Serodiagnosis of pericardial tuberculosis

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Summary

The AIDS epidemic has led to the resurgence of tuberculosis. Extrapulmonary manifestations may appear in over half of the patients who are dually infected. This has resulted in a rising incidence of tuberculous pericarditis in several parts of Africa such as Tanzania. We tested a solid-phase antibody competition sandwich ELISA (SACT-SE) as a potential means of diagnosing tuberculous pericarditis. Fifty-one African patients with clinically diagnosed tuberculous pericardial effusion (of whom 25 had confirmation by pericardial fluid culture) were tested using a monoclonal antibody (CDC/WHO ref. no. IT39) which was raised against a specific epitope on the Mycobacterium tuberculosis 30 kDa antigen. All but one patient had negative sputum microscopy for acid-fast bacilli. A sensitivity of 61% (at 96% specificity) was achieved. Sera from 25 African patients with smear-positive tuberculosis were also examined; of which 20 tested positive (sensitivity 80%). This is the largest study to date on the potential application of serology in diagnosing pericardial tuberculosis.

Introduction

The AIDS epidemic has led to the resurgence of tuberculosis (TB), both in the US and in Central Africa. The association of extrapulmonary tuberculosis with HIV infection has resulted in the rising incidence of pericardial disease in Africa, particularly in individuals who are dually infected with the HIV virus.1 The need for a rapid, simple and inexpensive test for pericardial TB has not been met so far. The diagnostic yields from the examination of sputum,2 pericardial fluid (smear/culture)2,3 and/or pericardium4 (histology) are far from satisfactory, and concomitant chest radiographic features of pulmonary TB are infrequent.2 Tuberculin skin testing is of little value in an African setting, due to the very high prevalence of primary tuberculosis, attempts at mass BCG immunization (which has occurred in Transkei) and the likelihood of cross-sensitization from atypical mycobacteria present in the environment. Polymerase chain reaction has been shown to be useful in detecting Mycobacterium tuberculosis DNA in pericardial fluid5 but the technique involved is labour-intensive and not yet suitable for large numbers of specimens. The frequent presence of blood in pericardial fluid in tuberculous effusion also leads to false negativity through enzyme inhibition.

By comparison, serological assays such as enzyme-linked immunosorbent assay (ELISA) are simple, and do not require a specimen from the site of disease, hence obviating the need for any invasive procedure. Of the purified species-specific protein antigens that have been evaluated in indirect ELISAs, the 38 kDa and 30/31 kDa antigens have been found to be most immunodominant in pulmonary tuberculosis.6 However, in the context of extrapulmonary disease, these antigens are insensitive in this test format. Greater sensitivity while preserving specificity has been achieved with solid-phase antibody competition sandwich ELISA (SACT-SE) based on monoclonal antibodies (MAbs) that are directed against specific epitopes on the 38 kDa antigen.7,8
We screened a panel of five MAbs (directed against the 38 kDa and 30/31 kDa antigens) for affinity to *M. tuberculosis* soluble extract (MTSE). The antibody showing the highest affinity (CDC/WHO reference no. IT39, an epitope-specific MAb against the 30 kDa antigen) was then evaluated in SACT-SE using sera from patients with pericardial and smear-positive pulmonary TB as well as appropriate controls.

**Methods**

**Patients and sera**

Sera from a total of 98 patients were tested. Among these, 51 (from a previously reported clinical trial) had clinically diagnosed tuberculous pericardial effusion (25 culture positive for *M. tuberculosis*; 26 with typical features and response to specific anti-TB therapy); 25 had smear-positive pulmonary TB, and 22 were non-TB controls. These sera were collected from Umtata Hospital, Transkei. The controls had diseases simulating tuberculosis (unspecified) and no history of recent or remote TB. All patients were HIV-1 and HIV-2 seronegative by Wellcome recombinant HIV-1 EIA and Biokit HIV 1 +2 ELISA. All TB sera were collected before the institution of antituberculous therapy. Sera were stored in aliquots at −20 °C and tested blind to eliminate operator bias.

**Materials**

The MTSE and two of the MAbs screened (TB71 and TB72; epitopes on the *M. tuberculosis* 38 kDa antigen) were generously provided by Professor J. Ivanyi (MRC Tuberculosis and Related Disease Unit, Hammersmith Hospital, UK). The remainder three MAbs (CDC/WHO reference nos. IT21, IT37 and IT39) were kindly provided by the UNPD/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

**Solid-phase antibody competition sandwich ELISA (SACT-SE)**

Flat-bottom flexible, polyvinyl microtitre plates (Falcon 1413 Microtest III; Becton Dickinson) were coated with 50 μl per well of 50 μg/ml MTSE in phosphate-buffered saline (PBS) overnight at 4 °C. After washing once with PBS containing 0.5% Tween 20 (BDH) (PBST), the wells were incubated with 200 μl 2% skimmed milk in PBST at 37 °C for 1 h. The liquid was tipped off and the plates were patted dry. Four dilutions (1:5, 1:25, 1:125, 1:625) of human sera in 3% bovine serum albumin solution containing 0.2% Tween (BSA-Tween), each 25 μl, were added to duplicate wells and incubated for 1 h at 37 °C. With no washing step, 25 μl per well of MAb IT39 (in ascitic fluid) diluted in BSA-Tween (at a concentration which gave 90% of maximum binding in wells without competing serum i.e. high control; 1/1000 in our assay) were added. The plates were shaken on an Orbital shaker (Stuart Scientific) for 30 s and incubated for 1 h at 37 °C. After five washes, 50 μl per well of affinity isolated Fc-specific goat anti-mouse IgG peroxidase conjugate (Sigma), diluted in BSA-Tween to 1/1000 were added and the plates incubated for 1 h at 37 °C. After a further five washes, the plates were dried and 50 μl per well of 2′2′-azinobis(3-ethylbenzthiazoline sulphonic acid) (ABTS) solution (0.5 mg/ml ABTS in phosphate-citrate buffer, pH 4 with 0.01% hydrogen peroxide) were added. The A405 nm was read (40 min after the addition of ABTS) in a Titertek Multiscan spectrophotometer. Antibody titres were calculated as the dilution of serum causing 50% inhibition of the MAb binding by comparison with the high control (IDso). Antibody titres of <5 were calculated by extrapolation from linear regression lines derived from the four serum dilutions (r>0.9 in most cases). All titres of <1 were treated as zero, and the cut-off titre was taken as the mean of all non-TB control titres + 2 SD.

**Statistical analysis**

We used the SPSS/PC+ Statistics 4.0 package as specified in the results section. Curve-fitting by linear regression was done using Fig.P (Biosoft).

**Results**

See Figure 1.

**Non-TB controls**

The cut-off titre was 8.9 (1.3 + 2 × 3.8). Of the 22 controls tested, one was found to have an IDso titre (17) above cut-off, giving a specificity of 96%.

**Tuberculous pericarditis patients**

The mean IDso titre was 24 (range 0–189; significantly different from that of controls: p < 0.001 by the Mann-Whitney test). The overall sensitivity was 61% (31/51) which increased to 64% (16/25) within the subset of patients who had microbiological confirmation, i.e. positive culture from pericardial fluid. Based on the results of pericardial fluid culture as the reference test, a positive serology had a positive predictive value (ppv) of 0.94 and negative predictive value (npv) of 0.7 within the study population.

Nineteen (37%) patients subsequently developed signs of pericardial constriction despite standard anti-tuberculosis therapy (time to development of...
ELISA serology for pericardial TB

Constriction ranged from 2 weeks to 4 months, mean 5.7 weeks). There was no correlation between positive serology and progression to constriction (Pearson χ² 1.07, p=0.59) or pericardial fluid culture positivity (Pearson χ² 0.97, p=0.62).

All but one had negative sputum microscopy for acid-fast bacilli. Five patients (10%) (including the smear-positive case) also had positive sputum cultures for *M. tuberculosis* with a mean ID₅₀ titre of 28.6 (range 0–91).

Smear-positive pulmonary TB

The mean ID₅₀ titre was 32 (range 0–154; significantly different from that of controls: p<0.001 by the Mann-Whitney test). The overall sensitivity was 80% (20/25) with a ppv of 0.95 and npv of 0.81. The difference between the mean ID₅₀ titres of the pericardial (24) and pulmonary TB (32) groups was not significant (p=0.08 by the Mann-Whitney test). The difference in seropositivity between pericardial and pulmonary disease was also analysed by Pearson χ² and found not to be significant (χ² 2.8; p=0.09).

Discussion

This competition assay based on MAb IT39, an epitope-specific antibody to the 30/31kDa antigen, had an overall sensitivity of 61% in pericardial TB (ppv 0.94, npv 0.7) and 80% (ppv 0.95, npv 0.81) in smear-positive pulmonary TB at a specificity of 96%. These results raise several issues.

First, the test has diagnostic application. Patients in this study came from a previously published clinical trial which involved a total of 240 patients with active tuberculous pericardial effusion. The microbiological profiles of our subset of patients were similar to those published in the original trial, and illustrated the difficulties involved in establishing a rapid, definitive diagnosis in these patients. For instance, 11% (21/184) of patients in the trial had positive sputum cultures for *M. tuberculosis* (cf. 10% among our patients) while 59% (111/189) had positive pericardial fluid cultures. Only one patient (2%) in our study had detectable acid-fast bacilli in sputum on microscopy. Chest radiographic features of active pulmonary tuberculosis were present in only 30% of patients and tuberculin skin testing had little diagnostic value for reasons already mentioned. Where pericardial fluid (or biopsy) may not be obtainable, the competitive assay has the potential to offer an alternative means of diagnosis which is simple, rapid and non-invasive.

The sensitivity achieved with this assay in pericardial tuberculosis is comparable to those (56–78% at 97% specificity) published for the MAb-TB72-based competition assay in extrapulmonary (non-pericardial) infections. Twenty-eight of the pericarditis sera in this study were also tested using the MAb TB72 assay; only three (11%) had an ID₅₀ titre above cut-off, illustrating the relative immunodominance of the 30/31 kDa antigen in pericardial disease (data not shown). On the contrary, an indirect ELISA based on the whole *M. tuberculosis* 30/31 kDa

Figure 1. Results of SACT-SE with MAb IT39 in patients with pericardial TB (PC), pulmonary TB (PTB) and controls. Negative cut-off titre indicated by a broken horizontal line.
antigen was relatively insensitive in extrapulmonary (miliary, pleural and lymphatic) tuberculosis. Tuberculous pericarditis is an inflammatory reaction initiated by the rupture of mediastinal or paratracheal lymph node into the pericardial sac where active proteolytic degradation and antigen processing (into individual peptides) of the mycobacterial detritus take place. It is therefore possible that a significant proportion of anti-30/31 kDa antibodies in the sera from patients with extrapulmonary disease are directed to sequential (rather than conformational) epitopes such as that defined by MAb IT39.

The smear-positive pulmonary TB patients in our study had on average higher seropositive rate as well as antibody titres than those with pericarditis. Our preliminary data (not presented) showed that the whole 38-kDa-binding antibody titres in multibacillary pulmonary TB were also, on average, five times higher than in pericardial TB. The apparent site-dependence of antibody response may however be due to the difference in bacillary load in sputum (hence smear positivity) between the two presentations.

This site-dependent property of humoral response in TB has also been demonstrated by other epitope-directed competition assays. For instance, in primary tuberculosis, the MAb-TB68-identified epitope (on the 14 kDa antigen) appears to be more important than the 38-kDa-directed probes, and this occurs similarly in the cerebrospinal fluid of patients with TB meningitis.

In summary, this is the first study documenting the potential value of serology in the diagnosis of tuberculous pericarditis. We have shown that an assay measuring the serum antibody titres against the IT39-defined epitope may be used to diagnose tuberculous pericarditis. Studies are being undertaken to evaluate its use in the context of HIV infection as well as other forms of extrapulmonary tuberculosis, smear-negative pulmonary disease and childhood TB.

References


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