

# Prospective study of mycobacterial infections in patients with cystic fibrosis

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## Abstract

Fifty four patients with cystic fibrosis, aged 3-67 years, were studied prospectively for pulmonary mycobacterial infection. Sputum smears and cultures were carried out and intradermal skin tests performed. Mycobacteria were cultured from six patients in association with clinical deterioration; four patients had positive direct smears. *Mycobacterium tuberculosis*, *M avium-intracellulare*, *M kansasii*, and *M goodii* were isolated. There were no deaths and all improved with chemotherapy. A third of the other 48 patients had positive skin test responses (>6 mm) to purified protein derivative (PPD) tuberculin and 21 to one or more antigens prepared from non-tuberculous mycobacteria. Sensitisation increased with age; before the age of 11 only one patient had a positive response to PPD tuberculin and none to any other antigen. This was less than in healthy control subjects of similar age. After age 11 the reactions in sensitised patients were stronger than in positive healthy control subjects. Our study indicates that it is important to consider mycobacterial infection in patients with cystic fibrosis who deteriorate without obvious cause.

In patients with cystic fibrosis the major cause of death is the progressive pulmonary disease with infections caused by *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas* species. Pulmonary infection with mycobacteria is a rare but potentially serious complication.<sup>1-3</sup> We reported a three year prospective study using sputum culture and skin tests for mycobacterial infection in patients with cystic fibrosis.

## Methods

### PATIENTS

We investigated 54 patients with cystic fibrosis who attended the Stockholm cystic fibrosis centre regularly. All had previously been vaccinated with BCG, and all lived in the metropolitan area. Ten patients had contact with domestic animals or horses and two had contact with birds (cases 4 and 5). The mean age of the patients was 18 years (range 3-67) years (mean 19 years for the 23 males and 18 for the 31 females). The mean Shwachmann

score was 78 (range 30-100).<sup>4</sup> All patients had an abnormal sweat test result (chloride (>80 mmol/l) and pulmonary and gastrointestinal symptoms and all required treatment with pancreatic supplements, mucolytic agents (including by inhalation), and physiotherapy. Thirty one patients were chronically colonised with *Pseudomonas aeruginosa* or other *Pseudomonas* species and 13 with *S aureus* in addition. Ten patients were colonised only with *S aureus*. Six patients had diabetes mellitus. One patient required prednisolone because of severe asthma.

### SKIN TESTS

The following antigens were used for intradermal injections: PPD-T (PPD tuberculin in Tween diluent, 2 TU/0.1 ml), PPD-A (*M avium* antigen RS 10, 0.1 µg/0.1 ml), PPD-I (*M intracellulare* antigen RS 0.1 µg/0.1 ml), PPD-S (*M scrofulaceum* antigen RS 95, 0.1 µg/0.1 ml), PPD-K (*M kansasii*, RS 30, 0.1 µg/0.1 ml). All antigens were obtained from Statens Serum Institut, tuberculin department, DK-2300 Copenhagen S, Denmark. Each antigen (0.1 ml) was injected on the volar side of the middle third of the forearm and read at 72 hours by two specially trained nurses. A palpable induration of more than 6 mm diameter was regarded as positive.

### SPUTUM ANALYSES

At least three direct smear examinations and three cultures for mycobacteria were performed for each patient except for eight patients not producing sputum. Gastric lavage was performed in six patients, five of whom had one or more strongly positive skin test responses. Smears were examined after being stained with auramine. Mycobacterial isolates were identified by standard biochemical methods.<sup>5</sup> Susceptibility to streptomycin, amikacin, isoniazid, ethambutol and rifampicin was determined on Löwenstein-Jensen medium by the resistance ratio method.<sup>6</sup> Routine laboratory haematological determinations, serum immunoglobulin measurements, and liver and renal function tests were performed. Spirometric values were compared with those of healthy children and adults.<sup>7,8</sup>

### STATISTICS

Statistical analyses were performed by means of the  $\chi^2$  test.

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Table 1 Bacteriological findings and skin test results in six patients with cystic fibrosis and positive sputum cultures of mycobacteria

Patient No	Sex	Age (y)	Chronic sputum colonisation		Direct smear*	Mycobacteria in sputum	PPD† skin test results (mm)				
			Psa	Sa			T	A	I	S	K
1	F	11	+	+	+	<i>M avium-intracellulare</i>	0	22	17	18	ND
2	F	19	+	+	+	<i>M kansasii</i>	14‡	16	ND	ND	15
							10	10	6	8	ND
							15§	13	ND	ND	11
3	F	16	+	-	+	Unclassified mycobacteria	19	15	9	11	ND
4	M	16	+	+	-	<i>M gordonae</i>	0	0	0	0	ND
5	F	25	-	+	-	<i>M tuberculosis</i>	13	0	0	0	ND
6	F	34	+	-	+	<i>M avium-intracellulare</i>	11	18	13	16	ND

\*Positive (+) or negative (-) for mycobacteria.

†Purified protein derivatives (PPD): T—PPD-tuberculin; A—PPD-avium; I—PPD-intracellulare; S—PPD-scrofulaceum; K—PPD-kansasii.

‡Six months after the first investigation.

§Eighteen months after the first investigation.

Psa—*Pseudomonas aeruginosa*; Sa—*Staphylococcus aureus*; ND—not done.

## Results

Sputum cultures grew *M tuberculosis*, *M avium-intracellulare*, *M kansasii* and *M gordonae* in six patients (table 1). All six patients had received BCG vaccination in the first weeks of life. At the time of diagnosis they had deteriorated clinically with weight loss, considerable progression of the shadowing on chest radiographs, and impairment of lung function (figure). They reported intermittent fever, and patient 6, the only one with lymphadenopathy, had nightly attacks of profound sweating. All patients improved with specific chemotherapy (streptomycin, rifampicin, isoniazid, and ethambutol in standard doses). No case was fatal and no contacts were identified.

The skin test results in the patients from whom mycobacteria were isolated from sputum are given in table 1. Four patients had positive direct smears. All but one (No 4) had at least

two positive sputum cultures. In one patient (No 2) two species of mycobacteria were isolated within 18 months. The largest skin test reaction corresponded to the mycobacterial species isolated in three patients (four instances).

The results of skin tests in the other patients with cystic fibrosis are given in table 2. One third of the patients had a positive response to PPD tuberculin and almost half to one or more antigens from non-tuberculous mycobacteria. Fifteen of the 21 patients who had a positive response to one or more non-tuberculous antigens had a positive response to PPD tuberculin. Only two patients had a positive response to PPD tuberculin and a negative response to all other antigens; one had BCG vaccination 18 months before. Of those with a positive response to any non-tuberculous antigen, 13 had a positive response to three of the four antigens, seven to two, and two to one.

Results of yearly spirometry in six patients with cystic fibrosis in whom mycobacteria were isolated in sputum at the time of clinical deterioration (indicated by arrow). VC—vital capacity; FEV<sub>1</sub>—forced expiratory volume in one second. ■ Patient 1; □ 2; ▲ 3; △ 4; ● 5; ○ 6.

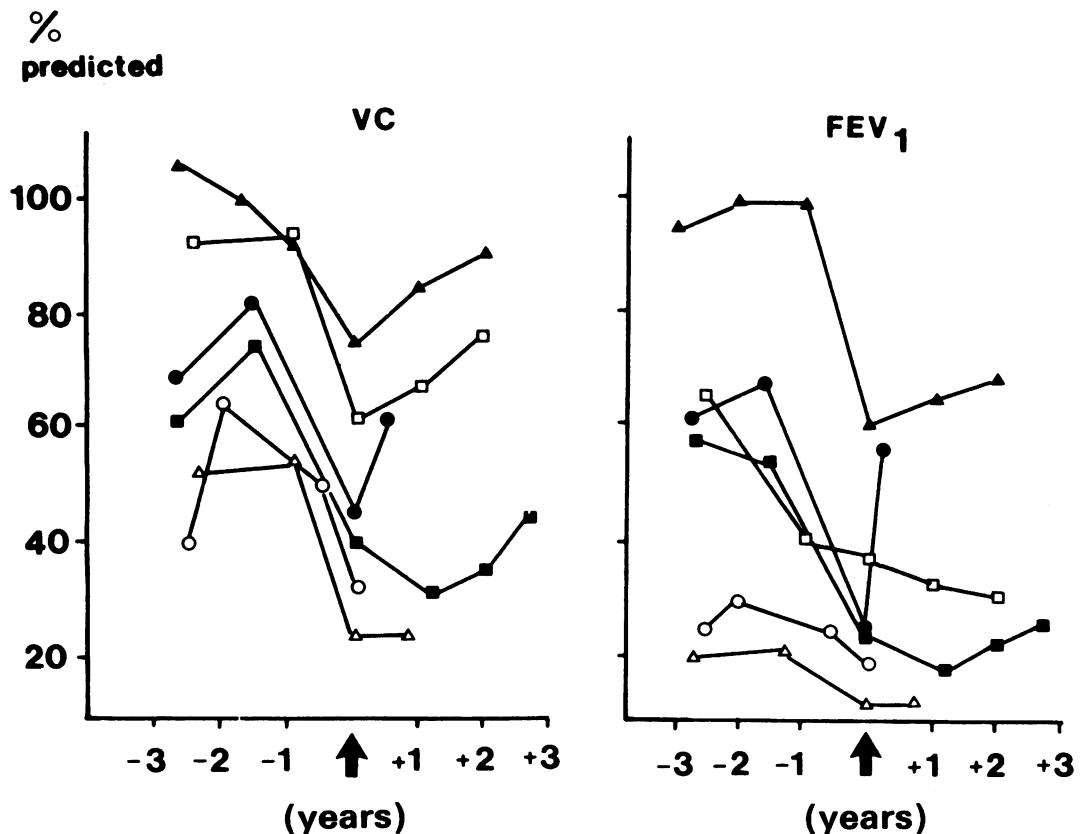


Table 2 Numbers of patients with cystic fibrosis and sputum cultures negative for mycobacteria who had positive responses (> 6 mm) to skin tests with mycobacterial antigens, by age

Age (y)	PPD-T	Other antigens*
0-10	1/11	0/11
11-15	2/10	4/10
16-20	3/12	8/12
> 21	10/15	9/15
All	16/48 (33%)	21/48 (44%)

\*One or more of PPD-A, PPD-I, and PPD-S (see second footnote to table 1).

Only one of 11 patients under the age of 11 had a positive response to any antigen (PPD tuberculin in the one case) despite prior BCG vaccination. Sensitisation to antigens increased with age (table 2).

Positive skin test responses to PPD-tuberculin occurred in 20% of patients aged 11-20 years with negative sputum cultures, compared with 44% of healthy 14 year old Swedish children who had BCG vaccination at birth<sup>9</sup> ( $p < 0.05$ ) and half of 8-9 year old Swedish children vaccinated with BCG ( $p < 0.05$ ).<sup>10</sup> No patient reacted to any of the non-tuberculous antigens before the age of 11, compared with 62-68% of healthy vaccinated 8-9 year old children<sup>10</sup> ( $p < 0.001$ ). A difference in the opposite direction was noticed when indurations of more than 10 mm were compared; these occurred in 38% (*M avium-intracellulare*) and 31% (*M scrofulaceum*) of patients with cystic fibrosis (data not shown) and in 10% and 13% of healthy children<sup>10</sup> ( $p < 0.001$  and  $p < 0.001$  respectively).

No relation was found between skin test results and erythrocyte sedimentation rate, total white blood cell count, differential white cell count, serum IgG, clinical state, FEV<sub>1</sub>, or chest radiographs (data not shown). Only two of the patients with positive mycobacterial cultures were in a poor nutritional state (< 2 SD for weight), and one was receiving corticosteroid treatment; none had diabetes mellitus.

### Discussion

Colonisation by non-tuberculous mycobacteria may be facilitated by bronchiectasis and cavitory disease.<sup>11</sup> It is surprising therefore that mycobacteria are isolated so infrequently from patients with cystic fibrosis.<sup>1</sup> Previous reported cases have usually been fatal.<sup>2,3</sup> A report of 16 years' experience at the Brompton Hospital, London, showed that during the first 10 years no mycobacteria were isolated.<sup>12</sup> In the six years after the introduction of routine direct smears and culture of sputum for mycobacteria in patients with cystic fibrosis seven of 223 patients were found to have positive sputum samples. In our screening of patients one proved to have tuberculosis, three had non-tuberculous mycobacterial infection, and two had equivocal infection (patients 3 and 4; table 1).

The relevance of mycobacteria isolated from sputum may be difficult to assess, especially in patients with cystic fibrosis or other chronic

pulmonary diseases. We found both an obligate pathogen (*M tuberculosis*) and one isolate (*M gordonae*) that is rarely associated with disease.<sup>13,14</sup> *M avium-intracellulare* and *M kansasii* form an intermediate group, causing either progressive lung disease or colonisation only.<sup>15</sup> The deterioration in lung function observed in all patients at the time of positive sputum culture suggests that these mycobacteria were causing disease (figure). Furthermore, infection is more probable than colonisation in cases with positive direct smears because these indicate a large number of mycobacteria in the sputum. Such findings with repeated isolation of the same bacterial strain and skin reactivity to the relevant antigen may be helpful for diagnosis.<sup>16,17</sup> As mycobacteria are known to possess cross reacting antigens, various PPD preparations should be used for comparison and chosen according to geographical distribution.<sup>18</sup> Our patients seemed to be less sensitised to non-tuberculous antigens than healthy young people in Sweden, but when positive the reaction seemed to be stronger than that in the healthy population. Our finding of a lower rate of sensitisation in patients with cystic fibrosis than in healthy children seems to be age related and might be related to biochemical changes during the progression of the disease. One progressive disturbance in cystic fibrosis is deficiency of essential fatty acids<sup>19</sup> and abnormal eicosanoid production (Strandvik *et al*, unpublished observations). Interestingly, administration of indomethacin, *in vitro* and *in vivo*, reverses subnormal immune reactivity to non-tuberculin mycobacterial antigens,<sup>20</sup> because patients with cystic fibrosis overproduce some eicosanoids.<sup>21</sup> T cell function has been considered to be normal in patients with cystic fibrosis,<sup>22</sup> but further studies are needed.

Our study indicates that mycobacterial infection should be considered in patients with cystic fibrosis who deteriorate without known cause. Sputum examination must be performed because skin tests are of limited value. In contrast to some earlier reports, none of our cases was fatal, indicating that chemotherapy may be life saving in non-tuberculous mycobacterial infection.

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