New blood tests for diagnosis of infection with *Mycobacterium tuberculosis*

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**Summary**

**Introduction** A major challenge in tuberculosis (TB) control is the diagnosis and the treatment of latent tuberculosis infection.

**State of art** At the present time, the diagnosis is based on tuberculin skin test (TST). TST is not specific, has poor sensitivity and is not easy to perform.

**Perspectives** Two interferon-based tests for the diagnosis of tuberculosis have just been licensed. These tests have some advantages on TST. They only require a blood sample and their results are not dependent on the examiner. Their specificity is higher than TST because they don’t cross-react with BCG vaccine and with most of the environmental *Mycobacterium* species. In addition, their sensitivity is higher for the diagnosis of active tuberculosis. In latent tuberculosis, the interferon-gamma assays show better correlation with exposure to *Mycobacterium tuberculosis* than TST. Both tests seem to show reduced ability to detect TB in immunocompromised patients, in particular the medically immunocompromised.

**Conclusions** Interferon-gamma assays seems to be useful tools in TB detection, but these good results have to be confirmed with larger studies of non selected patients.

**Key-words:** Blood tests • tuberculosis • diagnosis interferon-gamma • T-cell assay.
Introduction

With 8.8 million new cases and 1.7 million deaths each year [1], infection with Mycobacterium tuberculosis remains a major worldwide public health problem. The situation is also worrying in France; even though overall incidence is diminishing, it is increasing in persons born outside the country. Moreover, multi-resistant strains are appearing [2]. In countries where incidence is considered to be low, control of the disease is through screening and treatment of all tuberculosis infections; this includes the treatment of all latent tuberculosis infections in children and recent ones in adults. The diagnosis of tuberculosis is currently based on the concept of contagion, a body of clinical and radiographic findings, microbiology findings (presence of tuberculosis complex mycobacteria on direct examination or after culture), and the tuberculin skin test (TST). TST presents many disadvantages. Performing the test and reading the result are difficult and require two consultations. Cross-reactions with other mycobacteria are frequent, including with vaccine strains. For this reason TST interpretation is difficult in countries where BCG vaccination is widespread. The sensitivity of TST is frequently decreased in: cell-mediated immune deficiency (HIV, malnutrition, immunosuppressor treatment), viral infections, severe bacterial infections (including haematogenous tuberculosis), and the elderly. Finally, its positive predictive value (PPV) is extremely poor in low-prevalence countries. In a country with a prevalence of 5/100, the PPV of TST is 50%, whereas it is only 2% when prevalence is 1/10000 [3-5]. These new in vitro diagnostic tests, based on detection of interferon-gamma (IFN-γ) production or of cells producing IFN-γ, in the presence of specific tuberculosis complex antigens. We describe below the partial response they provide to our expectations.

Background

Robert Koch described the principle of TST in 1890; it was introduced as a diagnostic test early in the XXth century by Von Pirquet [4]. The test reveals a local delayed hypersensitivity reaction related to cytokine secretion (such as IFN-γ and interleukin 8, TNF-α) by specific T-cells in response to intradermal injection of tuberculosis antigens [3, 6].

To date, the antigen used has been tuberculin or purified protein derivative (PPD). Several types of tuberculin are used, all biologically equivalent to a batch produced in 1952: PPD-S [3, 5]. The World Health Organization recommends the use of tuberculin RT23 (at the dose of 2 units), but a different tuberculin is used in Europe and the United States. It is marketed as Tubertest® or Tubersol® [5].

The tuberculin is in fact a mixture of antigens not specific to the Mycobacterium tuberculosis complex (Mycobacterium tuberculosis, M. africanum, M. bovis and M. microti) [7]. Cross-reactions exist with all the other strains of mycobacteria, whether environmental or from vaccination [3, 5, 7].

Two advances in the 1990s paved the way for new diagnostic methods. The development of an in vitro diagnostic test based on detection of IFN-γ production by lymphocytes in the presence of PPD, in animals [8], then in human beings [9], suggested the possibility of simplifying the diagnosis of latent tuberculosis infection. A test based on this principle was approved for commercialization by the US Food and Drug Administration (FDA) in 2001 [10]. But it was the discovery of two new proteins, early secretary antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), that really opened the field for new diagnostic methods [11].

The specific antigens

Comparative analysis of Mycobacterium bovis, Mycobacterium tuberculosis and BCG using subtractive genomic hybridization has shown specific regions for virulent strains. These have been designated regions of difference (RD) 1, 2, 3, etc. [12]. Some RD1 genes encode two proteins, ESAT-6 and CFP-10, which have considerable antigenic properties [11, 13].

These two proteins were found in mycobacteria of the tuberculosis complex but were absent from the various BCG strains, in particular the M. bovis strain used for the Calmette-Guerin intradermal vaccine. These proteins are only found in 5 atypical mycobacteria out of the fifty strains involved in human infectious diseases [14]. They are Mycobacterium kansasii, M. marinum, M. szulagii, M. flavescens and M. gastri [3, 15, 16].

M. leprae also has a gene that encodes an ESAT-6 homologue, which is a possible source of cross-reaction [17].

The tests available

Two tests are currently commercialized. The QuantiFERON®-TB Gold test (Cellestis Ltd., Carnegie, Australia) was approved by the FDA in 2005 [18] and also by the European Union. The T-SPOT™ TB test (Oxford Immunotec, Oxford, UK) has been approved for use in Europe and is currently being assessed by the FDA. Thus both tests are available in France.

They are based on in vitro detection of specific cell immune response to tuberculosis infection [19]. The assay is performed on whole blood for the QuantiFERON test and on peripheral blood mononuclear cells for the T-SPOT test. The sample is incubated with a peptide mixture corresponding to the ESAT-6 and CFP-10 proteins [18, 19]. A positive control (phytohaemagglutinin) and a negative control (normal saline)
are systematically performed. The sample must be processed within 12 hours of collection and must be incubated in the presence of the specific antigens for 16 to 24 hours [18].

The QuantiFERON test uses ELISA to measure the quantity of IFN-γ produced. The T-SPOT test uses an enzyme-linked immunospot assay technique (ELISPOT) to detect the number of cells producing interferon [19].

Limitations of these tests

Three literature reviews have already been published on the subject [3, 19, 20]. Two of them reviewed all the studies using interferon-γ assay techniques, whatever the antigen used: specific antigens (ESAT-6 and CFP-10) or tuberculin [3, 19]. Our review was limited to studies evaluating the ELISA and ELISPOT techniques using the specific antigens. We identified 28 studies that met these criteria [21-48]. The study search was performed using the PubMed database.

From the outset, there were major difficulties in assessing test performance. In tuberculosis disease, test sensitivity can be calculated directly if only the number of bacteriologically-confirmed cases is taken into account. Evaluation of specificity is however impossible as there are currently no tests available that can definitively exclude tuberculosis infection. In latent tuberculosis infection, the diagnosis is currently based on the concept of contagion and TST results, with their known shortcomings. The diagnosis is thus presumed. Precise evaluation of diagnostic test sensitivity is thus impossible because we cannot identify all the patients. Studies are reduced to using tuberculin skin tests as their reference standard. A further difficulty was the variety of skin tests used in the studies that were not necessarily equivalent: TST with tuberculin RT23 at the dose of 1 [23], 2 [23, 25, 30, 33, 34, 37, 41] or 5 units [27], Tubertest® [38, 40, 42, 48], TST with tuberculin PPD-S at the dose of 1 [26] or 3 IU [32] or multiple puncture devices for studies in the United Kingdom [24, 26, 28, 43, 44]. It should be noted that the multiple puncture device is not a recognized diagnostic method in most countries (including France and the United States) [6, 7]. Specificity can only be calculated in tuberculosis-free populations. Here again, there are no tests available that can definitively exclude latent tuberculosis infection. Most of the time, we have to resort to approximations. We generally consider that all subjects living in low-endemic countries who do not present any risk factors for tuberculosis are not infected. The specificity of these new tests is thus probably slightly overestimated.

- With the tuberculin test, there are cross-reactions with all the other mycobacteria.
- Two new proteins, ESAT-6 and CFP-10, have paved the way for new diagnostic methods.
- These two proteins are present in mycobacteria of the tuberculosis complex but absent from the various BCG strains.
- The tests based on these proteins detect specific cell immune response to tuberculosis.

Results

Diagnosis of tuberculosis disease

The sensitivity of the in vitro tests in the diagnosis of tuberculosis disease has been evaluated in 13 studies which are summarized in Table I. Ten studies only involved adults, two were purely paediatric, and the last study included patients of all ages. The sensitivity varied from 66.7% [40] to 100% [27]. Two studies showed a sensitivity of less than 80% [40, 48]. The first study involved 11 adult subjects and compared the sensitivity of ELISA with that of TST (Tubertest®). The ELISA results were indeterminate in two patients. Test sensitivity with ELISA (calculated in the nine subjects with interpretable tests) was 66.7% while that of TST was only 33%. The difference was not significant [40]. The second study compared the sensitivity of the two in vitro tests respectively with the sensitivity of TST (Tubertest® in a population of 24 subjects. The sensitivity of the ELISPOT test was 83.3% while for ELISA it was only 70.8%. The sensitivity of both these tests remained superior to that of TST (66.7%). The authors did not indicate whether the difference was significant [48]. It should however be emphasized that the populations for these two tests were small. Three other studies found sensitivities between 80 and 85%. Two were paediatric studies [30, 41], while the third only used one antigen, ESAT-6, for the diagnosis [22]. In the eight other studies, in vitro test sensitivity was over 85%. Two studies in adult subjects using the ELISPOT test also took into account their HIV serology status. The sensitivity of the test did not seem to be modified by HIV-positivity [22, 27]. Moreover, disease localization (pulmonary or extrapulmonary) did not modify test sensitivity [21, 22, 26, 48].

Two were purely paediatric [30, 41] with large study populations. These were performed in South Africa. The first involved 262 young children (mean age 36 months); most of them were malnourished (mean Z score of –1.7), and nearly half of the children tested (46%) were HIV positive. The objective of this study was to compare the ELISPOT test and TST in the diagnosis of tuberculosis infection. The BCG vaccination coverage rate was 95% in the region concerned. The children referred for suspected tuberculosis (fever and cough for over a month, delayed weight gain or weight loss, signs suggestive of extrapulmonary tuberculosis) were classified in four groups: positive bacteriology-confirmed tuberculosis (n=57), high probability of tuberculosis defined by consistent radiographic, clinical and/or histology findings (n=76), possible tuberculosis defined as a diagnosis that
could not be excluded over a period of 6 months (n=116), and tuberculosis excluded (n=13). The ELISPOT test showed a sensitivity of 81% in the confirmed tuberculosis group and 84% in the high probability group (83% for the two groups combined). TST (2 units of R23) sensitivity was significantly lower, whatever the positivity threshold chosen (induration of 5, 10 or 15 mm, p < 0.001 for all 3 thresholds). It should be noted however that TST sensitivity was far better in the high probability group (80 or 81% depending on the threshold of positivity) than in the confirmed tuberculosis group (35 or 40%). But these results were biased because a positive TST was one of the inclusion criteria for the probable tuberculosis group. ELISPOT test sensitivity was not significantly modified by subject age (81% after the age of 3 and 85% before, p=0.53), HIV status (85% when negative against 73% when positive, p=0.12), or nutritional status (86% against 78% when malnourished, p=0.24). However, TST sensitivity was significantly reduced for each of these three parameters (51% before 3, 36% when HIV+ and 44% when malnourished). Finally, the sensitivity for the two tests when associated (TST and ELISPOT) was 91% for the confirmed and probable tuberculosis groups combined. The authors concluded that the ELISPOT test was superior to TST in the diagnosis of tuberculosis infection in this population of children, and that associating the two tests provided a diagnosis of tuberculosis with a higher sensitivity [30].

The second study involved 70 young children (mean age 32 months), all HIV negative and referred for suspected tuberculosis infection. They were classified in 3 groups, Table I.

<table>
<thead>
<tr>
<th>Study</th>
<th>Test</th>
<th>Antigens</th>
<th>Population</th>
<th>In vitro test sensitivity</th>
<th>Skin test sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arend et al. [21]</td>
<td>ELISA</td>
<td>ESAT-6 and CFP-10</td>
<td>37 adults with positive bacteriology</td>
<td>95%</td>
<td>NP</td>
</tr>
<tr>
<td>Chapman et al. [27]</td>
<td>ELISPOT</td>
<td>ESAT-6 and CFP-10</td>
<td>50 adults with positive bacteriology, of which 39 HIV+</td>
<td>100% for HIV- and 92% for HIV+</td>
<td>NP</td>
</tr>
<tr>
<td>Ferrara et al. [40]</td>
<td>ELISA</td>
<td>ESAT-6 and CFP-10</td>
<td>11 adults (5 confirmed with bacteriology)</td>
<td>66.7%</td>
<td>Multiple puncture 33%</td>
</tr>
<tr>
<td>Kang et al. [37]</td>
<td>ELISA</td>
<td>ESAT-6 and CFP-10</td>
<td>54 adults with high probability of tuberculosis infection</td>
<td>95.7%</td>
<td>TST with threshold of 10/15 mm=78/70%</td>
</tr>
<tr>
<td>Lalvani et al. [22]</td>
<td>ELISPOT</td>
<td>ESAT-6</td>
<td>50 adults with positive bacteriology, of which 6 HIV+</td>
<td>80% (100% in HIV + subjects)</td>
<td>NP</td>
</tr>
<tr>
<td>Lalvani et al. [26]</td>
<td>ELISPOT</td>
<td>ESAT-6</td>
<td>47 adults with positive bacteriology</td>
<td>96%</td>
<td>Multiple puncture 69%</td>
</tr>
<tr>
<td>Liebeschuetz et al. [30]</td>
<td>ELISPOT</td>
<td>ESAT-6 and CFP-10</td>
<td>262 children (HIV 46% and malnourished) distributed in 4 groups: proven tuberculosis infection (n=57), high probability (n=76), possible (n=116) or excluded</td>
<td>Proven: 81%, High probability: 84%</td>
<td>TST with thresholds of 5/10/15 mm (respectively) Proven: 39.5/37/35%, High probability: 81/81/80%</td>
</tr>
<tr>
<td>Mori et al. [32]</td>
<td>ELISA</td>
<td>ESAT-6 and CFP-10</td>
<td>118 adults with positive bacteriology</td>
<td>89%</td>
<td>TST with threshold of 5 mm=65.8%</td>
</tr>
<tr>
<td>Nicol et al. [41]</td>
<td>ELISPOT</td>
<td>ESAT-6 and CFP-10</td>
<td>70 children (VIH-) distributed in 3 groups: proven tuberculosis infection (n=12), high probability (n=47), possible (n=11)</td>
<td>83.3% proven, 72.3% probable, 45.5% possible</td>
<td>NP</td>
</tr>
<tr>
<td>Pathan et al. [46]</td>
<td>ELISPOT</td>
<td>ESAT-6</td>
<td>44 adult patients distributed in 3 groups: proven pulmonary tuberculosis (n=25), probable (n=11) and proven or probable lymph node tuberculosis (n=11)</td>
<td>92% proven, 88% probable and 91% with lymph node involvement</td>
<td>NP</td>
</tr>
<tr>
<td>Ravn et al. [39]</td>
<td>ELISA</td>
<td>ESAT-6 and CFP-10</td>
<td>48 adults with high probability of tuberculosis infection (27 with positive bacteriology)</td>
<td>85%</td>
<td>NP</td>
</tr>
<tr>
<td>Schölvink et al. [31]</td>
<td>ELISPOT</td>
<td>ESAT-6 and CFP-10</td>
<td>13 adults with positive bacteriology</td>
<td>ELISPOT 100%, ELISA 92.3%</td>
<td>NP</td>
</tr>
<tr>
<td>Ferrara et al. [48]</td>
<td>ELISPOT</td>
<td>ESAT-6 and CFP-10</td>
<td>24 patients</td>
<td>ELISPOT 83.3%, ELISA 70.8%</td>
<td>TST with threshold depending on patient 66.7%</td>
</tr>
</tbody>
</table>

using the same method as the previous study: confirmed tuberculosis (n=12), probable (n=47), and possible (n=11). The objective of the study was to evaluate the sensitivity of the ELISPOT test and to follow evolution of the results under treatment. The results were comparable with those of the previous study with a sensitivity of 83.3% in the confirmed tuberculosis group and 72.3% in the probable tuberculosis group. Here again, test sensitivity was not modified by the nutritional status of the children [41]. We return to the evolution of test results under treatment later in this article.

In the end, only four of the studies compared \textit{in vitro} tests with skin tests [26, 30, 32, 37]. The results were consistent and all supported the same findings: the sensitivity of \textit{in vitro} tests was always markedly superior to that of tuberculin skin tests.

- The sensitivity of the \textit{in vitro} tests in the diagnosis of tuberculosis infection varied from 66.7% to 100% depending on the study.
- The age of the subjects, their HIV status, and their nutritional status did not modify the sensitivity of the ELISPOT test.
- The sensitivity of the \textit{in vitro} tests was always markedly superior to that of the tuberculin skin tests.

\textbf{Diagnosis of latent tuberculosis infection}

\textit{In vitro} test effectiveness in the diagnosis of tuberculosis infection has been evaluated in 19 studies which are summarized in \textit{tables II and III}.

\textbf{Sensitivity}

As previously explained, sensitivity cannot be calculated directly. Evaluation of sensitivity can only be performed by analysing the concordance of \textit{in vitro} tests with TST, or relating the \textit{in vitro} test results to the probability of tuberculosis infection, evaluated by the proximity and intensity of exposure to an individual with tuberculosis.

Seven studies used index cases. The skin test used was TST for four studies [33-34, 37-38], and a multiple puncture device for the three others [24, 28, 44]. In the latter three studies, \textit{in vitro} (ELISPOT) test sensitivity seemed to be better than with the skin test. Thus, 50 contacts of index cases were classified in four groups for probability of infection depending on the degree of exposure. The probability of having a positive test increased by a factor of 9 between each group for the ELISPOT test, and a factor of 1.9 for the multiple puncture device [24]. In a similar study of 535 contacts (ELISPOT test with ESAT-6 and CFP-10), the probability of having a positive test increased by 2.78 between groups for the ELISPOT test, while it only increased by a factor of 2.33 for the multiple puncture device. The correlation with degree of exposure was better for the ELISPOT test than for the skin test (p=0.03). The concordance between the two tests was good (89%, kappa=0.72). When ELISPOT and multiple puncture device test results were different, an isolated positive ELISPOT test was a predictive factor of exposure to the index case, while an isolated positive skin test was not [28]. Finally, in a study of 75 subjects with close and prolonged contact with an index case strongly smear positive for bacilli, ELISA suggested considerable transmission as it was positive in 22% of the contacts while the skin test was positive in only 2.7%. However, there was no correlation between duration and proximity of contact with the ELISA results. Considering the intensity of the contact and the contagiousness of the index case, the authors thought the ELISA results were more consistent with the known contagiousness of the tuberculosis [44]. The results of the 4 studies using the reference skin test (TST) were not as clear-cut. In one study of 413 subjects with calculation of a contact score, correlation with degree of exposure was more marked with the ELISPOT test than with TST (Tuberculol®) but the difference was not significant. The concordance between the two tests was good (75%, kappa=0.49). The subjects with a negative ELISPOT test had a TST size smaller than those with a positive test (median=0 mm against 20 mm, p < 0.0001) [38]. In another study, 125 contacts of cases were tested with ELISA (ESAT-6, CFP-10 and tuberculin) and divided into four groups according to degree of exposure and history of previous vaccination. The 85 subjects without BCG vaccination also underwent TST (RT23, 2 units). ELISA was positive in 52.8% of the subjects at risk (high degree of exposure) and 5.5% of the subjects not at risk (low degree of exposure). In the non-vaccinated group, ELISA and TST showed similar results, in both the at-risk group (positive results of 53% and 56% respectively) and the not-at-risk group (negative results of 95% and 90% respectively). The concordance between ELISA and TST was excellent (94%, kappa=0.87) [34]. In a study performed in a country with intermediate endemicity (Korea), 219 subjects were divided into three groups: no risk factors, medical personnel with occasional contact with bacteriologically confirmed TB patients, and persons with close contact with a bacteriologically confirmed TB patient. All the subjects underwent TST (RT23, 2 units) and ELISA. The probability of having a positive test increased between each group, both for TST and ELISA (1.68 and 4.23 respectively). This probability was more marked for ELISA than for TST (p<0.001). Concordance between the two tests was poor (kappa between 0.08 and 0.17 depending on the group). The results were the same whether the TST positivity threshold was 10 or 15 mm [37]. Finally, a study of 735 contacts living in a high-endemicity country (Gambia). Though there was a positive correlation between the degree of exposure and the various tests used on the contacts (TST, ELISPOT with specific antigens and with tuberculin), this was more marked for TST than for the ELISPOT test. Moreover, the
probability of having a positive TST and a negative ELIS-POT test increased with the degree of exposure [33].

To be exhaustive, a further four studies which examined concordance between in vitro tests and skin tests should be mentioned [36, 40, 42, 48]. In a population of 726 health care workers in India, both tests (TST and ELISA) were correlated with years in the profession; concordance between the tests was good (81.4%, kappa=0.61) [36]. A study of a prison population in the United States showed concordance between the two tests of 90% (kappa=0.25) but this seemed to be slightly less in African-American individuals [42]. In a study of a non-selected population (adults and children, 20% immunosuppressed), concordance was 70.2%, kappa=0.4, markedly better in the non-vaccinated group (80.5%, kappa 0.56) than in the vaccinated group (41.5%, kappa=0.09) [40]. Similar results were found by the same team in another study using quite comparable methods. The concordances of ELISA (kappa=0.46) and the

### Table II.
Effectiveness of ELISA in the diagnosis of tuberculosis infection.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Vaccination with BCG</th>
<th>Positivity ELISA with specific antigens</th>
<th>Positivity of ELISA with PPD</th>
<th>Positivity with skin test</th>
<th>Concordance ELISA/skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. [44]</td>
<td>75 contact subjects</td>
<td>79%</td>
<td>22%</td>
<td>NP</td>
<td>TST 2.6%</td>
<td>NP</td>
</tr>
<tr>
<td>Arend et al. [23]</td>
<td>36 contact subjects divided into 3 groups:</td>
<td>None</td>
<td>TST+: 75% TST – and indeterminate: 0%</td>
<td>TST+: 100% TST – and indeterminate: 33.3%</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Brock et al. [34]</td>
<td>125 contact subjects, 4 groups according +to degree of exposure and BCG</td>
<td>32%</td>
<td>High exposure, BCG+: 50% High and BCG-: 53% Low exposure, BCG+: 6% Low exposure, BCG-: 5%</td>
<td>High exposure, BCG+: 38% High and BCG-: 47% Low exposure, BCG+: 44% Low exposure, BCG-: 5%</td>
<td>TST High exposure, BCG-: 55.5% Low exposure, BCG-: 10%</td>
<td>94%, k=0.86 High exposure: 93% Low exposure: 95%</td>
</tr>
<tr>
<td>Ferrara et al. [40]</td>
<td>307 non-selected subjects (adults and children, immunosuppressed 20%)</td>
<td>18.5%</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>70.2%, k=0.4 BCG+: 41.5%, k=0.09 BCG -: 80.3%, k=0.56</td>
</tr>
<tr>
<td>Kang et al. [37]</td>
<td>219 subjects in 3 groups: A: close contact, B: distant contact, C: no contact</td>
<td>87%</td>
<td>Group A: 44% Group B: 10% Group C: 4%</td>
<td>NP</td>
<td>TST with threshold of 10/15 mm Group A: 71/48% Group B: 60/43% Group C: 51/37%</td>
<td>NP</td>
</tr>
<tr>
<td>Mori et al. [32]</td>
<td>213 subjects without TRF</td>
<td>100%</td>
<td>1.9%</td>
<td>NP</td>
<td>TST 5 mm: 85.8% TST 10 mm: 64.6% TST 15 mm: 31.9%</td>
<td>NP</td>
</tr>
<tr>
<td>Pai et al. [36]</td>
<td>726 health care personnel</td>
<td>71%</td>
<td>40%</td>
<td>NP</td>
<td>TST 10 mm: 41% TST 15 mm: 23%</td>
<td>81.4%, k=0.61</td>
</tr>
<tr>
<td>Porsa et al. [42]</td>
<td>409 adults, all imprisoned, HIV -</td>
<td>Not specified</td>
<td>5.4%</td>
<td>NP</td>
<td>9%</td>
<td>90%, k=0.25</td>
</tr>
<tr>
<td>Ravn et al. [45]</td>
<td>39 subjects without TRF</td>
<td>Not specified</td>
<td>0%</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Ferrara et al. [48]</td>
<td>114 non-selected contact subjects (adults and children, immunosuppressed 38%)</td>
<td>18%</td>
<td>27.6%</td>
<td>NP</td>
<td>Not specified</td>
<td>k=0.46</td>
</tr>
</tbody>
</table>

ELISPOT test (kappa=0.508) with TST were similar. As expected, the concordance of these tests with TST was significantly less in the BCG vaccinated population [48].

**Specificity**

Once again, this is an approximation. The various specificities were calculated in populations that were in principle not infected, but without formal exclusion of infection being possible. The specificity of the *in vitro* tests varied between 92% [26] and 100% [22, 24, 27, 45-47]. In the only study showing specificity below 98%, only the ESAT-6 antigen had been used [26]. Estimation of specificity had been performed in populations where vaccination cover was high: 77% [26] to 100% [32, 47]. Unsurprisingly, in these populations, *in vitro* test specificity was far higher than with TST. Thus, in a population

**Table III.**

Effectiveness of the ELISPOT test in the diagnosis of tuberculosis infection.

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Vaccination with BCG</th>
<th>Positivity of ELISPOT with specific antigens</th>
<th>Positivity of ELISPOT with PPD</th>
<th>Positivity with skin test</th>
<th>Concordance ELISPOT/skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brock et al. [47]</td>
<td>22 healthy subjects without TRF</td>
<td>100%</td>
<td>0%</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Chapman et al. [27]</td>
<td>75 Zambian adults without tuberculosis infection (HIV+: 28%) and 40 British adults without TRF</td>
<td>Zambians: 76% British: 82%</td>
<td>Zambian HIV+: 43% Zambian HIV-: 69% British: 0%</td>
<td>Zambian HIV+: 29% Zambian HIV-: 83% British: 83%</td>
<td>Zambian HIV+: 36%</td>
<td>Zambian HIV-: 80%</td>
</tr>
<tr>
<td>Ewer et al. [28]</td>
<td>535 contact subjects divided into 4 groups according to duration and proximity of contact. Groups A to D in decreasing order of contact.</td>
<td>873%</td>
<td>Group A: 100% Group B: 53% Group C: 38% Group D: 17%</td>
<td>NP</td>
<td>Multiple puncture Group A: 62% Group B: 40% Group C: 27%</td>
<td>89%, k=0.72</td>
</tr>
<tr>
<td>Hill et al. [33]</td>
<td>735 contact subjects divided into 3 groups: group A: same room, B: same house, C: different house</td>
<td>45%</td>
<td>Group A: 38.3% Group B: 30.4% Group C: 23.5%</td>
<td>Group A: 72.5% Group B: 63.3% Group C: 61.7%</td>
<td>TST Group A: 62.4% Group B: 40% Group C: 27%</td>
<td>NP</td>
</tr>
<tr>
<td>Lalvani et al. [22]</td>
<td>100 healthy Indian adults and 40 healthy British adults</td>
<td>British: 82%, Indian &gt; 96%</td>
<td>Indian: 80% positive British: 0%</td>
<td>Indian: 98% British: 82%</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Lalvani et al. [24]</td>
<td>50 contact subjects divided into 4 groups according to duration and proximity of contact. Groups A to D in decreasing order of contact.</td>
<td>82%</td>
<td>Group A: 72.3% Group B: 37.5% Group C: 0% Group D: 0%</td>
<td>Group A: 90.9% Group B: 100% Group C: 91.7% Group D: 85.7%</td>
<td>Group A: 90.9% Group B: 100% Group C: 91.7% Group D: 85.7%</td>
<td>NP</td>
</tr>
<tr>
<td>Lalvani et al. [26]</td>
<td>47 subjects without TRF and 26 contact subjects</td>
<td>77% for healthy subjects and 100% for contacts</td>
<td>Healthy subjects: 8% Contact subjects: 85%</td>
<td>NP</td>
<td>Contacts: 100% but=inclusion criterion</td>
<td>NP</td>
</tr>
<tr>
<td>Pathan et al. [46]</td>
<td>32 healthy subjects and 27 contact subjects</td>
<td>87.5% healthy subjects and 85% contacts</td>
<td>Healthy: 0% Contacts: 85%</td>
<td>NP</td>
<td>Contacts: 100%</td>
<td>NP</td>
</tr>
<tr>
<td>Shams et al. [38]</td>
<td>413 contact subjects classified in groups according to degree of exposure, groups A to D in decreasing order of contact.</td>
<td>50%</td>
<td>Group A: 60% Group B: 43.7% Group C: 34% Group D: 30%</td>
<td>NP</td>
<td>TST Group A: 56.7% Group B: 52.4% Group C: 51.5% Group D: 40.8%</td>
<td>75%, k=0.49</td>
</tr>
<tr>
<td>Ferrara et al. [48]</td>
<td>114 non-selected contact subjects (adults and children, immunosuppressed 38%)</td>
<td>18%</td>
<td>37.8%</td>
<td>NP</td>
<td>k=0.508</td>
<td></td>
</tr>
</tbody>
</table>

of 113 student nurses beginning their course, without risk factors for tuberculosis, living in a low-endemicity country (Japan), and all vaccinated with BCG, specificity for ELISA was 98.1%, and for TST 35.4%, for a positivity threshold of 10 mm. Of note, even with a TST positivity threshold of 15 mm, specificity for ELISA remained far higher than for TST (98.1% against 68.1%) [32]. These results have been confirmed by a study in a country of intermediate endemicity (Korea, incidence=92/100000, BCG=87%). The specificity of ELISA was 96% whereas it was 49% for TST with a threshold of 10 mm (63% with a threshold of 15 mm) [37].

- The sensitivity of in vitro tests for tuberculosis infection cannot be calculated directly, but seems to be better than with skin tests.
- Concordance between ELISPOT and TST is good; between ELISA and TST it varies with the studies.
- The specificity of the in vitro tests varies between 92% and 100%, far higher than TST in the case of BCG vaccination.

Impact of immunosuppression on test results

The fact that skin tests are far less sensitive in immunodeficient patients has been acknowledged, whether the immunosuppression be secondary to HIV infection, treatment (long-term immunosuppressive and corticosteroid), or severe malnutrition [4, 6, 7]. Immunodeficiency seems to have less effect on the sensitivity of the two in vitro tests, even though less data are available. Three studies have examined the impact of HIV infection on test results [27, 30, 35]. In Zambia, in a population of 50 adults (including 39 HIV+), HIV positivity did not influence ELISPOT test sensitivity in the diagnosis of tuberculosis infection (92% if HIV+, 100% if HIV-, non-significant difference) [27]. Similar results were noted in South African children with suspected tuberculosis infection, with an ELISPOT test sensitivity of 85% in children that were HIV- or had not been tested, and 73% in VIH+ children (p=0.12). The same study found clearly reduced TST sensitivity (70% against 36% if HIV+, p=0.002) [30]. Seventy-five Zambian adults in good health (VIH +=28%) were tested with TST and ELISPOT in the context of diagnosis of tuberculosis infection. HIV positivity only slightly modified ELISPOT test performance (positive in 69% of HIV- subjects and 43% in HIV+ subjects, p=0.064) while TST performance was markedly reduced (80% against 36%, p=0.0057) [27]. Unfortunately, these two studies [27, 30] had only taken into consideration the HIV status of the patients and not their real immune status (CD4 cell count).

In another study [35], effectiveness of the ELISPOT test was related to HIV-induced immunosuppression. Nineteen HIV- and 29 HIV+ subjects without tuberculosis infection criteria were included. The variables studied were the presence of a positive control reaction to the test (phytohaemagglutinin) and its intensity according to the CD4 cell count. All the subjects included, except for one HIV+ subject, showed response. The mean cell counts were similar in both groups. However, HIV+ subjects with a CD4 cell count below 200/mm³ showed a significantly lower mitogen response than subjects with CD4 counts over 200/mm³.

Two studies included patients undergoing treatment with immunosuppressive agents or who presented chronic disorders causing immune suppression [40, 48]. The first was an Italian study using ELISA on a non-selected population of 318 subjects with suspected tuberculosis infection [40]. This population included 20.4% subjects undergoing treatment with immunosuppressive agents. One indeterminate result (insufficient or absent positive control reaction) was noted in 21.4% of these 318 patients. Indeterminate results were 3.5 times more frequent in patients undergoing immunosuppressive treatment compared with the other patients (p=0.00007). These results were corroborated by another study carried out by the same team [48]. With ELISA, indeterminate results were found in 11% of the 383 patients included, and only 3% with the ELISPOT test. The difference between the two tests was highly significant (p < 0.0001). Here again, the patients undergoing immunosuppressive treatment showed a higher risk of having indeterminate test results.

- Skin tests are markedly less sensitive in immunodeficient subjects.
- Immunodeficiency seems to have less effect on the in vitro tests.

Kinetics of in vitro test responses under antituberculosis treatment

Three studies examined the kinetics of in vitro test responses under antituberculosis therapy. The first [29] used the ELISPOT test (ESAT-6 antigen alone) in a population of 18 adults with proven tuberculosis infection (39% HIV+). Five of these patients were considered to be either clinical or bacteriological non-responders at 3 months (absence of clinical improvement or presence of acid-fast bacteria in sputum). All still had a positive ELISPOT test at 3 months, while the 11 patients showing clinical effectiveness of treatment had become negative. The second was a paediatric study [41] (70 children with suspected tuberculosis infection). The ELISPOT test was repeated 1 month after treatment in 42 children, then at 3 and 6 months in 10 children. At 1 month of treatment, the test results for
each antigen (ESAT-6 and CFP-10) were similar to those carried out at the time of diagnosis. There was perfect correlation of the results at 1 month and at diagnosis. In the 10 patients followed-up for a longer period, they observed an increase in the number of responsive cells at 1 month followed by a decrease at 3 and 6 months of treatment. These data are corroborated by the last study [43]. This study involved 41 adults with suspected tuberculosis infection, and also used the ELISPOT test. Thirty-three patients were treated (standard two-drug therapy) and 8 others were monitored with chest radiographs. In the treatment group, they observed a significant increase in the number of responsive cells to both antigens at 1 month of treatment. A decrease in the number of these cells was then observed. This decrease at the end of treatment was significant compared with the results at 1 month but not compared with the initial count. There were no modifications in test results in the group without treatment during the 3 months of follow-up.

Rate of indeterminate results

An indeterminate result is defined as absence or insufficient response to the positive control. This was rare as it was only reported in 5 of the studies [32, 36, 37, 40, 48] and the rate was in majority low. Two studies found a significant percentage of indeterminate tests (3 to 20%) [40, 48]. Interestingly, these were the only two studies carried out on non-selected populations (representative of current clinical practice). In the first study, the factors associated with an indeterminate test were immunosuppressive treatment or a negative TST. Of note, the presence of cancer, extremes of age (< 3 and > 80) and HIV positivity were not associated with indeterminate results [40]. The second study compared the two in vitro tests with each other. There were significantly fewer indeterminate results with the ELISPOT test than with ELISA (3% against 11%, p < 0.0001). Immunosuppressive treatment was associated with indeterminate results with both tests. Children under 5 also presented a risk factor for indeterminate test results, but only with ELISA [48].

• The factors associated with an indeterminate test are immunosuppressive treatment or a negative TST.
• Children under 5 also present a risk factor for indeterminate ELISA results.

Infection with atypical mycobacteria and in vitro tests

As previously explained, the ESAT-6 and CFP-10 antigens are only present in five atypical mycobacterial species. To date, no studies have demonstrated the absence of cross-reactivity with atypical mycobacteria that do not secrete these specific antigens. We can however suppose that, as with BCG, in vitro tests using specific antigens are not effected by an infection or disease caused by atypical mycobacteria (except for the five which secrete ESAT-6 and CFP-10 of course). Only one study, carried out in The Netherlands, has examined the sensitivity of these new tests in the diagnosis of infection with atypical mycobacteria [25]. Seven subjects with proven M. marinum infection and 5 subjects with M. kansasi infection were included. Three tests were used: TST, ELISA and the ELISPOT test. ELISA and ELISPOT showed similar sensitivities that were superior to that of TST. For example, for M. marinum, sensitivity was 85.7% with ELISA, 100% with the ELISPOT test and 80% with TST. The results were similar for M. kansasi, even though sensitivities were lower for all three tests.

• ELISA and ELISPOT show similar sensitivities, superior to that of TST for atypical mycobacteria.

Conclusions

The recently commercialized in vitro diagnostic tests for tuberculosis present several advantages compared with TST. They are easier to perform and the results obtained within 24 hours are less subjective. Their specificity for the tuberculosis complex makes them more effective than TST in the diagnosis of tuberculosis disease in vaccinated populations. Moreover, these in vitro tests show good sensitivity in the diagnosis of tuberculosis disease, including in populations where TST commonly shows drawbacks: young children, severe malnutrition, and HIV positivity. In the diagnosis of latent tuberculosis infection, the sensitivity of these tests seems to be identical or superior to skin tests. It should be noted however that the most significant results were obtained in comparison with multiple puncture devices which do not provide very good results. The value of these tests in the diagnosis of latent tuberculosis infection requires confirmation with large-scale studies using TST as the reference test.

Some other limitations require consideration. Few studies have been performed to date, in comparison with nearly a century of experience with TST. Excepting two studies, all were carried out in selected populations which are not representative of the general population. Finally, the cost of these tests is a drawback. Though benefit-cost studies have not been performed, their cost remains far higher than that of TST.
LEARNING POINTS

- Tuberculosis remains a major public health problem worldwide.
- Its overall incidence is decreasing in France, but is increasing in persons born outside the country.
- TST has many disadvantages: administering the test and reading the result are difficult; cross-reactivity is frequent with other mycobacteria, including vaccine strains; its sensitivity is reduced in cell-mediated immune deficiency, viral infections, severe bacterial infections, and in the elderly; its positive predictive value is poor.
- ELISA and ELISPOT show similar sensitivities, superior to that of TST for atypical mycobacteria.
- New tests exist based on detection of two new proteins: ESAT-6 and CFP-10.
- These tests detect specific cell immune response to tuberculosis in vitro.
- They have several disadvantages: their sensitivity is not always measurable; their specificity cannot be determined in the absence of a reliable reference test.
- In tuberculosis infection, the sensitivity of in vitro tests is always superior to that of skin tests.
- Their specificity can only be determined by approximation, but is superior to that of skin tests.
- Immunodeficiency seems to have less effect on the sensitivity of the two in vitro tests.

References


