The Use of Interferon-gamma Release Assays in HIV-positive Individuals

Matthias Hoffmann[1] and Pernille Ravn[2]

1. Clinical Research Fellow, Department of Internal Medicine, Division of Infectious Diseases, Cantonal Hospital St Gallen;
2. Senior Research Fellow, Clincal Infectious Disease Unit, Herlev University Hospital, University of Copenhagen

Abstract

Interferon-gamma release assays (IGRAs) are promising candidates to replace the tuberculin skin test (TST) in screening for latent tuberculosis infection (LTBI). However, there are some limitations: IGRAs are, like the TST, only an indirect marker for *Mycobacterium tuberculosis* (MTB) infection and sensitivity for diagnosing active TB is sub-optimal, with false-negative results. This article gives an overview of the performance of the two commercially available IGRAs in adult HIV-positive individuals. We will focus on sensitivity and indeterminate rates among HIV-positive patients and the potential influence of CD4+ cell count. Among the studies published using commercial IGRAs, we found that the sensitivity of IGRAs in HIV co-infected individuals with or without an indeterminate result is impaired compared with HIV-negative patients with TB. Indeterminate rates are higher in HIV-positive individuals and the number of CD4+ cells is significantly associated with the rate of indeterminate results, whereas the effect of CD4+ cell numbers on actual sensitivity is less pronounced. From our findings, both IGRAs are likely to give very high rates of indeterminate and probably unreliable results when CD4+ cell count is <100 cells/µl. However, the tests seem to give reliable results at a CD4+ cell count >200 cells/µl, but this needs to be confirmed in studies including a sufficient number of HIV-positive individuals with low CD4+ cell numbers to allow meaningful statistical calculations. IGRAs have a high negative predictive value in a low endemic but not in a high endemic setting. The high proportion of indeterminate results underlines the importance of positive control as a marker of anergy, helping to exclude false-negative results, which is not an option for the TST. The positivity rate increased by 10% when indeterminate results were excluded and therefore we strongly recommend full openness on the proportion and use of indeterminate results when reporting the performance and accuracy of IGRA.

Keywords

Tuberculosis, interferon-gamma release assay (IGRA), tuberculosis diagnosis, HIV infection, CD4 count

Disclosure: Matthias Hoffmann has been an invited speaker by Cellestis and received a travel grant in 2009, and has received a research grant from Roche. Pernille Ravn has been an invited speaker by Cellestis and served once as a consultant on clinical guidelines for the QFT test in 2006, has received a research grant from Abbott and Schering-Plough and has filed a patent on the use of IP10 as a diagnostic marker for tuberculosis.
Acknowledgements: We would like to thank M Aichelburg, M Balcells, K Dheda, C Lange, A Luetkemeyer, C Stephan, N Talati, T Wolf and RJ Wilkinson for providing us with more detailed information on their findings on CD4 cell count strata.
Received: 17 November 2009 **Accepted:** 25 February 2010 **Citation:** *European Infectious Disease*, 2010;4(1):23–9
Correspondence: Pernille Ravn, Department of Infectious Diseases, University of Copenhagen, Herlev Hospital, Herlev Ringvej 75, 2730, Denmark.
E: Herlev.Pravn@dadlnet.dk

Infection with *Mycobacterium tuberculosis* (MTB) remains one of the most challenging infectious disease problems worldwide. In most individuals the MTB infection is initially contained by the host's immune defence and remains latent. The World Health Organization (WHO) estimates that one-third of the world's population is latently infected with MTB.[1] For active tuberculosis (TB), the diagnosis is often complicated by unspecific clinical symptoms, slow growth of MTB bacteria and difficulties in obtaining adequate sample material and distinguishing between MTB and non-tuberculosis mycobacteria (NTM) infection before culture and polymerase chain reaction (PCR) results are available. MTB, which is dormant during latent TB infection (LTBI), can reactivate and cause active TB.[2,3] Reactivation of LTBI is most often seen in immune-compromised individuals,[4] especially in HIV-co-infected individuals even after the initiation of antiretroviral therapy (ART).[5–7] As reactivation can occur many decades after primary infection,[8] successful prevention of MTB spread on a population level and the prevention of disease on an individual level is a major challenge. Preventative therapy is recommended for HIV-infected individuals with LTBI in order to reduce the risk of progression to active TB disease, but is far from being implemented routinely.[9,10]

The main method for LTBI diagnosis has been the tuberculin skin test (TST). The TST is based on a delayed-type hypersensitivity reaction after stimulation with tuberculin purified protein derivate (PPD). Tuberculin PPD contains a broad range of cross-reactive antigens recognised both after bacille Calmette-Guérin (BCG) vaccination and after infection with MTB or non-tuberculous mycobacteria.[11,12] TST positivity is clearly associated with subsequent risk of developing active TB disease and has been used for decades to screen for LTBI.[13–15] The major downfalls are the impaired specificity due to cross-reactivity[11,12] and impaired sensitivity, especially in HIV-co-infected individuals.[9,16–20] Furthermore, the TST lacks an internal control to distinguish false-negative results due to anergy from true negative results, leading to a poor diagnostic accuracy, especially in HIV-positive individuals, due to a high rate of unresponsiveness.[16–20]

Table 1: Interferon-gamma Release Assay/Tuberculin Skin Test Sensitivity in Active Tuberculosis

First Author (ref.)	Test System	HIV-positive Individuals with Confirmed Active TB[a]					HIV-negative Individuals with Confirmed Active TB[a]			
		n	CD4+ Cell Count Median (IQR), Mean (range)[b]	Indeterminate Results % (n)	Positive Results (indeterminate results included) %	Sensitivity (indeterminate results excluded) %	n	Indeterminate Results % (n)	Positive Results (indeterminate results included) %	Sensitivity (indeterminate results included) %
Vincenti et al.[29]	QFT	13	N/A	Excluded	N/A	85				
Seshadri et al.[43]	QFT	13	N/A	31 (4)	23	33	40	18 (7)	70 (28/40)	85 (28/33)
OVERALL	QFT	26		N/A (n=4)	N/A (n=3)	63.5 (n=14) (95% CI 42.5–80.5)	40	N/A (n=7)	N/A (n=28)	N/A (n=28)
Tsiouris et al.[44]	QFT-IT	26	N/A	24 (5)	65	81	15	0	73	73
Kabeer et al.[45]	QFT-IT	105[c]	116 (48,209)[b]	17 (18)	65	78				
Raby et al.[46]	QFT-IT	59	212 (109,332)	17 (10)	63	76	37	13 (5)	84	97
Aabye et al.[47]	QFT-IT	68	272 (172,418)	22 (15)	65	83	93	9 (8)	81	88
Markova et al.[48]	QFT-IT	13	195 (15-450)[d]	0 (0)	92	92				
Leidl et al.[49]	QFT-IT	19	182 (N/A)	0 (0)	68	68				
OVERALL	QFT-IT	290		16.5% (n=48) (95% CI 12.5–21.5)	66 (n=191) (95% CI 60–71)	79 (n=191) (95% CI 73.5–83.5)	145	9% (n=13) (95% CI 5.5–14.5)	80.5 (n=117) (95% CI 73.5–86.5)	88.5 (n=117) (95% CI 85–94)
Markova et al.[48]	T-spot	13	195 (15-450)[d]	31 (4)	62	89				
Leidl et al.[49]	T-spot	19	182 (N/A)	11 (2)	89	100				
Vincenti et al.[29]	T-spot	13	N/A	Excluded	N/A	85				
Jiang et al.[50]	T-spot	32	N/A	0 (0)	66	66				
OVERALL	T-spot	77		9.5% (n=6) (95% CI 4.5–19)	72[e] (n=46) (95% CI 60–81.5)	80.5 (n=57) (95% CI 69.5–88)	–	–	–	–
Vincenti et al.[29]	TST[f]	13	N/A	–	46	–				
Seshedri et al.[43]	TST[f]	13	N/A	–	54	–	40	–	100	–
Tsiouris et al.[44]	TST[f]	26	N/A	–	85	–	16	–	94	–
Kabeer et al.[45]	TST[f]	105	116 (48,209)	–	31					
Raby et al.[46]	TST[f]	47	212 (109,332)	–	55	–	31	–	81	–
Jiang et al.[50]	TST	32	N/A	–	25	–		–		
OVERALL	TST[f]	236		–	43 (n=102) (95% CI 37–49.5)	–	87	–	92 (n=80) (95% CI 84.5–96)	–

a. Active TB defined as acid fast bacilli-positive sputum smear and/or positive Mycobacterium tuberculosis (MTB) culture, unless otherwise indicated; b. n=81 with available CD4+ cell count; c. Active MTB diagnosis based on positive sputum smear/culture result or radiological and clinical evidence; d. Mean CD4+ cell count (range); e. Vincenti et al. excluded from calculation; f. Tuberculin skin test (TST) cut-off ≥5mm. IQR = inter-quartile range; N/A = not available; QFT = QuantiFERON Gold®; QFT-IT = QuantiFERON Gold InTube®; tot = total; T-spot = T-spot TB®.

The development of new interferon-gamma (IFN-γ) release assays (IGRAs) is a promising attempt to overcome the problems with specificity and sensitivity.[21–24] Two IGRAs are now commercially available: the T.Spot TB® (Oxford Immunotech, UK) (T-Spot), and the QuantiFERON-TB gold® and QuantiFERON-TB gold InTube® (Cellestis, Australia) (QFT and QFT-IT, respectively). Both tests are based on *ex vivo* stimulation of T cells in either whole blood (QFT) or purified lymphocytes (T-spot) with MTB-specific antigens followed by quantification of IFN-γ using enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immunosorbent spot (ELISPOT) technology. The 6kDa early secretory antigenic target (ESAT-6) and culture filtrate protein 10 (CFP-10), used for stimulation, are encoded in the region of deletion (RD) 1, which is absent from the BCG vaccine strain[25–27] and most non-tuberculous mycobacteria.[21,28] The QFT-IT includes an additional specific epitope, TB7.7p4, and an optimised cell stimulation system based on vacutainer tubes pre-coated with the peptides, ensuring the immediate stimulation of T cells once the blood is drawn. IGRAs measure the TB-specific effector/memory T-cell responses directly *ex vivo* by their IFN-γ secretory capacity.[21,29]

Advantages and Limitations of Interferon-gamma Release Assays in HIV-positive Individuals

The obvious advantages of the IGRAs are that they are specific for MTB infections with hardly any cross-reactivity. The pooled specificity of the T-spot in healthy unexposed individuals was 97.7% (95% confidence interval [CI] 96–99%) and all QFT formats 92.5% (95% CI 86–99%).[22] As a further advantage, IGRAs contain an internal positive control allowing the reader to discriminate true negative from false-negative results. The readout is objective and results are presented as positive, negative or indeterminate based on an algorithm. The test requires only one visit, obviating the dismal return rates found with TST reading.[10,30]

Although the IGRAs are promising, there are still some limitations: the method is only an indirect marker for MTB infection and distinguishes neither between latent and active infection nor between recent, past or treated MTB infection. The sensitivity for diagnosing active TB is sub-optimal, with false-negative results in both HIV-negative and -positive TB patients and otherwise immunocompromised individuals.[21,22,31–38] IGRAs rely on functional CD4+ T cells[39–41] and their performance might be negatively influenced by low and impaired CD4+ cell counts in HIV-infected individuals. According to the manufacturer, the method is developed for the diagnosis of LTBI and is currently used to screen potentially infected individuals, but the evaluation is often performed in active TB as no gold standard is available for LTBI.[22,32,42]

This article provides an overview of the knowledge available on the performance of the two commercially available IGRAs in adult HIV-positive individuals. We will focus on the sensitivity of the commercially available IGRAs; the positivity and indeterminate rates among HIV-positive and -negative individuals without active TB; the influence of CD4+ cell count on positivity and indeterminate rates; and discussion of the preliminary evidence for the predictive value of

IGRAs. We found 30 studies,[29,43–71] of which 20 assessed commercially available IGRAs in adults.[29,43–61]

Sensitivity of Interferon-gamma Release Assays and Tuberculin Skin Test

TST sensitivity in immunocompromised patients and in HIV-positive individuals is impaired, with a high rate of false-negative results, especially in patients with low CD4+ cell counts at highest risk of LTBI reactivation.[4–7,9] We found eight published studies exploring the sensitivity of the commercially available IGRAs in HIV-positive individuals with active TB (see *Table 1*).[43–50] The gold standard for active TB was either a positive culture or a microscopy-positive sputum smear.

The overall sensitivity of the TST in six studies was 43% (range 25–85%, 95% CI 37–49.5).[29,43,44,46,50] Compared with the TST, both IGRAs had an equal or higher sensitivity but with marked differences between the studies (see *Table 1*). Excluding the indeterminate results, the sensitivity of T-spot was 80.5% (range 66–100%, 95% CI 69.5–88%) and of QFT-IT was 79% (range 68–92%, 95% CI 73.5–83.5)%) for the QFT-IT (indeterminate results T-spot 9.5%, QFT-IT 16.5%). Only two studies comprising 26 patients addressed the sensitivity of QFT and found very different results.[29,43] Interestingly, exclusion of indeterminate results increased the sensitivity by roughly 10% for both IGRAs (13% for the QFT-IT and 8.5% for the T-spot), underlining the importance of a positive control in immune-compromised individuals (see *Table 1*).

Two studies compared QFT-IT with the TST in populations similar in respect of CD4+ cell count and who were antiretroviral treatment (ART)-naïve.[44,46] Tsiouris et al. found that the sensitivity of the IGRA was similar to the TST (81 and 85%, respectively),[44] whereas Raby et al. showed a markedly increased sensitivity of QFT-IT compared with the TST (87 and 55%, respectively).[46] Only two studies, comprising 26 patients, addressed the sensitivity of QFT and they found very different results.[29,43]

Possible explanations for the differences between TST and IGRAs may be that IGRAs measure circulating T cells, whereas TST measures delayed-type immune responses dependent on the migration of immune-competent cells to the site of the TST application.[21,23] Studies including the comparison with TST are biased, as a significant number of individuals never return for their test result reading[30] or never have the TST applied, limiting the use of the TST in clinical practice and for study purposes. Three studies compared the IGRA sensitivity in HIV-infected and -uninfected patients directly and found a lower sensitivity in HIV-positive compared with HIV-negative TB patients, irrespective of the inclusion of indeterminate results in the analyses.[44,46,47] The two studies (comprising a total of 32 patients) that compared QFT-IT and T-spot found conflicting results.[48,49]

In summary, several studies on IGRAs and TST in other immune-compromising diseases and the studies presented here in HIV-co-infected individuals substantiate the perception of a higher sensitivity of either IGRA compared with the TST. However, IGRA sensitivity in HIV-infected individuals remains impaired compared with healthy controls, and IGRAs suffer the same constraints as the TST with a substantial number of false-negative results in patients with TB.

Given the impaired sensitivity and inability to discriminate between active and LTBI, IGRAs should not be used as an alternative to conventional microbiological and clinical investigations in the diagnosis of active TB.

Association of Probable Latent Tuberculosis Infection and Positive Interferon-gamma Release Assays

To establish the accuracy of IGRA in LTBI, investigators have used either TST as the gold standard or association with risk factors for TB infection such as exposure.[72,73] Using the latter approach, most studies in low-endemic regions find a higher agreement between IGRA positivity and clinical or epidemiological risk factors associated with LTBI than the TST,[35,60,73] and we have previously demonstrated in a low-endemic region that QFT and QFT-IT positivity was associated with long-term residency in high-endemic regions and with previous TB exposure,[35,56,74] whereas TST positivity was independent of TB risk factors.[35,75] *Table 2* gives an overview of the IGRA and TST positivity rates in HIV-positive and -negative individuals without active TB. In high- and low-endemic regions, overall IGRA positivity rates ranged from 39 to 68% and 5.3 to 13.2%, respectively, underlining the specificity of the tests. Exclusion of indeterminate results did not change the result (not shown). In contrast to the observation of impaired sensitivity for HIV-positive individuals in active TB (see *Table 1*) we did not see such clear differences in otherwise healthy HIV-positive individuals (see *Table 2*). In only four studies was a direct head-to-head comparison between the T-spot and the QFT or QFT-IT performed, reporting only a poor inter-test agreement (κ range 0.06–0.34).[49,51,55,57] The clinical relevance of this difference between the IGRAs remains to be elucidated.

Indeterminate Results

Indeterminate rates in active TB were higher (see *Table 1*) than in healthy controls, consistent with other observations demonstrating reduced T-cell reactivity in patients with severe TB.[75,77] The general perception is that T-spot seems to be more sensitive, especially in immunocompromised individuals, in comparison with the QFT/QFT-IT. We found a trend for higher sensitivity of the T-spot compared with QFT-IT in active TB, mainly explained by higher QFT-IT indeterminate rates (see *Table 1*), whereas the overall positivity rate in individuals with possible LTBI for both IGRAs was similar (see *Table 2*).

The overall rate of indeterminate results in individuals screened for LTBI was 4% (range 0.4–43.5%, 95% CI 1.8–4.7%), 4% (range 2–6%, 95% CI 3.5–5%) and 6.7% (range 1–14%, 95% CI 5.5–9%) for the QFT, QFT-IT and the T-spot, respectively. Five studies compared both IGRAs, with conflicting results: four studies found a higher rate of indeterminate T-spot results compared with the QFT/QFT-IT in active TB[48,49] or probable LTBI (see *Tables 1* and *2*);[48,49,52,55] Rangaka et al. found a higher rate of indeterminate QFT results[51] and Leidl et al. a similar rate (QFT-IT versus T-spot)[49] in screening for LTBI in high-endemic settings (see *Table 2*).

Impact of CD4+ Cell Count on Interferon-gamma Release Assay Performance

Several studies have evaluated the association between CD4+ cell count and indeterminate rates. Of 16 studies, two were biased in respect of CD4+ cell counts: patients with <100 CD4+ cells/µl were excluded or infrequent.[58,60] Fourteen of the 16 studies that addressed the effect of CD4 count on IGRA performances reported a trend towards a lower phytohaemagglutinin (PHA)-induced IFN-γ response with decreasing CD4+ cell count irrespective of the IGRA used, in addition looking at the consecutively increased risk of indeterminate results in association with CD4+ cell count; the effect seems to be more marked for the QFT/QFT-IT than for the T-spot, although most studies did not perform a direct head-to-head comparison.[45–49,51,52,54,55–59]

Table 2: Positivity, Negativity and Indeterminate Rates in Probable Latent Tuberculosis Infection in HIV-positive and -negative Individuals

First Author (ref.)	Test System	HIV-positive, TB-negative						HIV-negative, TB-negative			
		n	CD4+ Cell Count[a]	ART (%)	Positive (%)	Negative (%)	Indeterminate (%)	n	Positive (%)	Negative (%)	Indeterminate (%)
TB High/Medium-endemic Setting (TB prevalence >400/100,000)											
Rangaka et al.[51]	QFT	74	Median 392 (IQR 263;520)	0	43	50	7	84	46	51	2
Seshadri et al.[43]	QFT	16	N/A	n/a	19	37.5	43.5	14	14	50	36
OVERALL	**QFT**	**90**			**39 (n=35) (95% CI 29.5–49)**	**48 (n=43) (95% CI 37.5–58)**	**13 (n=12) (95% CI 8–22)**	**98**	**42 (n=41) (95% CI 32.5–52)**	**51 (n=50) (95% CI 41–61)**	**7 (n=7) (95% CI 3.5–14)**
Leidl et al.[49]	QFT-IT	109	Median 283 (N/A)	0	68	28.5	3.5	7	100	0	0
OVERALL	**QFT-IT**	**109**			**68 (n=74) (95% CI 58.5–76)**	**29.5 (n=32) (95% CI 21.5–38.5)**	**2.5 (n=3) (95% CI 1–8)**		**100 (–)**	**0 (–)**	**0 (–)**
Rangaka et al.[51]	T-spot	74	Median 392 (IQR 263;520)	0	52	47	1	86	59	41	0
Leidl et al.[49]	T-spot	109	Median 283 (N/A)	0	54	42.5	3.5	7	71.5	28.5	0
OVERALL	**T-spot**	**182**			**53.5 (n=97) (95% CI 46–60.5)**	**44 (n=80) (95% CI 37–51)**	**2.5 (n=5) (95% CI 1–6.5)**	**93**	**60 (n=56) (95% CI 50–69.5)**	**40 (n=37) (95% CI 30.5–50)**	**0 (n=0)**
Rangaka et al.[51]	TST	67	Median 392 (IQR 263;520)	0	52	48	–	77	86	14	–
Seshadri et al.[43]		16	n/a	n/a	19	81	–	14	50	50	–
Leidl et al.[49]	TST	89	Median 283 (N/A)	0	47	53	–	7	100	0	–
OVERALL	**TST**	**172**			**46.5 (n=80) (95% CI 39–54)**	**53.5 (n=92) (95% CI 46–61)**		**98**	**81.5 (n=80) (95% CI 73–88)**	**18.5 (n=18) (95% CI 12–27)**	–
TB Medium-endemic Setting (TB prevalence <200/100,000, >50/100,000)											
OVERALL	**QFT**	No study available									
OVERALL	**QFT-IT**	No study available									
Jiang et al.[50]	T-spot	68	N/A	9	67.5	32.5	0				
OVERALL	**T-spot**	**68**			**67.5 (n=46) (95% CI 56–77.5)**	**32.5 (n=22) (95% CI 22.5–44)**	**0**				
Jiang et al.[50]	TST	68	N/A	9	41	59	–				
OVERALL	**TST**	**68**			**41 (n=28) (95% CI 30–53)**	**59 (n=40) (95% CI 47–70)**					
TB Low-endemic Setting (TB prevalence ≤12/100,000)											
Stephan et al.[52]	QFT	275	Median 408 (range 7–1,510)	83	19	80.5	0.5				
Jones et al.[53]	QFT	201	Mean 453 (SD 312; range 1–1,886)	74	5.5	90	4.5				
OVERALL	**QFT**	**476**			**13.2 (n=63) (95% CI 10.5–16.6)**	**84.5 (n=402) (95% CI 80.9–87.4)**	**2.3 (n=11) (95% CI 1.3–4.1)**				
Aichelburg et al.[54]	QFT-IT	822[b]	Median 393 (IQR 264;566)	60	4	90	6				
Talati et al.[55]	QFT-IT	336	Median 335 (range 0–1,380)	69	3	95	2				
Brock et al.[56]	QFT-IT	590	Mean 523 (SD 278)	76	4.5	92	3.5				
Luetkemeyer et al.[57]	QFT-IT	294	Median 363 (IQR 214;581)	69	8.5	86.5	5				
Balcells et al.[58]	QFT-IT	115	Mean 393 (range 100–977)[c]	58	15	85	0				
OVERALL	**QFT-IT**	**2,157**			**5.3 (n=115) (95% CI 4.5–6.4)**	**90.6 (n=1,954) (95% CI 89.3–97.7)**	**4.1 (n=88) (95% CI 3.3–5.0)**				
Talati et al.[55]	T-spot	336	Median 335 (range 0–1380)	69	4	82	14				
Stephan et al.[52]	T-spot	275	Median 408 (range 7–1510)	83	24	73	3				
Dheda et al.[59]	T-spot	29	Median 361 (range 15–784)	N/A	7	90	3	19	5	95	0
Hoffmann et al.[4,60]	T-spot	85	Median 406 (range 50–1,080)[d]	N/A	10.5	80	9.5				
OVERALL	**T-spot**	**725**			**12.5 (n=91) (95% CI 10.3–15.2)**	**78.6 (n=570) (95% CI 76.7–82.0)**	**8.8 (n=64) (95% CI 7.0–11.1)**	**19**	**5 (n=1) (95% CI 1–25)**	**95 (n=18) (95% CI 75–99)**	**0**
Talati et al.[55]	TST	278	Median 335 (range 0–1,380)	69	2.5	97.5	–				
Stephan et al.[52]	TST	275	Median 408 (range 7–1,510)	83	12	88	–				
Jones et al.[53]	TST	201	Mean 453 (SD 312; range 1–1,886)	74	6.5	93.5	–				
Luetkemeyer et al.[57]	TST	205	Median 363 (214;581)	69	9.5	90.5	–				
Balcells et al.[58]	TST	110	Mean 393 (range 100–977)[c]	58	11	89	–				
Hoffmann et al.[e,60]	TST	85	Median 406 (IQR 285;560)	N/A	6	94	–				
OVERALL	**TST[f]**	**1,154**			**7.7 (n=89) (95% CI 6.3–9.4)**	**92.3 (n=1065) (95% CI 90.6–93.7)**	–				

a. Dependent on study: either median and interquartile range (IQR)/range or mean and standard deviation (SD)/range; CD4$^+$ cell counts as indicated for the whole study population; b. Individuals with active tuberculosis (TB) excluded; c. individuals with CD4$^+$ cell counts <100 cells/µl excluded; d. Swiss patients: median CD4$^+$ cell count 445 (IQR 300;650), sub-Saharan immigrants: median CD4$^+$ cell count 336 (IQR 273;447); e. Study design included individuals with high and low risk of previous TB exposure; f. Tuberculin skin test (TST) cut-off ≥5mm. IGRA = interferon-γ release assay; N/A = not available; QFT = QuantiFERON Gold®; QFT-IT: QuantiFERON Gold InTube®, T-spot: T-spot TB®.

Table 3: Rate of Indeterminate Interferon-gamma Release Assay Results Stratified by CD4+ Cell Count in HIV-positive Individuals without Active Tuberculosis

First Author (ref.)	Test System	CD4+ Cell Count[a]	Rate of Indeterminate Results (n/CD4 strata)			
			Overall[b]	CD4+ Cell Count (CD4+ cells/µl)		
				0–100	101–200	>200
Jones et al.[53]	QFT	Mean 453 (SD 312; range 1–1,886)	4.5%	37% (19)	13% (24)	0% (158)
Rangaka et al.[51]	QFT	Median 392 (IQR 263;520)	7%	50% (4)	16.5% (6)	3.5% (54)
Stephan et al.[57]	QFT	Median 408 (range 7–1,510)	0.5%	0% (14)	0% (28)	0.5% (244)
OVERALL	**QFT**		**3% (16/550) (95% CI 2–4.5)**	**24.5% (9/37) (95% CI 13.5–40) 13.5% (13/95) (95% CI 8–22)**	**7% (4/58) (95% CI 3–16.5)**	**0.5% (3/456) (95% CI 0.2–2)**
Brock et al.[56]	QFT-IT	Mean 523 (SD 278)	3.5%	24% (17)	3% (37)	2.5% (536)
Luetkemeyer et al.[57]	QFT-IT	Median 363 (IQR 214;581)	5%	16% (31)	8% (38)	3% (225)
Aichelburg et al.[c,54]	QFT-IT	Median 393 (IQR 264;566)	6%	22% (54)	16.5% (79)	3% (689)
Leidl et al.[49]	QFT-IT	Median 283 (N/A)	2.5%	10% (10)	0% (23)	4% (76)
Talati et al.[55]	QFT-IT	Median 335 (range 0–1,380)	2%	5.5% (56)	4.5% (46)	0.5% (234)
OVERALL	**QFT-IT**		**4% (91/2151) (95% CI 3.5–5)**	**15% (25/168) (95% CI 10.5–21) 11% (44/391) (95% CI 8.5–15)**	**8.5% (19/223) (95% CI 5.5–13)**	**2.5% (47/1760) (95% CI 2–3.5)**
Dheda et al.[59]	T-spot	Median 361 (range 15–784)	3%	0% (4))	0% (7)	5.5% (18)
Leidl et al.[49]	T-spot	Median 283 (N/A)	3.5%	0% (10)	8.5% (23)	2.5% (76)
Rangaka et al.[51]	T-spot	Median 392 (IQR 263;520)	1%	25% (4)	0% (6)	0% (54)
Hoffmann et al.[60]	T-spot	Median 406 (range 50–1,080)	9.5%	0% (2)	11% (9)	9.5% (74)
Stephan et al.[57]	T-spot	Median 408 (range 7–1,510)	3%	0% (14)	7% (28)	2.5% (244)
Talati et al.[55]	T-spot	Median 335 (range 0–1,380)	14%	21.5% (56)	17.5% (46)	11.5% (234)
OVERALL	**T-spot**		**7.5% (69/908) (95% CI 6–9.5)**	**15.5% (14/90) (95% CI 9.5–245.5) 13% (27/209) (95% CI 9–18)**	**11% (13/119) (95% CI 6.5–18)**	**6% (43/700) (95% CI 4.5–8)**

a. Dependent on study: either median and interquartile range (IQR)/range or mean and standard deviation (SD)/range; CD4+ cell counts as indicated for the whole study population; b. see Table 2*; c. Individuals with active tuberculosis (TB) excluded. QFT = QuantiFERON Gold; QFT-IT = QuantiFERON Gold InTube; T-spot = T-spot.TB.*

HIV-positive patients with active TB tend to have a higher rate of indeterminate results (see *Tables 1* and *2*) and might therefore not be directly comparable to HIV-positive patients without active TB. Aabye et al. have shown among 63 Tanzanian patients very high rates of indeterminate QFT-IT results of 45.5 and 19.5% in those with CD4+ cell counts <200 and >200 cells/µl, respectively.[47] However, the number of patients with active TB is limited and results between studies diverge. The higher rate of indeterminate results in patients with active HIV–TB co-infection can partly be explained by the lower mean/median CD4+ cell count, but an increased T-cell exhaustion due to the concomitant active HIV/TB co-infection might be an additional important factor influencing the rate of indeterminate results.

The most consistent results were found in HIV-positive patients without active TB, where an increasing rate of indeterminate results was found in patients with lower CD4+ T-cell counts (see *Table 3*). Overall indeterminate QFT-IT rates (n=2151) in patients with <100 and 100–200 CD4+ cells/µl were 15 and 8.5%, respectively, whereas the rate of indeterminate results in patients with CD4+ cells >200 cells/µl was 2.5% (see *Table 3*). For the T-spot (n=908), overall indeterminate rates were 16.5 and 11.5% for patients with CD4+ cell counts <100 and 100–200 cells/µl, respectively, and 6% among those with CD4+ cell count >200 cells/µl (see *Table 3*).

Thus, from these studies, the rate of indeterminate results in HIV-positive individuals with CD4 cell count >200 cells/µl is comparable to that reported from non-HIV-infected individuals,[22,83] with no significant difference between the two IGRAs. Interestingly, we did not find a difference between the IGRAs in patients with low CD4+ cell counts <200 or <100 cells/µl in terms of indeterminate results (see *Table 3*). It has previously been suggested that the T-spot might be more sensitive in very low CD4+ cell counts, because in contrast to the whole blood assay (QFT/QFT-IT) the T-spot is based on purified lymphocytes and cell numbers are quantitatively adjusted before incubation, which is thought to ensures a higher sensitivity by reducing the impact of lymphopenia and diurnal changes in number of circulating cells.[78–81] However, because of the variations between the studies and the limited number of patients with very low CD4+ cell counts (see *Table 3*), we could not confirm this hypothesis and should not draw any definite conclusions.

Irrespective of the IGRA used, several studies find[46,50,52,58] that the likelihood of positive results decreases with CD4+ cell count and diminished levels of antigen-specific-IFN-γ production, which raises concerns about increasing rates of false-negative IGRA results with decreasing CD4+ cell counts. In Aabye et al.[47] we found no effect of CD4 decline on the rate of false-negatives after exclusion of indeterminate rates, but studies with more patients in each CD4 stratum are needed in order to determine to what extent a decline in CD4+ cells affects the false-negative/sensitivity rate or only the indeterminate rate.

Some attempts have been made to adjust for low CD4+ cell counts. Rangaka et al.[51] found a better test performance by correcting the TB-specific IFN-γ response by CD4+ T-cell count, and a similar observation for the T-spot was made by Clark et al.[61] in a mixed population of HIV-positive patients. Goletti et al.[82] found no improvement using the corrected values by actual CD4+ cell count for the T-spot in a low-TB-endemic region.

Several publications have indicated that not only the absolute CD4+ cell number is of importance for test accuracy but also the nadir CD4+ cell counts.[9,53,54] Elzi et al. found an increase of anergic TST results

in patients with low CD4+ T-cell nadir even after successful ART initiation[9] and Aichelburg et al. reported a significant association between nadir CD4+ cell count and the rate of indeterminate QFT-IT results.[54] These findings indicate the probable impact not only of the CD4+ cell count but also of the CD4+ T-cell functionality on the IGRA performance.

In summary, analysis of the performance of IGRAs in HIV-infected individuals and the impact of CD4+ cell count suggests that IGRAs perform well in HIV-infected individuals with a high/normal CD4+ cell count of around 100–200 CD4+ cells/µl, whereas the performance in patients with a lower CD4+ cell count is impaired, with increased indeterminate results and potentially more false-negative results.

Positive Predictive Value

Several studies have demonstrated that a positive TST result is associated with subsequent development of active TB.[9,13–15] Elzi et al. found in a low-endemic setting (Switzerland) a significantly higher rate of consecutive active TB in HIV-positive individuals tested TST-positive; however, the TST had a negative predictive value <100%.[9]

A study following exposed contacts in a TB low-endemic setting found a progression rate to active TB in QFT-IT-positive individuals of 14.6% compared with 2.3% in individuals tested TST-positive.[83] The largest prospective study, conducted in the Gambia, used in-house ELISPOT and found an increased risk of developing active TB in IGRA-positive HIV-positive and -negative individuals but a poor negative predictive value, indicating either low sensitivity or high risk of new infection during follow-up.[84] Small studies in HIV-positive individuals from low-endemic regions have reported progression rates to active TB in HIV-infected QFT-GIT-positive individuals of two in 27 (7.4%)[56] and two in 20 (10%).[53] The largest study in a low-endemic region was by Aichelburg et al., who explored the positive predictive value of the QFT-GIT longitudinally in 830 HIV-positive individuals.[54] Of 37 who were QFT-IT positive, three (8.3%) developed active TB during the follow-up period of 19 months. None of 793 QFT-GIT-negative individuals progressed to active TB, indicating a very high negative predictive value. The patients were treated equally and the CD4+ cell count did not differ significantly between the patients testing QFT-GIT-positive or -negative.[54]

Finally, Elliot et al.[85] recently showed that the IFN-γ response to RD1 antigens increased significantly in the patients who developed active TB after ART initiation compared with the patients who did not, indicating the use of IGRA in diagnosing ART-associated development of active TB and confirming the hypothesis by Andersen et al.[86] Together, experience from the TST studies and these recent studies emphasises the role of IGRA as a more specific and potentially more sensitive tool for diagnosing LTBI in HIV-infected individuals. Several prospective studies in high- and low endemic regions are under way.

Improvement of Interferon-gamma Release Assays

A major challenge is to improve the accuracy of these assays for patients with immuno-suppression. Several attempts have been suggested, including different cut-off values for immunocompromised patients,[23,87,88] prolonged incubation time,[62,65,77,89,90] different peptides[64,91–96] and alternative read-out systems such as FACS.[97,98] Several cytokines, i.e. interleukin 2 (IL-2) and tumour necrosis factor alpha (TNF-α), as well as chemokines (i.e. IP-10, monocyte chemotactic protein 1 [MCP-1], MCP-2, MCP-3) and inflammation inhibitors (i.e. IL-1 receptor antagonist [sIL1-RA]), are induced antigen-specifically in high amounts.[99,100]

The combination of IFN-γ with IL-2 has been proposed in different viral infectious diseases as a marker of T-cell functionality and an (indirect) marker of disease state.[101,102] IL-2 production or its lack may help differentiate active disease and LTBI and may even be a follow-up marker to control for the effectiveness of anti-TB therapy.[97,103,104]

Promising results have been obtained with interferon-γ-induced protein 10 (IP-10). IP-10 is released specifically in high amounts after antigen stimulation and that the sensitivity and specificity are comparable to those of QFT-IT in HIV-positive and -negative TB patients and in healthy individuals with possible LTBI.[98,99,100,105–107] IP-10 and IFN-γ perform with similar sensitivity, but the two biomarkers do not detect the same responders. By combining IP-10 and IFN-γ, the detection rates among TB patients increased significantly without loss in specificity. Seemingly, the biomarkers complement each other and these as yet preliminary findings indicate that the combinatorial approach multiplexing information from several biomarkers is a way forward.[105,106] IGRA testing on cells obtained from the site of the infection (e.g. spinal fluid, pleural fluid) may be more sensitive and specific for extra-pulmonary TB.[108–110] Finally, a skin test based on ESAT-6 is currently under evaluation, but since this is based on a delayed-type hypersensitivity reaction, it may suffer the same lack of sensitivity in HIV-infected individuals as the TST.[111]

Limitations of the Studies To Date and Further Research Questions of Importance

Even though the IGRAs are principally considered for the diagnosis of LTBI, many studies use patients with active TB, as no standard exists for the diagnosis of LTBI. This approach assumes that test sensitivity in patients with active TB disease reflects the sensitivity in latently infected individuals. However, HIV-positive individuals with active TB will for several reasons (such as poor nutritional status, low CD4+ cell number, reduced cytokine responses, etc.) be more immune-compromised than individuals with LTBI and the T-cell assays will be more affected than those from healthy individuals. The influence of the CD4+ cell count on the negative predictive value is unknown, especially in high-endemic regions, and studies addressing the positive and negative predictive values in different study settings and in relation to ART initiation, CD4+ nadir and risk of infection are ongoing. The studies performed to date in HIV-positive individuals using commercially available IGRAs differ with respect to study design, where the majority are case–control studies or proof of concept studies, to the risk of infection and prevalence of TB in the study population, immune status (CD4+ cell count), if known, and the criteria used to define active TB and presumed LTBI. The number of patients included in many studies is too low to allow definite conclusions to be drawn.

Summary

The main conclusion of this article is that the sensitivity of IGRAs in HIV/TB co-infected individuals with or without indeterminate results is impaired compared with in HIV-negative patients with TB. Indeterminate rates are higher in HIV-positive individuals and the number of CD4+ cells is significantly associated with the rate of indeterminate results, whereas the effect of CD4+ cell numbers on actual sensitivity is less pronounced. We were not able to confirm the previous hypothesis that T-spot is more accurate than QFT-IT in HIV-positive individuals. In HIV-positive individuals without active TB, the overall rate of positive results varied depending on country of origin, confirming the high specificity of the tests.

Minor differences between the IGRAs were found, but we were not able to explain them by differences in sensitivity, specificity or host-related factors. The evaluation of the currently published studies on IGRAs is flawed by the heterogeneity of the studies summarised here. In low-endemic regions the negative predictive value in a well-treated HIV population is very high, whereas negative predictive value seems sub-optimal in a high-endemic settings, probably due to re-infection and an unknown proportion of false-negative results in immunocompromised untreated HIV-positive individuals. From our findings, both IGRAs are likely to give unreliable results when the $CD4^+$ cell count is <100 cells/µl. However, the tests give reliable results at $CD4^+$ cell counts >200 cells/µl, but this needs to be confirmed in studies including a higher number of immune-compromised HIV-positive individuals. The high proportion of indeterminate results underlines the importance of the positive control as a marker of anergy, helping to exclude false-negative results, which is not an option for the TST. When indeterminate results were excluded, the positivity rate increased by roughly 10%. Therefore, we strongly recommend full openness on the proportion and use of indeterminate results when reporting the performance and accuracy of IGRAs. ■

Matthias Hoffmann is a Clinical Research Fellow in the Division of Infectious Disease, Department of Internal Medicine at the Cantonal Hospital St Gallen, Switzerland. His research focuses on cellular immune responses to persistent pathogens in immune-compromised hosts. He has won several national awards for his work. He undertook professional training in internal medicine and infectious diseases at the Cantonal Hospitals of Münsterlingen and St Gallen and a medical research fellowship of the Swiss National Science Foundation (SNSF) at the University of Oxford.

Pernille Ravn is a Senior Research Fellow and part-time consultant in the Clinical Infectious Disease Unit at Herlev University Hospital in Copenhagen. Since 1994, she has been involved in developing and evaluating new specific tests for the diagnosis of tuberculosis infection. She has initiated a number of research projects involving improved diagnosis of tuberculosis in children, healthcare workers, HIV-positive and otherwise immunosuppressed patients in Denmark, Greenland, Nigeria, Tanzania and Guinea Bissau in collaboration with the Reference Laboratory for Mycobacteriology and Department for Infectious Diseases Immunology Staten Serum Insittue, University of Copenhagen and Liverpool School of Tropical Medicine.

1. Word Health Organiosation, *WHO report*, Geneva, 2009.
2. Russell DG, *Nat Rev Microbiol*, 2007;5:39–47.
3. Mack U, et al., *Eur Respir J*, 2009;33:956–73.
4. Horsburgh CR, *N Engl J Med*, 2004;350:2060–7.
5. Nunn P, et al., *Nat Rev Immunol*, 2005;5:819–26.
6. Lawn SD, et al., *AIDS*, 2005;19:1113–24.
7. Lawn SD, et al., *AIDS*, 2009;23:1717–25.
8. Lillebaek T, et al., *J Infect Dis*, 2002;185:401–4.
9. Elzi L, et al., *Clin Infect Dis*, 2007;44:94–102.
10. Rieder HL, *Clinical Infectious Diseases*, 2007;44:103–4.
11. Huebner RE, et al., *Clin Infect Dis*, 1993;17:968–75.
12. Farhat M, et al., *Int J Tuberc Lung Dis*, 2006;10:1192–204.
13. Comstock GW, et al., *Am J Epidemiol*, 1974;99:131–8.
14. American Thoracic Society, *MMWR Recomm Rep*, 2000;49:1–51.
15. Watkins RE, et al., *Int J Tuberc Lung Dis*, 2000;4:895–903.
16. Huebner RE, et al., *Clin Infect Dis*, 1994;19:26–32.
17. Chin DP, et al., *Am J Respir Crit Care Med*, 1996;153:1982–4.
18. Caiaffa WT, et al., *Arch Intern Med*, 1995;155:2111–7.
19. Duncan LE, et al., *Trans R Soc Trop Med Hyg*, 1995;89:37–40.
20. Markowitz N, et al., *Ann Intern Med*, 1993;119:185–93.
21. Andersen P, et al., *Lancet*, 2000;356:1099–104.
22. Menzies D, et al., *Ann Intern Med*, 2007;146:340–54.
23. Pai M, et al., *Lancet Infect Dis*, 2007;7:428–38.
24. Richeldi L, *Am J Respir Crit Care Med*, 2006;174:736–42.
25. Behr MA, et al., *Science*, 1999;284:1520–33.
26. Harboe M, et al., *Infect Immunol*, 1996;61:16–22.
27. Lewis KN, et al., *J Infect Dis*, 2003;187:117–23.
28. Mahairas GG, et al., *J Bacteriol*, 1996;178:1274–82.
29. Vincenti D, et al., *Clin Exp Immunol*, 2007;150:91–8.
30. Chaisson RE, et al., *J Acquir Immune Defic Syndr Hum Retrovirol*, 1996;11:455–9.
31. Menzies D, *Ann Intern Med*, 2008;148:298–9.
32. Mori T, *J Infect Chemother*, 2009;15;143–55.
33. Perkins MD, et al., *Int J Tuberc Lung Dis*, 2006;10:1.
34. Small PM, et al., *Lancet*, 2000;356:1048–9.
35. Søborg B, et al., *J Rheumatol*, 2009;36:1876–84.
36. Matulis G, Juni P, Villiger PM, et al., *Ann Rheum Dis*, 2008;67:84–90.
37. Kobashi Y, et al., *Eur Respir J*, 2007;30:945–50.
38. Piana F, et al., *Eur Respir J*, 2006;28:31–4.
39. Lalvani A, et al., *Proc Natl Acad Sci U S A*, 1998;95:270–75.
40. Lugos MD, et al., *Immunol Invest*, 2009;38:341–9.
41. Sutherland R, et al., *AIDS*, 2006;20:821–9.
42. Pai M, et al., *Semin Respir Crit Care Med*, 2008;29:560–68.
43. Seshadri C, et al., *East Afr Med J*, 2008;85:442–9.
44. Tsiouris SJ, et al., *J Clin Microbiol*, 2006;44:2844–50.
45. Kabeer SAB, et al., *PLoS One*, 2009;4:e5718.
46. Raby E, et al., *PLoS One*, 2008;3:e2489.
47. Aabye MG, et al., *PLoS One*, 2009;4:e4220.
48. Markova R, et al., *Biotechnol*, 2009;23:1103–8.
49. Leidl L, et al., *Eur Respir J*, 2009 (Epub ahead of print).
50. Jiang W, et al., *BMC Immunol*, 2009;10:31.
51. Rangaka MX, et al., *Am J Respir Crit Care Med*, 2007;175:514–20.
52. Stephan C, et al., *AIDS*, 2008;22:2471–9.
53. Jones S, et al., *Int J Tuberc Lung Dis*, 2007;11:1190–95.
54. Aichelburg MC, et al., *Clin Infect Dis*, 2009;48:954–62.
55. Talati NJ, et al., *BMC Infect Dis*, 2009;9:15.
56. Brock I, et al., *Respir Res*, 2006;7:56.
57. Luetkemeyer AF, et al., *Am J Respir Crit Care Med*, 2007;175:137–42.
58. Balcells ME, et al., *Int J Infect Dis*, 2008;12:645–52.
59. Dheda K, et al., *AIDS*, 2005;19:2038–41.
60. Hoffmann M, et al., *AIDS*, 2007;21:390–92.
61. Clark SA, et al., *Clin Exp Immunol*, 2007;150:238–44.
62. Lawn SD, et al., *BMC Infect Dis*, 2007;7:99.
63. Karam F, et al., *PLoS One*, 2008;3:e1441.
64. Goletti D, et al., *BMC Infect Dis*, 2008;8:11.
65. Rangaka MX, Diwakar L, Seldon R, et al., *Clin Infect Dis*, 2007;44:1639–46.
66. Hammond AS, et al., *J Infect Dis*, 2008;197:295–9.
67. Converse PJ, et al., *J Infect Dis*, 1997;176:144–50.
68. Chapman AL, et al., *AIDS*, 2002;16:2285–93.
69. Stavri H, et al., *Roum Arch Microbiol Immunol*, 2009;68:14–19.
70. Mandalakas AM, et al., *Int J Tuberc Lung Dis*, 2008;12:417–23.
71. Davies MA, et al., *AIDS*, 2009;23:961–9.
72. Ewer K, et al., *Lancet*, 2003;361:1168–73.
73. Girardi E, et al., *Euro Surveillance*, 2009;14:19373.
74. Hoffmann M, et al., *Swiss Med Wkly*, 2010 Feb 3 (pub ahead of print).
75. Søborg B, et al., *Scand J Infect Dis*, 2007;39:554–9.
76. Pathan AA, et al., *J Immunol*, 2001;167:5217–25.
77. Ravn P, et al., *J Infect Dis*, 1999;179:637–45.
78. Pai M, et al., *Lancet Infect Dis*, 2004;4:761–76.
79. Petrovsky N, et al., *J Immunol*, 1997;158:5163–8.
80. Pai M, et al., *Expert Rev Mol Diagn*, 2006;6:413–22.
81. Arend SM, Thijsen SFT, Leyten EMS, et al., *Am J Respir Crit Care Med*, 2007;175:618–27.
82. Goletti D, et al., *Clin Infect Dis*, 2007;45:1652–4.
83. Diel R, et al., *Am J Respir Crit Care Med*, 2008;177:1164–70.
84. Hill PC, et al., *PLoS One*, 2008;3:e1379.
85. Elliott JH, et al., *J Infect Dis*, 2009;200:1736–45.
86. Andersen P, et al., *Trends Mol Med*, 2007;13:175–82.
87. Veerapathran A, et al., *PLoS One*, 2008;3:e1850.
88. Harada N, et al., *J Infect*, 2008;56:348–53.
89. Leyten EMS, et al., *Clin Vaccine Immunol*, 2007;14: 880–85.
90. Munk ME, et al., *J Infect Dis*, 2001;183:175–6.
91. Ravn P, et al., *J Infect Dis*, 1999;179:637–45.
92. Aagaard C, et al., *J Infect Dis*, 2004;189:812–19.
93. Savolainen L, et al., *PLoS One*, 2008;3:e3272.
94. Andersen P, et al., *Lancet*, 2000;356:1099–1104.
95. Leyten EM, et al., *Microbes.Infect*, 2006;8:2052–60.
96. Dosanjh DPS, et al., *Ann Int Med*, 2008;148:325–36.
97. Sargentini V, et al., *BMC Infect Dis*, 2009;9:99.
98. Dinser R, et al., *Rheumatology (Oxford)*, 2008;47:212–18.
99. Petrucci R, et al., *Pediatr Infect Dis J*, 2008;27:1073–7.
100. Ruhwald M, et al., *BMC Res Notes*, 2009;2:19.
101. Pantaleo G, et al., *Nat Rev Immunol*, 2006;6:417–23.
102. Makedonas G, et al., *Springer Semin Immunopathol*, 2006;28:209–19.
103. Millington KA, et al., *J Immunol*, 2007;178:5217–26.
104. Biselli R, et al., *Clin Microbiol Infect*, 2009 (Epub ahead of print).
105. Ruhwald M, et al., *Microbes Infect*, 2007;9:806–12.
106. Ruhwald M, et al., *Expert Rev Resp Med*, 2009;3:387–401.
107. Kabeer BS, et al., *AIDS*. (2010), 24:323–5.
108. Thomas MM, et al., *Int J Tuberc Lung Dis*, 2008;12:651–7.
109. Kösters K, et al., *Infection*, 2008;36:597–600.
110. Jafari C, et al., *Am J Respir Crit Care Med*, 2009;180:666–73.
111. Arend SM, et al., *Tuberculosis (Edinb)*, 2008;88:249–61.