivity for either test (table 1).

Impaired performance of the QFT-IT in HIV-positive patients with a low CD4 cell count has been well documented and we have previously demonstrated that QFT-IT sensitivity decreases with decreasing CD4 cell count in HIV-positive patients, which is mediated by an increasing proportion of indeterminate results. Interestingly, for the IP-10 test, we found no such trend with CD4 cell count for rate of positive ($p=0.44$) or for indeterminate ($p=0.55$) results (fig. 1). Another recent study in HIV-infected patients with active TB reported that, while QFT-IT yielded indeterminate results when CD4 cell count was <200 cells·μL^{-1}, an IP-10-based test yielded indeterminate results only when CD4 cell count was <50 cells·μL^{-1} [4]. Thus, it seems that IP-10 is not, or at least is much less, affected by CD4 cell count than is the QFT-IT. Although IFN-γ is directly involved in inducing IP-10 production, IP-10 is produced primarily by the monocyte and might be induced by CD4 T-cell- and IFN-γ-independent pathways (*e.g.* induction

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TABLE 1 Distribution of QuantiFERON®-TB Gold In-Tube (QFT-IT) and interferon-γ-inducible protein (IP)-10 test results and comparison between tests

Test result	Single-marker tests		Combined test
	IP-10 test[#]	QFT-IT[¶]	
HIV-negative[+]			
Positive	75 (82; 72–89)	74 (80; 71–88)	86 (93; 88–99)[##]
Sensitivity	86 (79–93)	88 (81–95)	96 (91–99)[##]
Negative	12 (13; 7–22)	10 (11; 5–19)	4 (4; 0–9)[##]
Indeterminate	5 (5; 2–12)	8 (9; 4–16)	2 (2; 0–5)[¶¶]
HIV-positive[§]			
Positive	41 (63; 50–75)[f]	41 (63; 50–75)[f]	46 (71; 60–82)[##]
Sensitivity	76 (65–87)	82 (74–95)	88 (80–97)[##]
Negative	13 (20; 11–32)	9 (14; 7–25)	6 (9; 2–16)[++]
Indeterminate	11 (17; 9–28)[f]	15 (23; 14–35)[f]	13 (20; 10–30)

Data are presented as n (%; 95% CI) or % (95% CI). Sensitivity was calculated after excluding indeterminate results. Algorithms for test results can be seen in online supplement 1. In the combined approach, the test was deemed positive if either test was positive, negative if both tests were negative and indeterminate if one test was indeterminate and the other was negative. All p-values were calculated using McNemar's test. [#]: cut-off 673 pg·mL^{-1}, as determined in [5]; [¶]: cut-off 0.35 IU·mL^{-1} (17.5 pg·mL^{-1}); [+]: n=92; [§]: n=65; [f]: $p<0.05$ compared with HIV-negative patients; [##]: $p<0.05$ compared with either single-marker test; [¶¶]: $p<0.05$ compared with the QFT-IT alone; [++]: $p<0.05$ compared with the IP-10 test alone.

by other cytokines) [7]. We found no effect of monocyte count on IP-10 test performance in either HIV-positive or -negative patients (data not shown). Another possible explanation for the finding is the larger amounts of biomarker produced, which may render an IP-10-based test less sensitive to the effect of immune suppression. Antigen-induced IP-10 levels were 21-fold higher than IFN-γ (interquartile range 7–48; $p<0.001$). Lastly, the mitogen cut-off was rather low, giving less indeterminate IP-10 test results and, thereby, possibly obscuring CD4 dependency. However, increasing the mitogen cut-off did not change these findings (data not shown). We found no further connection between clinical characteristics (listed in online supplement 2) and indeterminate or false negative results by either test.

Agreement between the tests was 0.73 (κ=0.67) for HIV-negative and 0.78 (κ=0.60) for HIV-positive patients (online supplement 3). Combining the tests significantly increased sensitivity to 96% in HIV-negative and 88% in HIV-positive patients (table 1). We and others have found a similar improvement in sensitivity among patients from low-endemic setting without a compromise in specificity [8, 9]. Thus, combining tests appears to be a feasible way to improve performance of the antigen-specific assays. We found no connection between clinical characteristics (listed in online supplement 2) and discordant test results.

It is important to remember that the diagnostic sensitivity obtained in a population with active and severe TB may be lower than the sensitivity obtained among otherwise healthy individuals with LTBI. Also, a limitation of the present study is the lack of a control group, which prevents estimation of specificity. None of the tests discriminates between active TB and LTBI and, given the prevalence of LTBI in the background population, we could not identify a valid control group in this setting. Inclusion of a control group from another geographic region would not have been a valid approach due to potential influences of genetic background, comorbidity, nutritional status, *etc.* However, a very high specificity of both QFT-IT and the IP-10 test has been convincingly demonstrated in healthy, unexposed individuals from low-endemic regions. Unfortunately, TST was not performed in this study population.

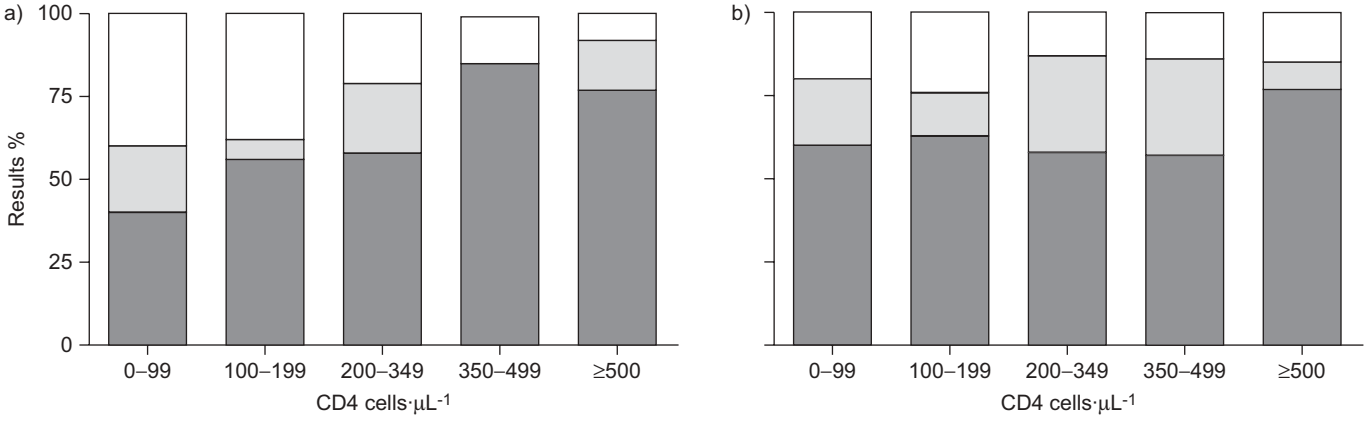

FIGURE 1. Influence of CD4 cell count on performance of the a) QuantiFERON®-TB Gold In-Tube (■: positive results (p=0.05); ■: negative results (p=0.99); □: indeterminate results (p=0.04)) and b) interferon-γ-inducible protein 10 tests (■: positive results (p=0.44); ■: negative results (p=0.72); □: indeterminate results (p=0.55)) in HIV-positive patients. p-values are for Cochrane–Armitage test for trend. Test results were grouped by the individual number of CD4 cells·μL^{-1}. The numbers of patients in each CD4 cell group were as follows. 0–99 cells·μL^{-1}: n=5; 100–199 cells·μL^{-1}: n=16; 200–349 cells·μL^{-1}: n=24; 350–499 cells·μL^{-1}: n=7; >500 cells·μL^{-1}: n=13. a) Modified from [3].

In conclusion, the IP-10 test performs with equal sensitivity to the QFT-IT in a TB/HIV-endemic setting. Combining the tests significantly improves sensitivity, even in HIV-positive patients. The IP-10 test seems less affected by a low CD4 cell count than the QFT-IT. Further approaches for improvement of TB diagnosis are needed, especially in TB/HIV-endemic settings, and the IP-10 biomarker represents a promising example.

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REFERENCES

1 World Health Organisation. Global tuberculosis control: epidemiology, strategy, financing. Geneva, WHO, 2009.
2 Domínguez J, Latorre I, Altet N, *et al.* IFN-γ-release assays to diagnose TB infection in the immunocompromised individual. *Expert Rev Resp Med* 2009; 3: 309–327.
3 Aabye MG, Ravn P, PrayGod G, *et al.* The impact of HIV infection and CD4 cell count on the performance of an interferon gamma release assay in patients with pulmonary tuberculosis. *PLoS ONE* 2009; 4: e4220.
4 Kabeer BS, Sikhamani R, Raja A. Comparison of interferon gamma and interferon gamma-inducible protein-10 secretion in HIV-tuberculosis patients. *AIDS* 2010; 24: 323–325.
5 Ruhwald M, Bodmer T, Maier C, *et al.* Evaluating the potential of IP-10 and MCP-2 as biomarkers for the diagnosis of tuberculosis. *Eur Respir J* 2008; 32: 1607–1615.
6 Ruhwald M, Petersen J, Kofoed K, *et al.* Improving T-cell assays for the diagnosis of latent TB infection: potential of a diagnostic test based on IP-10. *PLoS ONE* 2008; 3: e2858.
7 Korpi-Steiner NL, Bates ME, Lee WM, *et al.* Human rhinovirus induces robust IP-10 release by monocytic cells, which is independent of viral replication but linked to type I interferon receptor ligation and STAT1 activation. *J Leukoc Biol* 2006; 80: 1364–1374.
8 Lighter J, Rigaud M, Huie M, *et al.* Chemokine IP-10: an adjunct marker for latent tuberculosis infection in children. *Int J Tuberc Lung Dis* 2009; 13: 731–736.
9 Syed Ahamed KB, Raman B, Thomas A, *et al.* Role of QuantiFERON-TB gold, interferon gamma inducible protein-10 and tuberculin skin test in active tuberculosis diagnosis. *PLoS ONE* 2010; 5: e9051.

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New insight into extremely drug-resistant tuberculosis: using atomic force microscopy

To the Editors:

We have recently documented the emergence of new forms of resistant tuberculosis (TB) bacilli (totally drug resistant (TDR)-TB or extremely drug-resistant (XXDR)-TB strains) among multidrug-resistant (MDR)-TB patients [1]. XXDR-TB defines any case of TB with resistance to all first- and second-line anti-TB drugs whose smears and cultures remain positive despite prolonged therapy [1–3]. At the cellular level of XXDR-TB strains, adaptation was observed and evaluated using transmission electron microscopy (TEM) [4, 5]. In the exponential phase, three different cell populations were clearly distinguished: one displayed an ordinary pattern (70–80%), one exhibited a round or oval shape (15–20%), and the third displayed an extraordinarily thick cell wall (21–26 nm) with features similar to stationary or anaerobic dormant bacilli (5–7%) [3, 4]. These adapted forms were detected in all XXDR-TB isolates, irrespective of their super families or genotype patterns. We tried to evaluate the different cell population of XXDR-TB strains in comparison to susceptible cells using atomic force microcsopy (AFM). To achieve this goal, we included sputum and culture positive specimens of the same