

➔ Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study

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Summary

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Background Two commercial blood assays for the diagnosis of latent tuberculosis infection—T-SPOT.TB and QuantiFERON-TB Gold—have been separately compared with the tuberculin skin test. Our aim was to compare the efficacy of all three tests in the same population sample.

Methods We did a prospective study in 393 consecutively enrolled patients who were tested simultaneously with T-SPOT.TB and QuantiFERON-TB Gold because of suspected latent or active tuberculosis. 318 patients also had results available for a tuberculin skin test.

Findings Overall agreement with the skin test was similar (T-SPOT.TB $\kappa=0.508$, QuantiFERON-TB Gold $\kappa=0.460$), but fewer BCG-vaccinated individuals were identified as positive by the two blood assays than by the tuberculin skin test ($p=0.003$ for T-SPOT.TB and $p<0.0001$ for QuantiFERON-TB Gold). Indeterminate results were significantly more frequent with QuantiFERON-TB Gold (11%, 43 of 383) than with T-SPOT.TB (3%, 12 of 383; $p<0.0001$) and were associated with immunosuppressive treatments for both tests. Age younger than 5 years was significantly associated with indeterminate results with QuantiFERON-TB Gold ($p=0.003$), but not with T-SPOT.TB. Overall, T-SPOT.TB produced significantly more positive results (38%, $n=144$, vs 26%, $n=100$, with QuantiFERON-TB Gold; $p<0.0001$), and close contacts of patients with active tuberculosis were more likely to be positive with T-SPOT.TB than with QuantiFERON-TB Gold ($p=0.0010$).

Interpretation T-SPOT.TB and QuantiFERON-TB Gold have higher specificity than the tuberculin skin test. Rates of indeterminate and positive results, however, differ between the blood tests, suggesting that they might provide different results in routine clinical practice.

Introduction

Eradication of tuberculosis in low-prevalence countries is judged a realistic aim.^{1,2} Even in areas with a decreasing prevalence of the disease, however, several important concerns need to be resolved before the disease can be controlled.^{3,4} A key factor is the number of asymptomatic individuals with latent disease, who are a reservoir of future cases. As the prevalence of tuberculosis, and the risk of future re-infection, decreases, diagnosis and treatment of these individuals becomes increasingly important. There are an estimated 9–14 million people in the USA who have latent infection,⁵ and the number continues to increase as immigrants from high-prevalence areas move to America and person–person transmission of *Mycobacterium tuberculosis* occurs. Evidence suggests that treatment of latent infection reduces the risk of progression to active tuberculosis, especially in high-risk groups, such as patients infected with HIV.^{6,7} Individuals with radiological evidence of inactive tuberculosis, close contacts of patients with active disease, patients receiving infliximab and other immunosuppressive therapy, and children aged 5 years and younger are also priority groups that could benefit from treatment of latent disease.⁸ Unfortunately, the standard diagnostic test for latent tuberculosis infection—

the tuberculin skin test (also known as the intradermal Mantoux test)—is unreliable, often providing false-negative results in these high-risk groups, especially in immunosuppressed patients and young children.⁹ Furthermore, the specificity of the test is low; false-positive results in individuals vaccinated against BCG and in those infected by non-tuberculous mycobacteria are common.^{9,10} As such, high-risk patients who stand to benefit most from preventive chemotherapy might not be diagnosed, and resources are being wasted on treatment of individuals incorrectly identified as having latent infection.

Two new blood tests, based on detection of interferon γ produced by T cells in response to antigens specific to *M tuberculosis* and encoded by the RD1 region, might be more accurate than the tuberculin skin test.^{11,12} Both tests are commercially available in regulatory agency-approved formats. T-SPOT.TB (Oxford Immunotec, Abingdon, UK) is based on the ex vivo overnight enzyme-linked immunospot (ELISPOT) assay. It has been approved for in-vitro diagnostic use in Europe and is being assessed by the US Food and Drug Administration (FDA). QuantiFERON-TB Gold (Cellestis, Carnegie, Australia) is based on a whole-blood ELISA and has been approved for in-vitro diagnostic use by the FDA. Guidelines for the

use of QuantiFERON-TB Gold have been released by the US Centers for Disease Control and Prevention,¹³ and both tests are included in the UK guidelines on tuberculosis published by the National Collaborating Centre for Chronic Conditions.¹⁴

T-SPOT.TB and QuantiFERON-TB Gold offer clear operational advantages over the tuberculin skin test: no follow-up visit is required, results are available within 24 h, and the boosting effect is not a concern with repeated testing. Both tests are more specific than their predecessor in BCG-vaccinated populations.¹⁵ Data for individuals with latent infection and at high risk of disease progression are scarce, but an RD1-ELISPOT assay did better than the tuberculin skin test in children co-infected with *M tuberculosis* and HIV.¹⁶ Finally, these tests have a positive internal control and can produce an indeterminate result—ie, a result judged unreliable owing to the failure of the internal positive control for interferon γ production in the absence of a clearly positive response to a specific antigen. Although indeterminate results are a limitation of these new methods of diagnosis, in particular in immunosuppressed patients,¹⁷ the advantage is that they caution clinicians to consider the possibility of a false-negative result with the tuberculin skin test. The tuberculin skin test does not have any internal control; instead, concomitant skin tests with *Candida* or other common antigens are often done in immunosuppressed people, but with little success.¹⁸

In theory, the higher diagnostic accuracy of these new blood tests should assist tuberculosis control programmes, especially in non-endemic areas where elimination of the disease is a realistic target. However, studies that have assessed the diagnostic importance of these blood tests compared with the tuberculin skin test have mostly been undertaken in preselected populations, such as the epidemiological setting of contact tracing.¹⁵ Our aim was to compare the specificity and efficacy of the new diagnostic assays in routine clinical practice, assessing the degree of concordance between T-SPOT.TB, QuantiFERON-TB Gold, and the tuberculin skin test.

Methods

Participants

Between Oct 15, 2004, and Aug 15, 2005, we prospectively enrolled consecutive individuals with suspected tuberculosis (active or latent) and tested them with T-SPOT.TB and QuantiFERON-TB Gold at the Policlinico University Hospital of Modena, Italy. T-SPOT.TB and QuantiFERON-TB Gold were used for outpatients and inpatients in any hospital ward.

The ethics committee of the University of Modena and Reggio Emilia approved the study. All patients provided oral consent. Written consent was not deemed necessary since both diagnostic tests are already used in clinical practice.

Procedures

A form used to identify the main reason for testing was completed for each participant; prespecified items included: radiological suggestion of active tuberculosis, diagnosis of cancer, immigration to Italy within the past 2 years from a country with a high prevalence of tuberculosis, recent close contact with someone with active pulmonary tuberculosis (based on written records provided by the local public-health service), solid organ transplantation, current treatment with immunosuppressive agents, chronic renal failure, and HIV infection. From clinical records, we obtained results of tuberculin skin tests (only when done at the same time as the blood testing), BCG vaccination status (as reported by the prescribing physician and based on questions about past BCG vaccination and scar inspection), and demographic, clinical, radiological, and microbiological data for all patients.

The tuberculin skin test was done with 5 tuberculin units of purified protein derivative (Biocine Test PPD, Chiron, Siena, Italy), according to the intradermal Mantoux method: 48–72 h after inoculation, the main diameter of skin induration was recorded in millimetres. The results of the test were interpreted by hospital staff on the basis of the patients' degree of risk, according to current guidelines¹⁹—the lower cut-off of 5 mm for a positive test was used for individuals who were positive for HIV, had had recent contact with a person with tuberculosis, had changes on their chest X-ray consistent with previous tuberculosis, or had had an organ transplant or were immunosuppressed for other reasons.

Two trained technicians who were unaware of the results of any blood test done, the tuberculin skin test, and the final diagnosis of the patient, tested blood with commercially available versions of T-SPOT.TB and QuantiFERON-TB Gold in the microbiology and virology laboratory of the hospital, according to the manufacturers' recommendations. Peripheral blood samples for the two tests were obtained simultaneously and processed within 4 h. Each test included a negative control well (no mitogen or antigens), a positive control well (phytohaemagglutinin), and two antigen wells (*M tuberculosis*-specific early secretory antigen target 6 and culture filtrate protein 10 antigens).

For T-SPOT.TB, peripheral blood mononuclear cells were separated by centrifugation from an 8-mL (4 mL for children aged 6 years or younger) sample of peripheral venous blood and plated (2.5×10^5 per well) on a plate precoated with antibody against interferon γ . Plates were incubated overnight at 37°C in 5% carbon dioxide. After incubation, wells were washed and developed with a conjugate against the antibody used and an enzyme substrate. Spot-forming units (SFUs) were counted with an automated ELISPOT reader (AID Systems, Strassberg, Germany), according to the protocol provided by the manufacturer of T-SPOT.TB. If the negative control well contained 5 SFUs or less, this value was subtracted from

	Number (%) or median (range)
Male	212 (54%)
Age (years)	
Overall	43 (0-93)
≤5	25 (6%)
≥80	26 (7%)
BCG vaccinated	72 (18%)
Diagnosis of cancer	69 (18%)
Ongoing cancer chemotherapy	28 (7%)
Ongoing treatment with systemic steroids	35 (9%)
Ongoing treatment with drugs against TNFα	10 (3%)
Reason for test	
Clinical or radiological suggestion of active tuberculosis	166 (42%)
Recent contact with patient with bacillary pulmonary tuberculosis	119 (30%)
Recent (<2 years) immigration from tuberculosis-endemic country	48 (12%)
Pretransplantation screening	9 (2%)
Other	51 (13%)

Table 1: Patients' characteristics (n=393)

	Site of disease	Tuberculin skin test	T-SPOT.TB	QuantiFERON-TB Gold
1	Pulmonary	-	-	-
2	Pulmonary	NA	+	+
3	Pulmonary	+	+	+
4	Pulmonary	-	-	-
5	Pulmonary	+	+	+
6	Pulmonary	+	+	+
7	Pulmonary	+	+	+
8	Pulmonary	+	+	+
9	Pulmonary	+	+	+
10	Pulmonary	NA	-	-
11	Pulmonary	-	-	-
12	Pulmonary	NA	+	+
13	Pulmonary	-	+	+
14	Extrapulmonary	+	+	+
15	Extrapulmonary	+	+	+
16	Extrapulmonary	+	+	+
17	Extrapulmonary	-	+	+
18	Extrapulmonary	+	+	+
19	Extrapulmonary	+	+	+
20	Extrapulmonary	NA	+	-
21	Extrapulmonary	+	+	+
22	Extrapulmonary	+	+	+
23	Extrapulmonary	-	+	-
24	Extrapulmonary	+	+	Indeterminate

NA=not available.

Table 2: Results of the three tests in patients with a diagnosis of active tuberculosis

the number of SFUs counted in the wells stimulated by the *M tuberculosis*-specific antigens and we interpreted the result of the test as: positive, if there were 6 SFUs or more in either of the antigen wells, irrespective of the result of the positive-control well; negative, if there were

less than 6 SFUs in both antigen wells and 20 SFUs or more in the positive-control well; and indeterminate, if there were less than 6 SFUs in both antigen wells and less than 20 SFUs in the positive-control well. If the negative-control well contained 6 SFUs or more, the result of the test was interpreted as positive if the number of SFUs in either of the antigen wells was more than twice the number in the negative control well, irrespective of the result of the positive-control well. We considered all tests with 10 SFUs or more in the negative-control well as potential technical errors, according to the manufacturer's recommendations.

For QuantiFERON-TB Gold, 1-mL aliquots from a 9-mL (5 mL for children aged 6 years or younger) sample of whole blood were transferred to wells of a plate and incubated overnight at 37°C in a humidified chamber. After incubation, plasma aliquots were harvested, and concentrations of interferon γ measured in international units (IU) with a DSX Automated System ELISA reader (Dinex Technologies, West Sussex, UK). We interpreted the result of the test, after subtraction of the value of the negative-control well from the amount of IU of interferon γ measured in the wells stimulated by the *M tuberculosis*-specific antigens, as: positive, if the concentration of interferon γ in either of the antigen wells was 0.35 IU/mL or more, irrespective of the result of the positive-control well; negative, if the concentration of interferon γ in both antigen wells was less than 0.35 IU/mL and the concentration in the positive-control well was 0.5 IU/mL or more; and indeterminate, if the concentration of interferon γ was less than 0.35 IU/mL in both antigen wells and less than 0.5 IU/mL in the positive-control well. We recorded as a potential technical error any test in which the concentration of interferon γ in the negative-control well was 2 IU/mL or more, according to the manufacturer's recommendations.

Statistical analysis

We calculated proportions of indeterminate and positive results for both blood tests by dividing the number of indeterminate and positive results by the number of patients undergoing tests. We used the McNemar²⁰ test to compare the proportion of indeterminate, negative, and positive results among the three tests. We used logistic regression analysis to assess the association between indeterminate results obtained with each blood test and the main risk factors for immunosuppression. The kappa (κ) statistics measure was used to assess agreement among T-SPOT.TB, QuantiFERON-TB Gold, and tuberculin skin test. This statistic provides values of +1 (perfect agreement) via 0 (no agreement above that expected by chance) to -1 (complete disagreement).²⁰ We also calculated agreement among tests in a subgroup analysis, for which we stratified patients by BCG vaccination status. We judged a p value of less than 0.05 significant. We did all analyses with SPSS for Windows (version 13.0).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We enrolled 393 people who were simultaneously tested with T-SPOT.TB and QuantiFERON-TB Gold. Table 1 shows their demographic characteristics and the most frequent reasons for testing. Hospital wards that requested the tests were those of respiratory diseases (47%, n=184), paediatrics (24%, n=96), and oncology (13%, n=53). 149 tests (38%) were done in patients with immune suppression—ie, those with any form of cancer (with or without concurrent chemotherapy), HIV infection, or chronic renal failure, those receiving chronic systemic steroid therapy or agents against TNF α , those aged 5 years or younger or 80 years or older, or those in need of solid organ transplantation. Most individuals were assessed on the basis of clinical or radiological suggestion of active tuberculosis or a recent close contact with a patient with active pulmonary tuberculosis (table 1). 24 patients were eventually confirmed as having active tuberculosis, 11 of whom had extrapulmonary disease (table 2).

We excluded the results of five (1%) QuantiFERON-TB Gold and five (1%) T-SPOT.TB tests from analysis because of high background values in the negative-control wells; these were judged potential technical errors. Based on the FDA-approved criteria for QuantiFERON-TB Gold, a value of more than 0.7 IU/mL in the negative-control well should also be considered a potential technical error; thus, according to these criteria, two more test results (included in the present analysis) should have been excluded (both were negative, according to the European Union-approved package insert). 383 tests were, therefore, included in the analysis. A tuberculin skin test result was available for 83% (n=318) of participants.

All (n=10) indeterminate T-SPOT.TB test results and 26 of 32 (81%) indeterminate QuantiFERON-TB Gold test results were in participants who tested negative on the tuberculin skin test (significantly different from those who tested positive; p=0.0080 for QuantiFERON-TB Gold and p=0.0097 for T-SPOT.TB). Figure 1 shows the results of the blood tests stratified according to the results of the tuberculin skin test. Overall, agreement with the skin test was good (T-SPOT.TB κ =0.508 [standard error 0.050]; QuantiFERON-TB Gold κ =0.460 [0.052]). However among BCG-vaccinated participants agreement between the skin test and either T-SPOT.TB (κ =0.155) [0.097] or QuantiFERON-TB Gold (κ =0.170 [0.063]; for both tests p<0.0001) was significantly lower. Table 3 shows degree of agreement between the skin test and the blood tests in individuals tested because of contact with patients with active tuberculosis. The tuberculin skin test

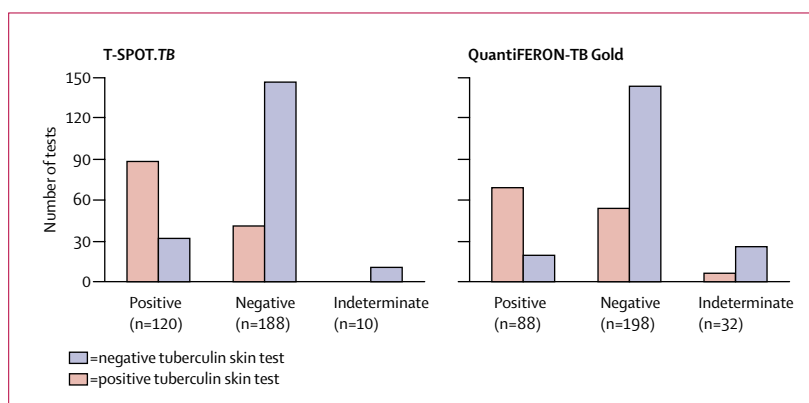


Figure 1: Results of blood tests versus results of tuberculin skin test (n=318 [83%])

	Tuberculin skin test	QuantiFERON-TB Gold	T-SPOT.TB
All contacts (test results available for 114 of 119)	62 (54%)	25 (22%)	39 (34%)
p for comparison between blood test and skin test	..	<0.0001	<0.0001
Non-BCG-vaccinated contacts (test results available for 84 of 85)	36 (43%)	21 (25%)	29 (35%)
p for comparison between blood test and skin test	..	0.0001	0.0923

Table 3: Positive results obtained with tuberculin skin test, QuantiFERON-TB Gold, and T-SPOT.TB in participants recently in close contact with a patient with active tuberculosis

scored more positive results than either blood test, irrespective of BCG vaccination status.

The overall agreement between the two blood tests was high (κ =0.699), irrespective of BCG vaccination status (data not shown). However, the proportion of positive and indeterminate results scored by T-SPOT.TB and QuantiFERON-TB Gold were significantly different (p<0.0001). The blood tests identified 383 patients as definitely positive or negative, with T-SPOT.TB finding significantly more individuals positive (38%, n=144) than QuantiFERON-TB Gold (26%, n=100; p<0.0001) (figure 2 and figure 3).

Individuals tested after close contact with an individual with pulmonary tuberculosis (n=114) were significantly more likely to be scored positive with T-SPOT.TB than with QuantiFERON-TB Gold (34%, 39 of 114 vs 22% [25 of 114]; p=0.0010). Table 2 shows the results of the three tests in patients with a diagnosis of active tuberculosis. 11 (46%) patients with tuberculosis were culture-positive: eight (73%) were positive and two (18%) were negative with both blood tests, and one (9%) was T-SPOT.TB-positive/QuantiFERON-TB Gold-negative. T-SPOT.TB correctly identified all patients with extrapulmonary tuberculosis as positive, whereas QuantiFERON-TB Gold was positive in eight (73%), negative in two (18%), and indeterminate in one (9%).

Overall, T-SPOT.TB provided significantly fewer indeterminate results (3% [n=12]) than did QuantiFERON-TB Gold (11% [n=43], p<0.0001; figure 2). After logistic regression analysis, we noted that indeterminate results for both blood tests were significantly more frequent in patients undergoing cancer chemotherapy (T-SPOT.TB

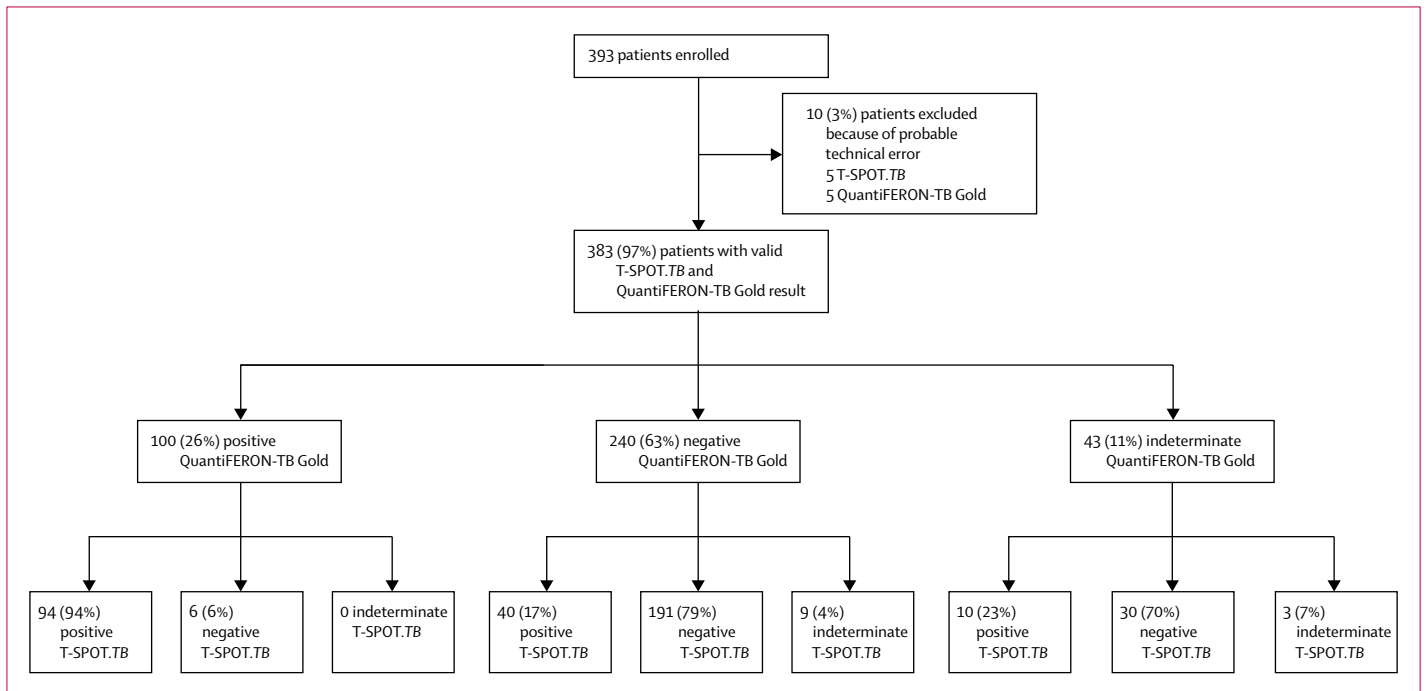


Figure 2: Distribution of T-SPOT.TB results, according to results obtained with QuantiFERON-TB Gold

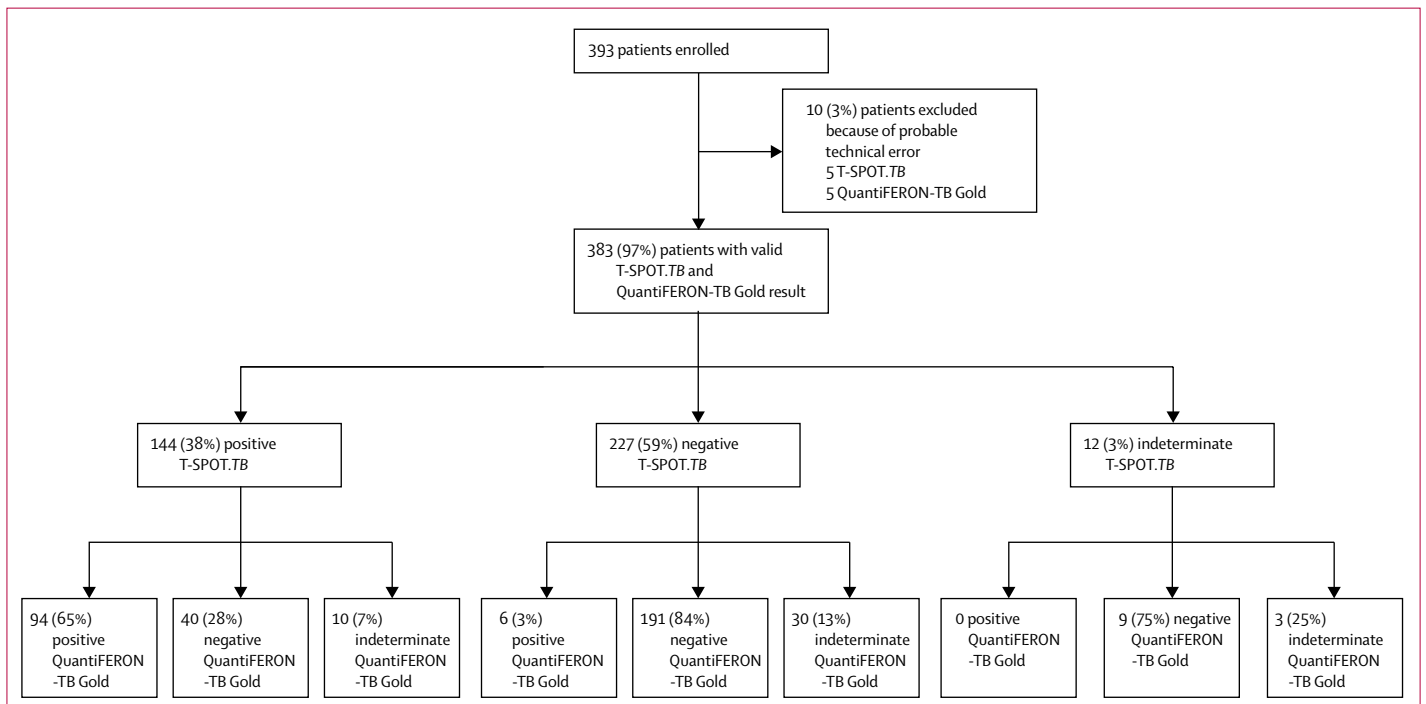


Figure 3: Distribution of QuantiFERON-TB Gold results, according to results obtained with T-SPOT.TB

21% [6 of 28] vs 2% [6 of 355]) than in participants not treated with chemotherapy ($p < 0.0001$; QuantiFERON-TB Gold 36% [10 of 28] vs 9% [33 of 355], $p = 0.0001$). Indeterminate results with QuantiFERON-TB Gold were significantly over-represented in children aged 5 years

and younger (32% [7 of 22] vs 10% [36 of 361] in individuals aged older than 5 years, $p = 0.0034$), whereas T-SPOT.TB provided definite results in all of these young children.

We also compared the proportions of indeterminate results between the two blood assays with the McNemar

test. The number of indeterminate results with T-SPOT.TB was lower than with QuantiFERON-TB Gold in patients with cancer (3% [1 of 40] vs 15% [6 of 40], $p=0.125$), children younger than age 5 years (0% vs 32% [7 of 22], $p=0.0156$), individuals aged 80 years or older (4% [1 of 26] vs 12% [3 of 26], $p=0.625$), patients treated with systemic corticosteroids (3% [1 of 34] vs 18% [6 of 34], $p=0.0625$), and patients undergoing cancer chemotherapy (21% [6 of 28] vs 36% [10 of 28], $p=0.344$).

Discussion

Our findings indicate that, despite overall good agreement, T-SPOT.TB and QuantiFERON-TB Gold might provide different results when used in routine clinical practice. They might not, therefore, have equal validity as a substitute for the tuberculin skin test¹³ or when used to confirm diagnosis of tuberculosis infection.¹⁴

A limitation of our study is the high proportion of individuals enrolled who were immunosuppressed and at high risk of tuberculosis. Our results are not, therefore, necessarily generalisable to the general population. It is noteworthy, however, that such high-risk populations are likely to be the best candidates for testing with the blood tests assessed.

The two blood tests differed in terms of the proportions of unequivocal and indeterminate results. More patients tested positive with T-SPOT.TB than with QuantiFERON-TB Gold. Unfortunately, since there is no diagnostic gold standard for latent tuberculosis infection, we could not assess whether T-SPOT.TB was more sensitive or less specific than QuantiFERON-TB Gold. The findings of two subgroup analyses, however, could help answer this question. First, patients with active tuberculosis, already used as positive controls in other studies to assess the sensitivity of both QuantiFERON-TB Gold^{21,22} and T-SPOT.TB,^{23,24} tested positive more often by T-SPOT.TB than by QuantiFERON-TB Gold; because of our prospective study design though enrolment of patients with active tuberculosis during the predefined study period was limited and this difference was not significant. As such, no firm conclusions can be drawn. Second, T-SPOT.TB diagnosed more individuals recently exposed to a patient with pulmonary tuberculosis as positive for the disease than did QuantiFERON-TB Gold, thus suggesting a higher diagnostic accuracy. However, fewer contacts tested positive with QuantiFERON-TB Gold or T-SPOT.TB than with the tuberculin skin test after exclusion of the confounding factor of BCG vaccination. This result might indicate a selection bias, since most contacts were referred to our clinic on the basis of a positive skin test result, but it might also indicate a difference in sensitivity between these assays for the diagnosis of latent infection among exposed contacts. Findings of other studies of the ELISPOT technique^{25–28} indicate that the interferon γ blood test is better than the skin test at identifying individuals with tuberculosis infection after exposure to patients with culture-proven

disease. However, despite its limitations,⁹ the tuberculin skin test is the only test able to predict progression to active disease in all risk groups, and there is evidence for the efficacy of preventive treatment for individuals with a positive skin test who are at risk of progression.¹⁹ With the exception of a few confounding factors (such as BCG vaccination), the clinical relevance of discordant results obtained with the tuberculin skin test and the blood tests is unknown. Longitudinal and powered studies are needed to assess the predictive value of the new tests, although encouraging data already exist.²⁹ Our data seem to lend support to the use of both skin and blood tests in contact-screening procedures.

The rate of indeterminate test results was significantly higher for QuantiFERON-TB Gold than for T-SPOT.TB, and indeterminate results of both tests were associated with immunosuppression. These findings are important in consideration of the increasing clinical relevance of pulmonary infections (including those caused by mycobacteria) in patients treated with cancer chemotherapy.³⁰ Furthermore, they confirm those previously reported¹⁷ in a study that enrolled a high proportion of patients with cancer undergoing chemotherapy. Our finding of an over-representation of indeterminate results with QuantiFERON-TB Gold among individuals who tested negative on the tuberculin skin test corroborates our earlier finding.¹⁷ We extend this finding to T-SPOT.TB. These results lend further support to the notion that the skin test is often unreliable among immunosuppressed patients⁹ and that an indeterminate blood test (in particular, an indeterminate T-SPOT.TB) usually does not mask a positive tuberculin skin test result. This finding is relevant for practical clinical use and for the effective application of these new tests in the routine setting. However, our data also suggest that, in a proportion of cases, the two blood tests provide discordant results.

The blood tests were affected by factors potentially associated with reduced functioning of the cellular immune system, such as age (very young or very old)^{9,31} or immunosuppressive treatments. In children aged 5 years or younger, T-SPOT.TB performed better than QuantiFERON-TB Gold, which might be clinically relevant, since young children with recent primary infection are at increased risk of progressing to tuberculosis⁸ and can test as false-negative with the tuberculin skin test.^{9,32} Systemic steroids had a similar, though non-significant, effect on the accuracy of QuantiFERON-TB Gold and T-SPOT.TB.

To help control and possibly eliminate tuberculosis in low-prevalence areas, a specific and sensitive test for latent infection is needed, especially in populations at high risk for progression from infection to disease. The tuberculin skin test alone is inadequate. QuantiFERON-TB Gold and T-SPOT.TB show good diagnostic agreement with the skin test and have higher specificity. As such, either in combination with or as a substitute for the skin test, they could increase the diagnostic sensitivity of

testing for latent infection. It is noteworthy, however, that there are differences between the two blood assays when they are used in routine practice, especially in the diagnosis of high-risk individuals, such as immunosuppressed patients and young children. The choice of which diagnostic test to use should depend on the population being tested, the purpose of testing, and the resources available.

Contributors

G Ferrara and M Losi contributed equally to this article. G Ferrara, M Losi, and L Richeldi participated in study design, data collection, data analysis, data interpretation, and writing of the report. R D'Amico participated in data analysis, data interpretation, and writing of the manuscript. P Roversi, R Piro, A Andreani, and B M Bergamini participated in data collection, data analysis, data interpretation, and writing of the manuscript. M Meacci, B Meccugni, I Marchetti Dori, C Mussini, F Rumpianesi, and L M Fabbri participated in data collection and interpretation.

Conflict of interest statement

L Richeldi received consultancy fees in 2005 for participation in an Oxford Immunotec (manufacturer of T-SPOT.TB) advisory board meeting; his institution (the University of Modena and Reggio Emilia) received money in 2005 from the Italian representative of Cellestis and the distributor of the QuantiFERON-TB Gold test in Italy (ADA, Padova, Italy). All other authors declare that they have no conflict of interest.

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