

Clinical value of the measurement of *Mycobacterium tuberculosis* specific antibody in pulmonary tuberculosis

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Abstract

Background A serological test that could help to diagnose tuberculosis, especially smear negative disease, would contribute to patient management.

Methods Levels of antibody to distinct antigens of *Mycobacterium tuberculosis* were assessed for their value in the diagnosis and management of pulmonary tuberculosis. Serum was taken from 52 patients who were smear positive, from 27 patients who were smear negative but with evidence of active tuberculosis (sputum culture positive in 16, response to antituberculosis chemotherapy in 11), from 11 patients with old healed tuberculosis (pre-antibiotic era), and from 39 healthy subjects vaccinated with BCG.

Results In smear positive tuberculosis an enzyme linked immunosorbent assay using a single 38 kDa antigen gave a diagnostic sensitivity of 80% with a 100% specificity. In smear negative pulmonary tuberculosis, however, combination of the 19 kDa antigen, lipoarabinomannan (ML 34 epitope), and hsp 65 (TB 78 epitope) was needed to achieve a sensitivity of 64% with a specificity of 95%. Recurrent and extensive radiographic disease with a poor prognosis was associated with high anti-38 kDa and low anti-14 kDa antibody levels in patients with active disease. Patients with less pulmonary cavitation had high anti-19 kDa titres. Bacteriological relapse during treatment was indicated by a rise in anti-14 kDa (TB68 epitope) antibodies. Four patients with non-tuberculous mycobacterial infection showed no anti-38 kDa antibody.

Conclusion Antigen or epitope specific serology may help in the diagnosis, assessment of prognosis, and monitoring of chemotherapy in patients with pulmonary tuberculosis.

Tuberculosis remains an important health problem in the United Kingdom.^{1,2} The decline in incidence has slowed³ and diagnosis has become more difficult because of atypical presentations and a lack of familiarity with the disease among doctors.⁴ The mortality from tuberculosis has remained constant for the past decade at about 10%¹ (A Fenton-Lewis, personal communication). The diagnosis of

tuberculosis is readily made when a sputum smear contains acid fast bacilli. Subsequent culture will usually identify those patients with tuberculosis and those infected with other mycobacterial species after a delay of three to nine weeks. Clinical features that identify patients with a risk of greater morbidity include increasing age, alcoholism, neutrophilia and lymphopenia, and delayed diagnosis.^{5,6} Severe or extensive disease is associated with a reduced tuberculin response.⁷ Short course chemotherapy for tuberculosis still lasts for six months and poor compliance with treatment is a common problem.⁸⁻¹⁰ A serological test capable of diagnosing smear negative tuberculosis, and also cases in which sputum is difficult to obtain, would be very helpful.

The usefulness of determining antibodies in tuberculosis has been largely dismissed since Mitchison *et al*¹¹ could not reproduce the results of one such assay.¹²⁻¹³ Serological tests using crude antigens derived from tubercle bacilli have been unsuccessful owing to the broad cross reactivity of mycobacterial antigens.¹⁴ This problem has been overcome by the development of monoclonal antibodies that recognise antigenic determinants or epitopes specific for the *Mycobacterium tuberculosis* complex.¹⁵ These monoclonal antibodies have been used to measure levels of antibody to specific epitopes¹⁶ and to purify antigens for use in diagnostic assays.¹⁷⁻¹⁹ This study examined the diagnostic value of *M tuberculosis* specific antibody and its association with the extent of disease, prognosis, and compliance with chemotherapy to investigate whether serological tests might have a role in the management of patients with pulmonary tuberculosis.

Methods

PATIENTS

1 Serum was obtained from 56 patients with acid fast bacilli detected in their sputum before the start of treatment; in 52 patients *M tuberculosis* was cultured from sputum, six strains being resistant to isoniazid. In four patients non-tuberculous mycobacteria were identified. Fourteen of the smear positive patients had had tuberculosis before but had been free from disease for at least two years before the present episode. Thirty one of the patients with a positive smear were followed during treatment and serum samples were obtained seven, 14,

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and 28 days and two, three, four, and six months after the start of treatment.

2 Serum samples were also obtained from 27 patients whose sputum smear and bronchial washings had shown no acid fast bacilli; in 16 *M. tuberculosis* was subsequently identified by culture and the other 11 were treated on the basis of radiographic appearance alone and showed a good symptomatic and radiological response to antituberculous chemotherapy after non-tuberculous antibiotic treatment had failed.

Eleven patients with smear positive and three with smear negative tuberculosis had received BCG vaccination.

3 Eleven further patients had a documented history of smear positive tuberculosis before the introduction of chemotherapy and had recovered without antibiotic treatment; repeated sputum samples in this group were consistently culture negative for *M. tuberculosis*.

4 Control serum was obtained from 39 healthy individuals vaccinated with BCG.

Radiographs were obtained before the start of treatment in all patients in addition to a full blood count and erythrocyte sedimentation rate. The radiographic extent of disease was assessed by dividing each lung field into three zones and giving a score of 1 for infiltration or 2 for infiltration and cavitation. A clinical scoring system⁶ designed to predict mortality from tuberculosis was evaluated and compared with serological criteria.

MONOCLONAL ANTIBODIES

We used five monoclonal antibodies of restricted species specificity, TB68 (14 kDa antigen), TB23 (19 kDa antigen), TB71 and TB72 (38 kDa antigen) and TB78 65 kDa antigen), and one (ML34) that binds to lipoarabinomannan, a glycolipid common to mycobacteria.¹⁵⁻¹⁷

SOLID PHASE ANTIBODY COMPETITION TEST

Wells of polyvinyl flexible microtitre plates (Dynatech, Billingshurst, West Sussex) were coated with 50 μ l of a soluble extract of *M. tuberculosis* H37Rv 30 μ g/ml overnight at 4°C. Non-specific binding was prevented by incubation with 3% bovine serum albumin for one hour at 20°C. Four dilutions of serum (1:5, 1:25, 1:125, and 1:625) were prepared in 3% bovine serum albumin and 25 μ l incubated in duplicate wells at 20°C for four hours. Without being washed, 25 μ l of ¹²⁵I labelled monoclonal antibody, diluted to give 1000–2000 cpm when incubated in wells without competing serum (the high control), was added to each well and incubated overnight at 4°C. After repeated washing wells were counted in an LKB 1260 Multigamma counter. Antibody titres were calculated as the dilution of serum causing 50% inhibition of binding of the labelled antibody (ID₅₀) by comparison with the high control after non-specific binding to wells coated with phosphate buffered saline had been subtracted from all values.¹⁸ This method measures antibody with the same or overlapping specificity as the monoclonal antibody without reference to class; early IgM responses are detected as readily as later IgG ones.

ENZYME LINKED IMMUNOSORBENT ASSAY WITH PURIFIED ANTIGENS

The 14, 19, and 38 kDa antigens were purified by affinity chromatography, with the monoclonal antibodies TB68, TB23, and TB71.¹⁹ The 65 kDa antigen, a recombinant protein, and lipoarabinomannan were also used. Microtitre plates (Immulon M129, Dynatech) were coated with 50 μ l of 0.2 μ g/ml 14 or 19 kDa antigens, 0.35 μ g/ml 38 kDa antigen, 1 μ g/ml 65 kDa antigen, 0.1 μ g/ml lipoarabinomannan or with phosphate buffered saline overnight at 4°C. Non-specific binding was blocked with phosphate buffered saline containing 0.05% Tween 20 (Sigma Chemical Company, Poole, Dorset) and 1% skimmed milk (Sainsbury PLC: PBSTM). Fifty microlitres of four dilutions of serum (1:5, 1:25, 1:125, and 1:625) in PBSTM were incubated for one hour at 37°C in duplicate wells and after repeated washing 50 μ l of goat anti-human IgG peroxidase conjugate (Bio-Rad Laboratories) diluted 1:3000 were incubated for one hour at 37°C. After washing, bound peroxidase activity was measured with 0.1 μ g/ml tetramethylbenzidine in 0.1 M citrate buffer pH 5.1 containing 0.01% hydrogen peroxide (v/v) for 10 minutes, the reaction being stopped by the addition of 0.5 M sulphuric acid. Optical densities were measured at 450 nm and the antibody titre was calculated as that dilution of serum giving 30% of the plateau of a standard hyperimmune serum (ABT₃₀).

STATISTICAL ANALYSIS

Comparisons between groups were made by using the Mann-Whitney U test. Antibody titres and clinical measures were compared by means of the Spearman rank correlation test. Antibody titres within the same group were compared by using Student's *t* test. Values of *p* were corrected for the number of tests (*n*) by using the formula $p_{\text{corr}} = 1 - (1 - p)^n$. Positive antibody titres were defined as those greater than log mean + 2 SD of control values and the frequency of positive titres was compared by the χ^2 test. The sensitivity, specificity, and negative and positive predictive values were calculated by the method of Toman.²⁰

Results

DIAGNOSTIC VALUE OF SEROLOGY

Patients with smear positive pulmonary tuberculosis had raised titres of antibody to all the antigens and epitopes tested, but the response in smear negative disease was more restricted (table 1). The frequencies of positive titres of antibody in smear positive and smear negative patients to the 19 kDa antigen (61% and 58%) and ML34 epitope (54% and 32%) did not differ (table 1; $\chi^2 = 0.01$ and 2.6, *p* > 0.1). A combination of positive titres of antibody to these two antigenic determinants and to the TB78 epitope gave the best diagnostic sensitivity in patients with smear negative pulmonary tuberculosis (table 2). A positive antibody titre in patients with smear negative, culture positive pulmonary tuberculosis was obtained on average 36 (median 33, range 19–

Table 1 Antibody titres in controls and patients with pulmonary tuberculosis

Antigen or epitope†	Antibody titres (median (range Q1-3) : % positive‡)			
	Controls (n = 39)	Self healed (n = 11)	Smear negative (n = 27)	Smear positive (n = 52)
14 kDa TB68	1 (0-3); 5 4 (3-6); 0	23 (6-43); 36 6 (5-11); 11	25 (1-40)**; 46 6 (3-12); 11	78 (12-241)**; 72 14 (9-21)**; 50
19 kDa TB23	0 (0-4); 3 6 (3-9); 0	11 (0-49)*; 55 8 (4-9); 0	12 (2-25)**; 58 5 (3-12); 4	41 (4-106)**; 61 17 (8-36)**; 46
38 kDa TB71 TB72	0 (0-0); 0 1 (0-2); 0 2 (1-4); 3	0 (0-0); 9 2 (1-2); 0 4 (1-5); 0	0 (0-0); 15 2 (1-4)**; 11 5 (2-8); 18	60 (12-287)**; 83 7 (2-26)**; 56 24 (8-67)**; 73
65 kDa TB78	79 (23-181); 0 0 (0-1); 0	317 (37-514); 18 1 (0-4); 18	107 (19-290); 0 1 (0-4); 14	172 (71-566)*; 11 3 (1-5)**; 48
LAM LAM (IgM) ML34	48 (3-127); 0 10 (0-25); 0 15 (10-20); 3	9 (0-307); 0 0 (0-12); 0 18 (14-30); 11	69 (31-440); 19 29 (4-63)*; 0 18 (12-46)*; 32	1013 (165-3125)**; 40 34 (7-108)**; 11 41 (25-126)**; 54

Patients were compared with controls by the Mann-Whitney U test: *p < 0.01; **p < 0.001.

†Antibodies to purified antigens were IgG unless indicated otherwise; antibodies to individual epitopes were not class specific.

‡Positive titres were defined as > log mean + 2SD of control values.

63) days before the results of culture were available.

Although the frequencies of positive titres of antibody to the 38 kDa antigen and its TB72 epitope did not differ, the use of lipoarabinomannan as a diagnostic reagent gave poorer results than the ML34 competition test (table 1). More than half (54%) the patients with smear negative tuberculosis had an IgM antibody titre greater than the titre of IgG antibody to lipoarabinomannan, compared with 20% of those with smear positive disease ($\chi^2 = 5.5$, $p = 0.04$).

EXTENT AND SEVERITY OF DISEASE

Survival

Four deaths occurred in patients with smear positive pulmonary tuberculosis, tuberculosis being the main cause in three. Titres of antibody to the TB71 epitope (38 kDa antigen) were greater ($t = 3.04$, $p = 0.03$) and of IgG antibody to the 14 kDa antigen were lower ($t = -3.26$, $p = 0.03$) in the four who died than in survivors (figure).

No patient was identified as being likely to die from tuberculosis on the basis of clinical

criteria,⁶ though a more unfavourable score was associated with lower anti lipoarabinomannan IgM antibody ($r = 0.43$, $p = 0.002$, $p_{\text{corr}} = 0.03$). Alcoholism and increasing age were associated with death ($\chi^2 = 8.75$, $p < 0.01$ and 0.002 respectively): 15 patients with smear positive tuberculosis were over 65 or had an alcohol problem and three of these died during treatment. Serological criteria (TB71 titre > 25, or < 25 with a titre of < 11 for the 14 kDa antigen), however, would have been significantly better than clinical criteria for predicting mortality ($\chi^2 = 5.6$, $p = 0.02$).

Radiographic extent of disease

Despite the crude scoring of the extent of radiographic disease there were correlations with anti-38 kDa antibody levels in smear positive tuberculosis ($r = 0.39$, $p = 0.005$, $p_{\text{corr}} = 0.049$) and with anti-19 kDa and anti lipoarabinomannan antibody titres in smear negative disease (for both $r = 0.50$, $p = 0.004$, $p_{\text{corr}} = 0.039$). There was a negative correlation between cavitation and titres of antibody to the 19 kDa antigen ($r = -0.32$, $p = 0.017$, $p_{\text{corr}} = 0.05$) in those with smear positive tuberculosis.

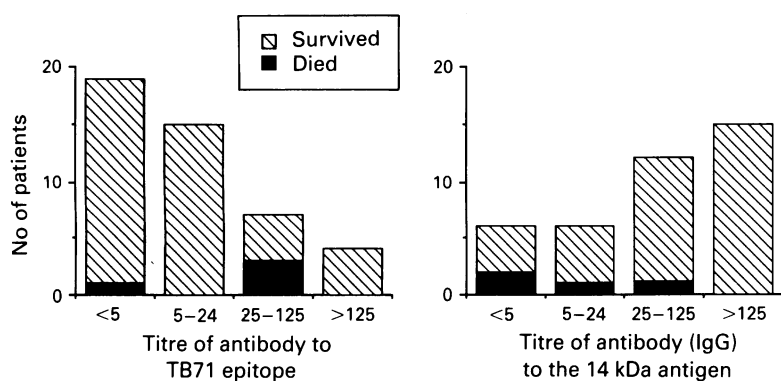
Table 2 Serodiagnostic potential of Mycobacterium tuberculosis specific reagents

Test	Sensitivity (%)	Specificity (%)	Predictive value* (%)	
			+	-
SMEAR POSITIVE PULMONARY TUBERCULOSIS				
Smear	100	94	71	100
Chest radiograph†	41	99	93	94
Tuberculin‡	87	83	43	98
38 kDa antigen	80	100	100	97
TB72 epitope	73	100	100	96
Six epitopes	90	92	62	99
SMEAR NEGATIVE PULMONARY TUBERCULOSIS				
38 kDa antigen	15	100	100	90
19 kDa antigen	58	97	74	95
ML34 epitope	32	100	100	92
19 kDa/ML34/TB78	72	95	65	96

*Predictive values have been calculated with Toman's formulae and data from the London Chest Hospital, where 1462 individuals were culture positive for *M. tuberculosis* out of 11 455 patients tested during 1969-88.

†Data from Toman.²¹

‡Data from Chaparas *et al.*²²



Relation between prognosis and titres of antibody to the TB71 epitope (38 kDa antigen) and 14 kDa protein antigen in patients with pulmonary tuberculosis. The titres of antibody to the TB71 epitope in the three patients whose tuberculosis was the main cause of death were 80, 38, and 0 and to the 14 kDa antigen 63, 11, and 4, compared with 27 and 4 respectively in the patient whose tuberculosis was merely a contributory factor in his death.

Previous disease

Patients who had a recurrence of tuberculosis had higher titres of antibody to both epitopes (TB71 and TB72) of the 38 kDa antigen than patients with their first infection with tuberculosis ($p = 0.01$ and 0.03 respectively). In contrast, patients with old (pre-antibiotic) healed tuberculosis that had been smear negative for more than 40 years had high titres of antibody to the 14 and 19 kDa antigens (table 1) and showed a negative correlation between the area of pulmonary scarring visible on the chest radiograph and titres of antibody to the 14 kDa antigen ($r = -0.75$, $p = 0.007$, $p_{\text{corr}} = 0.048$).

ANTIBODY TITRES AND TREATMENT IN SMEAR POSITIVE TUBERCULOSIS

Culture positivity during treatment

After two months of antituberculous chemotherapy sputum samples from most patients were smear and culture negative; in four patients with extensive pulmonary cavitation a positive smear persisted for up to four months but culture for *M. tuberculosis* was negative after two months; thus treatment was effective in all patients. In five patients a positive culture occurred during treatment after a period of negative cultures. There was no change in the antibiotic sensitivities of the cultured organism in any of these cases, suggesting that the patient had failed to continue treatment. All five patients, none of whom had a positive antibody

titre for the TB68 epitope of the 14 kDa antigen when they had become smear and culture negative, developed a positive titre of antibody to this epitope at the time that the culture positive sputum was obtained. Titres of antibody to the TB68 epitope were significantly greater in those who became culture positive than in those who did not during the same periods (table 3).

Isoniazid resistance

Isoniazid resistant strains of *M. tuberculosis* were grown from six patients, four of whom had recurrent tuberculosis. Titres of antibody to all the epitopes except for TB23 rose more slowly after treatment in these patients than in those from whom fully sensitive strains were cultured; significant differences were observed at two weeks (in all cases $t < -2.3$, $p < 0.04$). Titres of antibody to the TB72 epitope of the 38 kDa protein at two weeks had fallen below pretreatment levels in all subjects with isoniazid resistant strains, a fall that occurred in only two patients with fully sensitive strains of *M. tuberculosis* ($\chi^2 = 4.2$, $p = 0.04$).

Non-tuberculous mycobacteria

Four patients were infected with atypical mycobacteria (one with *M. avium-intracellulare*, one with *M. kansasii*, and two with *M. xenopi*). One patient had a myeloma, but the other three had no evidence of immunosuppression and all were negative for HIV antigen and antibody. Two patients had weakly positive titres of antibody (2-3 SD above the log mean of control values—to the TB68 and TB78 epitopes respectively) at the start of treatment; none was positive for the TB71 and TB72 epitopes of the 38 kDa antigen. No patient with atypical mycobacterial infection had a positive titre of antibody to any of the six epitopes two weeks after treatment had begun.

Discussion

This study has shown that *M. tuberculosis* restricted antigens can be used in the serological diagnosis of pulmonary tuberculosis. The serological test was as sensitive as but more specific than the use of tuberculin and could distinguish non-tuberculous mycobacterial disease from tuberculosis by the absence of anti-38 kDa antibody and no rise in levels of antibody to other *M. tuberculosis* specific epitopes and could detect patients with smear negative pulmonary tuberculosis before the results of sputum culture were available. In smear positive pulmonary tuberculosis, most patients had high titres of antibody to the 38 kDa antigen, confirming the results of previous studies.^{18-19, 23-24} The 38 kDa antigen is also contained in the antigen 5 preparation²⁵⁻²⁶ and is of value in serological diagnosis and screening for infectious tuberculosis.²⁷⁻³² Serological tests in smear negative pulmonary tuberculosis have been disappointing.³³⁻³⁵ Our study is the first to show that a combination of antigens can achieve a sensitivity in smear negative pulmonary tuberculosis similar to that

Table 3 Antibody titres in five patients considered to be non-compliant with treatment

Patient No	Relative antibody titre†					
	TB23	TB68	TB71	TB72	TB78	ML34
10	1.19	1.62	1.50	0.75	0.84	0.81
15	2.31	4.00	1.64	1.08	2.00	1.32
34	2.09	13.30	8.14	2.41	2.50	2.82
140	1.37	7.86	2.05	1.22	1.05	1.17
163	1.50	1.67	2.45	2.95	0.50	1.79
Other patients*	1.68	1.19‡	2.17	2.17	1.80	1.58

*Mean relative antibody titres for the same periods in patients who did not become culture positive again during treatment.

†Ratio between antibody titre when sputum was culture positive and the titre of the preceding sample, when sputum had been culture negative.

‡Significantly less than the mean value for the five patients who had become culture positive during treatment (Student's t test; $t = -3.25$; $p = 0.03$).

Table 4 Positive (+) and negative (-) associations between antibody specificity and clinical feature (associations with smear negative disease in bold type)

Clinical feature	Antigen (epitope)			
	14 kDa (TB68)	19 kDa (TB23)	38 kDa (TB71, TB72)	LAM (ML34)
Diagnosis	NS	+	+	+
Poor prognosis	-*	NS	+	-
Recurrent disease	-	-	+	NS
Radiographically extensive disease	-	+	+	+
Cavitation	NS	-	NS	NS
Non-compliance	+†	NS	NS	NS
Isoniazid resistance	NS	NS	-‡	NS

*A poor prognosis was associated with low titres of IgG antibody to the 14 kDa antigen but not of antibody to the TB68 epitope.

†Non-compliance was associated with a rise in the titre of antibody to the TB68 epitope.

‡Isoniazid resistance was associated with a fall in the titre of antibody to the TB72 epitope.

LAM—lipoarabinomannan; NS—no significant association.

of tuberculin testing but with greater specificity.

The use of monoclonal antibodies is an advance in the serological diagnosis of tuberculosis because they are readily prepared in a standard form, are free from the problems of cross reactivity, permit the measurement of both IgM and IgG antibodies in a single assay, and obviate the need to purify antigens from *M tuberculosis*. Indeed, the TB72 competition assay fared as well as the use of purified 38 kDa antigen in the serodiagnosis of smear positive tuberculosis. An ELISA modification of the competition assay³⁶ can give a result in four hours with precoated plates and has been used successfully in the diagnosis of extrapulmonary tuberculosis.³⁷ The cost of such an assay would be similar to that of an autoantibody screen. In smear negative pulmonary tuberculosis, however, purified 19 kDa antigen will be needed until the epitope, which is not that defined by the MAB TB23, has been identified.

High titres of antibody to the 38 kDa antigen were associated with more extensive and recurrent tuberculosis and increased mortality as well as being largely confined to those with smear positive disease whereas antibody to the 14 kDa antigen was associated with a good prognosis and less radiographic infiltration in patients with self healed pulmonary tuberculosis (table 4). Antibody titres for these two antigens were more successful than a scoring system based on clinical criteria,⁶ or the factors of advanced age and alcoholism, in predicting who would be at greatest risk from tuberculosis. Inpatient treatment of tuberculosis is increasingly rare and the identification of patients at greatest risk would be valuable in deciding which patients require hospital admission.

The treatment of tuberculosis is necessarily prolonged and compliance is a problem. This study suggests that titres of antibody to the TB68 epitope of the 14 kDa antigen could be used to monitor compliance by indicating those who had become culture positive again; such patients might then electively receive supervised chemotherapy. Isoniazid resistant strains of *M tuberculosis* were associated with a failure of antibody titres to rise after two weeks' treatment; adding other drugs to the standard regimen of rifampicin, isoniazid, and pyrazin-

amide could be considered in such patients. Non-tuberculous mycobacterial infections were associated with an absence of anti-38 kDa antibody despite a positive sputum smear. *M kansasii* and *M xenopi* are the two most commonly isolated non-tuberculous mycobacterial species in the London area³⁷ and are usually resistant to pyrazinamide and isoniazid. While bacteriological confirmation is awaited, this group of patients could also receive ethambutol or streptomycin, or both—drugs recommended for the treatment of non-tuberculous mycobacterial infections³⁸⁻⁴⁰—in addition to standard triple chemotherapy.

The measurement of titres of antibody to *M tuberculosis* specific antigens may be of value in the diagnosis of both smear positive and smear negative pulmonary tuberculosis. Antibody titres may also help to determine which patients should be treated in hospital, which require supervised chemotherapy, and which might require the addition of other drugs to the standard regimen for the treatment of tuberculosis. This study has established which antigens or their epitopes might be of value in the management of patients with tuberculosis, together with cut off titres. Prospective evaluation of the role of serology, comparing patients whose antibody titres affected clinical management with patients where antibody levels were not known, together with a cost-benefit analysis, is essential before widespread use of these tests can be recommended.

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- Horne NW. Problems of tuberculosis in decline. *BMJ* 1984;288:1249-51.
- Cook GC. Tuberculosis—certainly not a disease of the past! *QJ Med* 1985;56:519-21.
- Davies PDO. The slowing of the decline in tuberculosis notifications and HIV infection. *Respir Med* 1989;83:321-2.
- McNichol M. Trends in the epidemiology of tuberculosis—a physician's view. *J Clin Pathol* 1983;36:1087-90.
- Humphries MJ, Byfield SP, Derbyshire JH, Davies PDO, Nunn AJ, Citron KM, et al. Deaths occurring in newly notified patients with pulmonary tuberculosis in England and Wales. *Br J Dis Chest* 1984;78:149-58.
- Barnes PF, Leedom JM, Chan LS, Wong SF, Shah J, Vachon LA, et al. Predictors of short-term prognosis with pulmonary tuberculosis. *J Infect Dis* 1988;158:366-71.
- Howard WL, Klopfenstein MD, Steinmenger WJ, Woodruff CE. The loss of tuberculin sensitivity in certain patients

- with active pulmonary tuberculosis. *Chest* 1970;57:530-4.
- 8 British Thoracic Society. A controlled trial of 6 months' chemotherapy in pulmonary tuberculosis. Final report: results during the 36 months after the end of chemotherapy and beyond. *Br J Dis Chest* 1984;78:330-6.
 - 9 American Thoracic Society and the Centers for Disease Control. Treatment of tuberculosis and tuberculosis infection in adults and children. *Am Rev Respir Dis* 1986;134:355-63.
 - 10 Committee on Treatment of the International Union against Tuberculosis and Lung Disease. Anti-tuberculosis regimens of chemotherapy. *Bulletin of the International Union Against Tuberculosis and Lung Disease* 1988;63:60-4.
 - 11 Mitchison DA, Aber VR, Ahmed FJ, Allen BW, Devi S. Evaluation of a serological test for tuberculosis. *BMJ* 1977;i:1383-7.
 - 12 Nicholls AC. A serodiagnostic test for tuberculosis. *J Clin Pathol* 1975;28:850-3.
 - 13 Nicholls AC, Horsefield K. Serological diagnosis of tuberculosis; a report of 12 months' clinical experience. *Thorax* 1976;31:289-93.
 - 14 Grange JM. The humoral immune response in tuberculosis: its nature, biological role and diagnostic usefulness. *Adv Tuberc Res* 1984;21:1-78.
 - 15 Coates ARM, Hewitt J, Allen B, Ivanyi J, Mitchison DA. Antigenic diversity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* detected by means of monoclonal antibodies. *Lancet* 1981;ii:167-9.
 - 16 Ivanyi J, Morris JA, Keen M. Studies with monoclonal antibodies to mycobacteria. In: Macario AJL, Conway de Macario E, eds. *Monoclonal antibodies against bacteria*. Vol 1. New York: Academic Press, 1985:59-90.
 - 17 Engers HD, Houba V, Bennedsen J, Buchanan TM, Chaparas SD, Kadival G, et al. Results of a World Health Organization sponsored workshop to characterise antigens recognised by mycobacteria-specific monoclonal antibodies. *Infect Immun* 1986;51:718-20.
 - 18 Hewitt J, Coates ARM, Mitchison DA, Ivanyi J. The use of murine monoclonal antibodies in the serodiagnosis of tuberculosis. *J Immunol Meth* 1982;55:205-11.
 - 19 Jackett PS, Bothamley GH, Batra HV, Mistry A, Young DB, Ivanyi J. Immunodominance and specificity of mycobacterial antigens and their epitopes in the serology of tuberculosis. *J Clin Microbiol* 1988;26:2313-8.
 - 20 Toman K. Sensitivity, specificity and predictive value of diagnostic tests. *Bulletin of the International Union Against Tuberculosis* 1981;56:18-28.
 - 21 Toman K. Casefinding. In: *Tuberculosis, case-finding and chemotherapy: questions and answers*. Geneva: World Health Organisation, 1979:3-65.
 - 22 Chaparas SD, MacVandiviere H, Melson I, Koch G, Becker C. Tuberculin test: variability with the Mantoux procedure. *Am Rev Respir Dis* 1985;132:175-7.
 - 23 Ivanyi J, Krambovitis E, Keen M. Evaluation of a monoclonal antibody (TB72) based serological test for tuberculosis. *Clin Exp Immunol* 1983;54:337-45.
 - 24 Hoepfner V, Jackett PS, Swanson Beck J, Kardjito T, Grange JM, Ivanyi J. Appraisal of the monoclonal antibody-based competition test for the serology of tuberculosis in Indonesia. *Serodiagnosis and Immunotherapy* 1987;1:69-77.
 - 25 Olds GR, Sanson AJ, Daniel TM. Characterisation of *Mycobacterium tuberculosis* antigen 5 epitopes using a panel of 19 monoclonal antibodies. *J Clin Microbiol* 1987;25:471-5.
 - 26 Wiker HG, Harboe M, Bennedsen J, Closs O. The antigens of *Mycobacterium tuberculosis* H37Rv studies by crossed immunoelectrophoresis. *Scand J Immunol* 1988;27:223-39.
 - 27 Benjamin RG, Daniel TM. Serodiagnosis of tuberculosis using the enzyme-linked immunosorbent assay (ELISA) of antibody to *Mycobacterium tuberculosis* antigen 5. *Am Rev Respir Dis* 1982;126:1013-6.
 - 28 Balestrino EA, Daniel TM, de Latini MDS, Laini OA, Ma Y, Scocozza JB. Serodiagnosis of pulmonary tuberculosis in Argentina by ELISA of IgG antibody to *Mycobacterium tuberculosis* antigen 5 and tuberculin purified protein derivative. *Bull WHO* 1984;62:755-61.
 - 29 Daniel TM, Debanne SM, van der Kuyp F. Enzyme-linked immunosorbent assay using *Mycobacterium tuberculosis* antigen 5 and PPD for the serodiagnosis of tuberculosis. *Chest* 1985;88:388-92.
 - 30 Ma Y, Wang Y-M, Daniel TM. Enzyme-linked immunosorbent assay (ELISA) using *Mycobacterium tuberculosis* antigen 5 for the diagnosis of pulmonary tuberculosis in China. *Am Rev Respir Dis* 1986;134:1273-5.
 - 31 Alde SLM, Pinasco HM, Pelosi FR, Budani HF, Palma-Beltran OH, Gonzalez-Montaner LJ. Evaluation of an enzyme-linked immunosorbent assay (ELISA) using IgG antibody to *Mycobacterium tuberculosis* antigen 5 in the diagnosis of active tuberculosis in children. *Am Rev Respir Dis* 1989;139:748-51.
 - 32 Daniel TM, de Murillo GL, Sawyer JA, Griffin AM, Pinto E, Debanne S, et al. Field evaluation of any enzyme-linked immunosorbent assay for the serodiagnosis of tuberculosis. *Am Rev Respir Dis* 1986;134:662-5.
 - 33 Turner M, van Vooren J, de Bruyn J, Serruys E, Dierckx P, Yernault J. Humoral immune response in human tuberculosis: Immunoglobulins G, A and M directed against the purified P32 protein antigen of *Mycobacterium bovis* Bacillus Calmette-Guerin. *J Clin Microbiol* 1988;26:1714-9.
 - 34 Sada E, Ferguson LE, Daniel TM. An ELISA for the serodiagnosis of tuberculosis using a 30,000-Da native antigen of *Mycobacterium tuberculosis*. *J Infect Dis* 1990;162:928-31.
 - 35 Sada E, Brennan PJ, Herrera T, Torres M. Evaluation of lipoarabinomannan for the serological diagnosis of tuberculosis. *J Clin Microbiol* 1990;28:2587-90.
 - 36 Wilkins EL, Bothamley G, Jackett P. A rapid, simple enzyme immunoassay for detection of antibody to individual epitopes in the serodiagnosis of tuberculosis. *Eur J Clin Microbiol Infect Dis* 1991;10:559-63.
 - 37 Wilkins EGL, Ivanyi J. Potential value of serology for the diagnosis of extrapulmonary tuberculosis. *Lancet* 1990;336:641-4.
 - 38 Smith MJ, Citron KM. Clinical review of pulmonary disease caused by *Mycobacterium xenopi*. *Thorax* 1983;38:373-7.
 - 39 Banks J, Hunter AM, Campbell IA, Jenkins PA, Smith AP. Pulmonary infection with *Mycobacterium kansasii* in Wales 1970-9: a review of treatment and response. *Thorax* 1983;38:271-4.
 - 40 Wolinsky E. Nontuberculous mycobacteria and associated diseases. *Am Rev Respir Dis* 1979;119:107-59.