REVIEW

Serodiagnosis of tuberculosis: Due to shift track

Juraj Ivanyi*

Department of Clinical and Diagnostic Sciences, Kings College London at Guy's Hospital, 28 Floor, Tower Wing, London SE1 9RT, UK

1. Introduction

The technical advantages and low cost of serology appeared initially attractive for developing a diagnostic test for tuberculosis (TB), when the first set of Mycobacterium tuberculosis (Mt) specific antigens has first been identified using monoclonal antibody (mAb) and recombinant gene expression technologies. However, the finding that antibody levels are considerably higher and more frequent in the multibacillary than in the paucibacillary forms of the disease was noted as an obstacle to clinical application. Since only the 'smear-negative' TB attracted interest for improving the diagnosis, many studies over the past 20 years have hoped to increase the diagnostic sensitivity for paucibacillary TB, by expanding the range of test antigens. However, this approach failed to expand the detection of smear-negative cases substantially, because patients apparently produce elevated antibody levels only in multibacillary forms of TB; this outcome is similar to the high antibody levels in lepromatous, but not in tuberculoid forms of leprosy.

2. Antigens recognised in HIV−, sputum+, adult pulmonary TB

The antigens used for serodiagnosis have been selected on the grounds of optimal specificity and sensitivity. The PstS1 secreted glycolipoprotein was used by most investigators and achieved approximately 80% sensitivity in sputum-positive patients, by a number of laboratories. Antibodies from patients bind mostly conformational epitopes, while abundant T cells recognise...
a number of HLA-DR permissively binding peptide epitopes, TLR2/4 dependent cytokine production and apoptosis of monocytes could play a role in the prominent immunogenicity of PstS1. Nevertheless, poor diagnostic sensitivity of PstS1 was also reported. This could have been due to lower bacillary load or lesser disease severity of the tested patients, HIV co-infection, genetic differences in the population or technical problems with the assay.

The Mtb specificity of PstS1, when using healthy tuberculin positive controls was found to be high in Europe, America and Asia. However, poor specificity due to elevated antibody levels in pulmonary diseases excluding TB, was observed in Uganda.23 This could have been due to cross-sensitization by environmental mycobacteria, considering that PstS1 shares significant sequence with a homologous antigen from Mycobacterium intracellulare. Similar cross-sensitization interfered with the diagnostic use of the strongly immunogenic 19 kDa glycolipoprotein in Indonesia. In view of these concerns about specificity in TB endemic countries, it is important to note the high specificity as well as sensitivity of antibody levels to the Rv0222 (enol-CaA hydrolyase) antigen in Uganda.23

Combining PstS1 with other antigens was reported to increase the diagnostic sensitivity by about 10% (to ~ 85–95%) using Mtb11 secreted protein, MTB48, AcR-1 and a number of other antigens (Table 1). However, the diagnostic value of AcR-1, CFP-10 and ERL-1 is diminished by raised antibody levels in latently infected individuals. Several other antigens have been evaluated excluding PstS1, (hence probably overlapping with PstS1 responsiveness) of which reached high diagnostic sensitivity (Table 1). The potential of a very large number of serologically active protein antigens identified by microarrays requires further clinical evaluation.

### 3. Antibody repertoire related to bacterial load and clinical forms of TB

The load of tubercle bacilli in the pathological lesions influences which antigens are secreted within infected cells or released from killed macrophages. Serum antibody levels are profoundly higher in smear-positive than in smear-negative pulmonary TB and correlate with the extent of cavitation when testing for PstS1 or Ag85. Of the extrapulmonary forms of TB, antibody levels are relatively high when combined with the multibacillary pulmonary form, i.e. in patients with pleural, peritoneal or pericardial effusions, intermediary in bone and joint disease, and low in genitourinary and lymphatic disease. These findings, corroborating those from lung disease, generally confirm that anti-protein antibody levels associate with the bacterial load.

The relatively low anti-PstS1 levels in paucibacillary TB led to the search for new antigens, which might be more immunogenic in paucibacillary forms of TB. Antibodies to AcR-1 were found more frequently in paucibacillary TB, but the highest levels were still reached in multibacillary cases. In contrast, antibodies the PGL-TB1 glycolipid, were even higher in sputum-negative, than sputum +ve patients. However, antibodies to glycolipids can be increased also in infected healthy individuals, which interferes with diagnosing active TB. Diagnosis of TB meningitis, extrapulmonary and child TB are of special clinical interest. They are unlikely to disseminate infection, except by adolescent children, who develop cavitary TB. Early diagnosis of TB meningitis is of life-saving importance and can benefit from testing antibodies in the cerebrospinal fluid (CSF) against LAM or Acr-1, Ag85, or other antigens.33

In HIV-coinfected TB patients (even with more than 200 CD4 cells per ul), serodiagnosis was found to be of low sensitivity, when testing several of the best performing protein antigens (PstS1, AcR-1, CFP-10, Mtb11, Mtb12, PGL-Tb1) alone or in combinations, including a polyprotein fused from 4 constituents or a panel of 10 protein antigens. Glycolipid antigens DAT (2,3 di-o-acylrelose) and PGL-Tb1 (triglycerol phenyl dimycolcerosate), or immune-complexes have high sensitivity, but fail diagnostically by being elevated also without clinical TB symptoms. Antibodies to glycolipids, unlike proteins, are not related to CD4 T cell counts, probably due to their recognition by CD1-restricted T cells, which persist in AIDS patients.

### 4. Technical aspects of antibody serology

The technical details of ELISA based serology play an important role in the definition of cut-off points between TB patients and controls. They determine both the sensitivity of diagnosis and the robustness (i.e. reliability) of the test. The initially used competition test, where human sera are titrated for inhibition of binding by a labelled mouse mAbs, is of high sensitivity because serum dilutions as low as 1:5 (ELISA needs about 1:100 dilution) can be

### Table 1

<table>
<thead>
<tr>
<th>Population Geography</th>
<th>No. subjects tested*</th>
<th>Technique</th>
<th>Protein antigens</th>
<th>% Sensitivity**</th>
<th>First Author (Ref. no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indonesia</td>
<td>100/34</td>
<td>Competition RIA</td>
<td>+ PstS1</td>
<td>93</td>
<td>Hoepnner, 1987</td>
</tr>
<tr>
<td>East London, UK</td>
<td>46/38</td>
<td>ELISA</td>
<td>+ AcR-1</td>
<td>83</td>
<td>Jackett, 1987</td>
</tr>
<tr>
<td>North London, UK</td>
<td>30/53</td>
<td>Competition ELISA</td>
<td>+ -</td>
<td>85</td>
<td>Wilkins, 1990</td>
</tr>
<tr>
<td>China</td>
<td>54/30</td>
<td>Membrane device (IC)</td>
<td>+ -</td>
<td>89</td>
<td>Cole, 1996</td>
</tr>
<tr>
<td>New York, USA</td>
<td>31/83</td>
<td>ELISA</td>
<td>MtB11, MPT32, Ag85A, Ag85C</td>
<td>81</td>
<td>Samanci, 2006</td>
</tr>
<tr>
<td>South Africa</td>
<td>65/57</td>
<td>ELISA, fusion protein</td>
<td>MtB11, Mtb8, Mtb48</td>
<td>85</td>
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<td>California, USA</td>
<td>60/117</td>
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<td>+ MPT64, Glu-S</td>
<td>nt/93</td>
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<td>New York, USA</td>
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<td>ELISA</td>
<td>PPE55</td>
<td>97</td>
<td>Singh, 2005</td>
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<td>Denmark</td>
<td>38/30</td>
<td>ELISA</td>
<td>Rv0222</td>
<td>82</td>
<td>Rosenkrands, 2008</td>
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<td>Uganda</td>
<td>64/100/30</td>
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<td>PstS1</td>
<td>98/30</td>
<td>-</td>
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<td>Agra, India</td>
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<td>ELISA, Stata-7 analysis</td>
<td>Ag85C</td>
<td>84</td>
<td>Kumar, 2008</td>
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<td>Beijing, China</td>
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<td>ELISA</td>
<td>Mtb48, Mtb11</td>
<td>90</td>
<td>Wu, 2010</td>
</tr>
</tbody>
</table>

Acr: 16 kDa, Rv0211c, hsp16.3 or X, -crystallin Ag85A (Rv3804c); Ag85C (Rv0129c): 30/32 kDa, mycolyl transferase; Glu-S: glucose synthase; MPT32: DPEP; MPT51: 27 kDa, Rv3803c; MPT64: 25 kDa, Rv1980; Mtb: NP214893; Mtb11: Rv3874, CFP-10, MtsA-10; Mtb48: AL02210, proline rich; Mtb81: 88 kDa, Rv1837c, malate synthase; PPE55: Rv3347c; PstS1: 38 kDa, Rv0934, antigen 5 or 78; Rv0222: enol-CoA hydrolyase.

* Smear-positive TB/PID +ve healthy/non-TB pulmonary symptoms.

** Proportion of TB patients positive above cut-off derived from PPD +ve healthy subjects, or/non-TB pulmonary patients from the same area; nt – not tested.

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used. Fine tuning of cut-off points can be achieved by testing different serum dilutions, i.e. antibody titrations. The use of OD values from a single serum dilution is cruder, although it satisfies the demand for labour and time efficiency.

In response to the demands for rapid diagnosis at the point-of-care, a number of diagnostic kits have been developed using the PstS1 antigen and Ag85. However, ‘all’ commercial kits have been criticised on the grounds of their performance which included poor sensitivity for smear-negative cases and was aggravated by publicity to profit-motivated abuse. New lateral flow techniques resulting in the precipitation of antigen-attacked gold particles, which have successfully been used for point-of-care HIV serology, seem worth consideration for TB serodiagnosis. These problems could be avoided by performing serology at professionally run laboratories. Good laboratory standards seem to be mandatory in achieving high sensitivity and reliability, as well as high-throughput testing of samples. These requirements are feasible to achieve operationally, are cost effective in district or regional laboratories, and are probably of overriding priority when compared with the benefits of the use of test kits at points-of-care. To achieve this goal, the worldwide supply of quality-controlled antigens and standardised ELISA procedures from commercial sources, would be required.

5. Alternative diagnostic techniques

The self-enclosed PCR based DNA amplification kit Xpert MTB/RIF (Cepheid, USA) is a rapid test, detecting also rifampicin resistance at high sensitivity for both pulmonary and extrapulmonary active TB. It has prominently been endorsed by WHO (http://whqlibdoc.who.int/publications/2011/9789241501545_eng.pdf) by quoting, that it is expected ‘to revolutionize the diagnosis of tuberculosis’. However, its currently high cost ($US20–55 thousand for the apparatus, $55 for the cartridges and $16 per test) could be a limiting factor, though the price could be subsidised or reduced in the future. Thus, serology might still compete on the grounds of lower perceived costs. Moreover, the daily performance of Xpert MTB/RIF two-module instrument is limited to only 20 tests, while in principle, hundreds of samples could be tested by ELISA.

Antigen detection techniques claimed time and cost advantages. Use of anti-LAM mAb for capture and polyspecific antibody could detect 10,000 organisms/ml of sputum. Dot-ELISA detected a 55 kDa antigen in serum at about 90% sensitivity in both pulmonary and extrapulmonary TB. A bacteriophage based test kit performed at higher sensitivity than smear microscopy for both sputum and urine. Rapid detection of LAM by reverse passive agglutination or glycolipids by a liposomal agglutination card test have been used for testing the cerebrospinal fluids. Importantly, sputum smear examination has recently been improved using LED microscopy.

Interferon gamma release assays (IGRA) specifically detect M. tuberculosis infection, with merely discrete differences between latent infection and active disease. Hence, calling IGRA results summarily as ‘diagnostics for tuberculosis’, can be misleading. Diagnosis of active disease may also be confounded by inhibition of IFN-γ production by the Th2 cytokines. Interestingly, T cell anergy, combined with high antibody responsiveness, has been particularly pronounced in some populations. Potentially, identifying those infected individuals who are most likely to develop active TB, including HIV-ve populations is of great interest.

Recently, a ‘telehealth’ approach has been explored for making an electronic diagnosis of smear –ve/culture –ve active TB. Digitalised images of X-rays and a brief clinical history obtained at remote locations in Pakistan and the Philippines were sent by Email to radiologist and pneumologist experts for evaluation. This approach speeded-up the process of diagnosis by 2–4 weeks when compared with face-to-face diagnosis by experts, and correlated well with the ‘gold standard’ sputum culture & 2 month follow-up evaluations.

6. What are the reasons for the late diagnosis of TB

Tuberculous infection is transmitted to susceptible contacts at home and in the community before TB is suspected and diagnosed. Late diagnosis delays contributes the greatest number of infectious days, it delays the onset of chemotherapy and makes its prognosis worse. The reasons for delayed diagnosis concern a number of socio-cultural factors, which associate with life in impoverished conditions, poor sanitation, and create confounding behavioural barriers against medical examination. Delays can relate to: poor perception of the cause of illness, the relevance of clinical symptoms, stigma and attitudes to females or older age in the family, distance from clinic and attendance to private clinic. As an example of frightful attitudes, 5.2% of sputum positive individuals in Pakistan defaulted even after diagnosis, hence treatment could not be started. In developed countries, the vulnerable populations (homeless, drug users, prison populations) in London with 20 times higher TB incidence than the general population showed chaotic behaviour, while in Arkansas, social behavioural factors influenced transmission more than the infectiousness of the source.

Health services in TB endemic regions rarely use sputum culture despite its superior sensitivity, while microscopy needs to be restricted to suspected cases with pronounced clinical symptoms. Health providers can be dysfunctional and confounded by profit motivation and the use of traditional healers, leads to circuitous help-seeking and referral patterns. In Kenya, 80% of patients reported relevant complaints on several occasions without their names recorded, before being diagnosed. Delay of diagnosis has been related to fractured communications between private doctors, health centres. Patients ‘shopping’ has been due to the perception that X-ray testing by private doctors is more effective and accessible than by public providers. As those in the lowest social hierarchy are more likely to neglect their health, access to free consultation by TB suspects seems mandatory for reducing the delay in diagnosis.

7. Which patients are infectious?

Patients with sputum-positive pulmonary TB are considered to be the key source of transmissible infection. Infection is transmitted by floating droplet nuclei and depends on their composition and on the cession and location of cavities in relation to bronchi. Vigour and frequency of coughing are essential, although sneezing, speaking and singing can also spread the infection. Thus individual variations in infectivity are not correlated only with the bacterial counts.

Patients with extrapulmonary or sputum smear negative forms of TB are a lesser hazard for transmitting infection, making the need for their diagnosis less urgent. The rate of conversion to smear-positivity in a proportion of cases, though most relevant to transmission, has been poorly documented. HIV-positive patients manifest either pulmonary disease, which mostly lacks cavitation, and consequently the sputum is smear negative, or often manifest extrapulmonary forms; hence, both instances represent only a very small risk for transmitting infection. Child TB is often of the pulmonary form and about 20% of cases are sputum-positive, but children under the age of 12 rarely produce sputum and therefore are less likely to disseminate the infection.

It is generally assumed that sputum-positive patients infect approximately 10 times more contacts, than smear-negative patients. Therefore, the incidence of infection (PPD conversion) is calculated from the prevalence of smear-positive cases, and their reduction is expected to reduce infectiousness.

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8. Could serological screening help to diagnose TB earlier than current procedures?

Harnessing serology to improve the current delays in the diagnosis of infectious TB appears to be a mandatory, yet so far an unacknowledged objective. Its importance for public health is represented by the potentials for reducing the transmission of infection to susceptible contacts, of whom about 5–10% is then expected to develop active TB. While the current diagnostic procedures ultimately do help save patients lives by therapy, they often come too late to prevent the transmission of infection to the susceptible contacts (Figure 1).

One of the causes of delayed diagnosis is reporting the symptoms only after they become aggravated. Thus, screening of suspects who report ‘mild’ symptoms could result in earlier diagnosis. Such extension of the initial screening could be sustained by facilities and cost for serology better than for other laboratory tests or chest X-ray. Obviously, seropositive suspects would need to be examined comprehensively by clinical and other laboratory tests to confirm the diagnosis.

Serology could be used for testing large numbers of samples. Low cost is a key factor, particularly when proposing an approximately 10-fold increase in the number of tests, using finger-prick blood samples, which can be easily transported on filter paper. Serology being amenable to automation is suitable to high throughput testing, in professionally equipped laboratories. They could attain satisfactory quality standards, which could not be achieved using kits intended for point-of-care use. This strategy is in accord with recent initiatives and expanded funding to laboratory testing facilities.91,92 It is acknowledged that evaluation and endorsement by the New Diagnostics Working Group of WHO has become necessary for acceptance to national TB control programmes.

There are limitations to the early detection of infectious cases by serology. If a proportion of sputum positive patients simply do not come to see a doctor then they can only be found by ‘active case-finding’, possibly involving some form of pre-selection of a community for testing. Although serology can be positive in a fraction of sputum-negative patients, it is not known if this fraction represents patients who will change over a period of time into sputum-positive infectious cases.

As most TB patients are poor, they should not need to spend money on getting the diagnosis. This is of particular concern for the several countries where the majority of diagnosed patients reported first seeking help from private doctors, rather than public health services, and where the pre-diagnostic out-of-pocket expenditure to patients is higher than the post-diagnostic expenditure.85 Reduction in the expenditure of patients for diagnosis has been suggested as an important approach for fighting the delay in diagnosis, and also on the grounds of facilitating a positive attitude towards the health provider. The economy factor should apply to the cost to patients for giving a blood sample, rather than to the cost of the laboratory test, which should come from public funds considering the benefits from reduced transmission and drug resistance for community health.

9. Prospective diagnostic trials

The potentials of serological screening for leading to early diagnosis need to be evaluated in controlled prospective trials. This is required, because almost all of the previously reported serodiagnostic studies, represented merely case-control surveys of sera from patients with a confirmed diagnosis. An exception is the study, which tested antibodies to PstS1, Mtbl1, ESAT-6, Acr and Mtbb1 in a cohort of 30 HIV-coinfected TB patients for up to 30 months prior to clinic and bacteriological diagnosis.93 Antibodies to PPE55(Rv3347c) were also found to be increased for an undefined period before diagnosis40 without documentation, if the suspects were infectious already at the time when the blood samples were drawn.

Geographical staging of diagnostic prospective trials would need to match the conflicting requirements of combining a population with high TB incidence, with sufficiently reliable clinical support and laboratory facilities. The positive predictive value of the screening test would be highly dependent from the prevalence of active TB in the tested population. Therefore, assuming at least 80% sensitivity and 95% specificity of the serological test, it would be necessary to carry out the trial in a test population of about 50,000 individuals with a higher than 100 per 100,000 incidence of active TB. These requirements could be met by studying selected populations in TB endemic regions or possibly in urban immigrant populations in developed countries.

The greatest diagnostic benefit would be for individuals with the lowest degree of suspicion. These would probably be individuals manifesting only mild/vague clinical symptoms, and consequently individuals who would not qualify for testing by the conventionally used clinical criteria. Their volume would not be feasible to test by the sputum smear, though could be accommodated by the high-throughput serology approach. When aiming to test suspects with mild clinical symptoms, defining a set of grading criteria could benefit from an active case finding study in Kenya.94 Extension from the 3-week cough, recommended by WHO to fewer, tiredness and chest pain, would increase the number of suspects considerably. These criteria are expected to differ greatly between countries, urban or rural populations, gender, and on the basis various social, economic, cultural or ethnic factors, which determine patient attitudes. Differences in local health services, and disparities between immigrant and resident populations, could also be significant. An important element of the trial should be the follow-up of individuals.
combined use in ELISA based serology can compensate for individual community. Hence, it retained as the gold standard of TB diagnosis in clinical medicine, aims for the cessation of tuberculosis in the community. 

Conclusions

The ethos of National Tuberculosis Programmes, unlike the goal of clinical medicine, aims for the cessation of tuberculosis in the community.

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Ethical approval:

References


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