Evaluation of IgA-Mediated Humoral Immune Response Against the Mycobacterial Antigen P-90 in Diagnosis of Pulmonary Tuberculosis*

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Background: Serologic methods for diagnosis of tuberculosis have been widely investigated owing to their low cost and rapid technical execution. Sensitivity and specificity of different tests have been reported to be largely variable.

Study objectives: To evaluate the IgA-mediated humoral immune response against the mycobacterial antigen P-90 as a tool for diagnosis of pulmonary tuberculosis.

Participants: Eighty-eight patients with microbiologically confirmed diagnosis of pulmonary tuberculosis (32 with positive sputum smears and 56 with negative sputum smears), 28 patients with a definite nontuberculous lung disease, 12 subjects with healed tuberculosis, and 47 healthy volunteers (24 purified protein derivative negative and 23 positive).

Measurements and results: Detection of anti-P-90 IgA was performed by enzyme-immunoassay. At a cutoff of 0.221 optical density, determined by a receiver operating characteristic curve, the overall sensitivity and specificity of the test were 70.4% and 91.9%, respectively. Patients with active tuberculosis showed significantly higher titers of anti-P-90 IgA compared with other groups (p<0.05).

Conclusions: The evaluation of IgA-mediated humoral immune response against the antigen P-90 might constitute a useful tool for presumptive diagnosis of pulmonary tuberculosis.

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Key words: antigen P-90; IgA; serodiagnosis; tuberculosis

Abbreviations: BCG=bacille Calmette-Guérin; EIA=enzyme-immunosorbent assay; OD=optical density; PPD=purified protein derivative; pTB=pulmonary tuberculosis; ROC=receiver operating characteristic; TB=tuberculosis

Tuberculosis (TB) is one of the most serious public health problems worldwide. It is estimated that 400,000 new cases occur in the industrialized world each year; in the underdeveloped world, the incidence is at least 8 million cases per year.1 The epidemic of HIV infection1-4 and several social and economic factors (such as poverty, homelessness, illicit use of drugs)1,5,6 seem to be responsible for this resurgence of TB. Moreover, the increasing frequency of multiple drug-resistant strains and incomplete or inadequate treatments play an important role in this phenomenon.1,7

Rapid identification of cases, allowing a timely beginning of antituberculous chemotherapy, is one of the most effective infection control measures.7-9 The detection of alcohol acid-resistant bacilli on sputum samples is hampered by a low sensitivity, especially in noncavitary forms of the disease.10-12 Cultural isolation of Mycobacterium tuberculosis remains the gold standard for diagnosis of TB; however, this procedure requires 4 to 6 weeks and its sensitivity is not absolute.10

Serologic methods have been proposed in the past decades as well as recently;13-19 several tests have been set up for detection of antibodies against different mycobacterial antigens. However, a large variability in diagnostic accuracy has been reported, depending on the antigen or the immunoglobulin class employed.15-22

The aim of the present work was to define the diagnostic accuracy of detection of IgA against the mycobacterial antigen P-90 in pulmonary TB (pTB).
**Materials and Methods**

**Subjects**

One hundred seventy-five consecutive subjects were enrolled into the study (Table 1); all were HIV-negative and previously nonvaccinated with bacille Calmette-Guérin (BCG). None had received oral or parenteral steroids in the previous 6 months.

The healthy control population consisted of 47 volunteers [24 purified protein derivative-negative (PPD−) and 23 PPD-positive (PPD+)] with no signs of clinical impairment and normal chest radiograph. Twenty-eight patients with a definite diagnosis of lung disease other than TB and 12 subjects treated for active pTB (microbiologically confirmed) in the past 8 to 10 years and with no signs of disease reactivation in the preceding 6 years were also studied.

In 88 patients, the diagnosis of active pTB was established according to clinical and radiologic data and cultural growth of *M. tuberculosis*; they were divided into two groups according to the results of searching for acid-fast bacilli on sputum smears. Patients with active pTB were all symptomatic for 10 to 24 days (mean, 16 days); none had been treated with antituberculous drugs.

**Technique for IgA Measurement**

The detection of IgA against the mycobacterial antigen P-90 was performed by the enzyme-immunosorbent assay (EIA) technique, using a commercially available kit (Kreatech EIA-TB; Amsterdam, the Netherlands). P-90 is prepared by the manufacturer from BCG according to a standard protocol.25 Sonicated French-pressed broken bacilli are centrifuged at 90,000 g for 2 h at 4°C; the pellet represents the antigenic complex indicated as P-90.

In our protocol, 100 μL of each patient’s serum, diluted 1/400 in phosphate-buffered saline solution, was distributed in duplicate in microtiter wells coated with P-90 and incubated at 37°C for 1 h. Subsequently, a washing cycle with phosphate-buffered saline solution was performed and 100 μL of peroxidase-conjugate antihuman IgA was added. A second 1-h incubation period at 37°C was started and another washing cycle was performed prior to the addition of the enzymatic substrate (H₂O₂) mixed with the chromogen tetramethylbenzidine. The colorimetric reaction was then prolonged for 15 min at room temperature in the dark and stopped with the addition to each well of 100 μL of 4N H₂SO₄. The absorbance values at 450 nm were recorded with an automatic reader system. Two serum samples provided by the manufacturer as the negative and the positive standards were also analyzed.

**Data Analysis**

Demographic data of subjects enrolled into the study were analyzed by one-way analysis of variance test. Results of determinations of titers in different groups were compared by Student’s *t* test (Epistat Statistical Package; IBM PS/2 Model 30); statistical significance was accepted at a level of *p*<0.05.

True-positive rates (sensitivity) and false-positive rates (100% minus specificity) were calculated at different cutoff values and plotted on a graph to obtain a receiver operating characteristic (ROC) curve.26 In this kind of analysis, the point that encloses the largest area, or, less formally, that lies farthest to the “northwest” of the graph, is believed to be the most accurate cutoff value,25 and was chosen for this purpose.

**Results**

Subjects of different groups were matched by age and sex (Table 1). Patients with active pTB showed significantly higher titers of anti-P-90 IgA (0.436±0.421 optical density [OD] in smear-negative patients and 0.577±0.642 in smear-positive patients) compared with healthy control subjects (0.113±0.063 in PPD− and 0.131±0.110 in PPD+), patients with nontuberculous lung disease (0.150±0.110), and subjects with healed TB (0.129±0.049) (*p*<0.05; Fig 1 and Table 1).

Among tuberculous patients, mean antibody levels were higher in patients with positive sputum smears than in patients with negative sputum smears; however, this difference did not reach statistical significance. No statistically significant differences were found among values of different control groups (Table 1).

With a cutoff of 0.221 OD, the test was positive in 62 of 88 patients with active pTB (38/56 with negative sputum smears and 24/32 with positive sputum smears), in 2 of 47 healthy volunteers (1 PPD− and 1 PPD+), in 4 of 28 patients with nontuberculous lung disease, and in 1 of 12 subjects with healed TB (Table 1).

Data about sensitivity and specificity at different cutoff values are reported in Table 2 and in Figure 2 (ROC curve).

### Table 1—Characteristics of Groups Included Into the Study and Results of Measurement of Anti-P-90 IgA*

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Cases</th>
<th>No. of Men</th>
<th>Mean Age, yr (SD)</th>
<th>Mean OD (SD)</th>
<th>No. Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC PPD−</td>
<td>24</td>
<td>15</td>
<td>46.2 (12.3)</td>
<td>0.113 (0.063)</td>
<td>1</td>
</tr>
<tr>
<td>HC PPD+</td>
<td>23</td>
<td>14</td>
<td>41.6 (12.6)</td>
<td>0.131 (0.110)</td>
<td>1</td>
</tr>
<tr>
<td>N-TB</td>
<td>28</td>
<td>17</td>
<td>44.6 (11.4)</td>
<td>0.150 (0.110)</td>
<td>4</td>
</tr>
<tr>
<td>hpTB</td>
<td>12</td>
<td>7</td>
<td>47.0 (9.1)</td>
<td>0.129 (0.049)</td>
<td>1</td>
</tr>
<tr>
<td>pTB smear−</td>
<td>56</td>
<td>30</td>
<td>45.3 (10.3)</td>
<td>0.436 (0.421)</td>
<td>38</td>
</tr>
<tr>
<td>pTB smear+</td>
<td>32</td>
<td>21</td>
<td>42.1 (9.5)</td>
<td>0.577 (0.642)</td>
<td>24</td>
</tr>
</tbody>
</table>

*HC PPD− = healthy control subjects, PPD negative; HC PPD+ = healthy control subjects, PPD positive; N-TB = nontuberculous lung disease (bronchial asthma, *n* = 7, bronchietasis, *n* = 2, lung cancer, *n* = 6, acute pneumonia, *n* = 4; exacerbation of COPD, *n* = 5; cryptogenic fibrosing alveolitis, *n* = 2; idiopathic pulmonary hypertension, *n* = 2); hpTB = healed pulmonary TB; pTB smear− = pulmonary TB with negative sputum smears; pTB smear+ = pulmonary TB with positive sputum smears. No. positive = number of positive results at the cutoff of 0.221 OD.

1. *p*<0.05 vs HC PPD−, HC PPD+, N-TB, hpTB.

2. *p* = NS vs pTB smear−.

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**Discussion**

P-90 represents an antigenic complex common to both *M. tuberculosis* and BCG; a protocol for its purification has been described. It has been shown to elicit a humoral immune response in rabbit; furthermore, spleen and lymph node cells from mice immunized intraperitoneally with BCG or *M. leprae* show a net increase in incorporation of tritiated thymidine when P-90 is added to culture medium. The synthesis of antibodies against P-90 has been studied in patients with leprosy and, less extensively, with TB. In particular, it has been reported that high levels of IgG against P-90 are present in the course of both leprosy and TB; high IgM titers seem to be more specific of leprosy and, more exactly, of the lepromatous form. However, synthesis of IgA against P-90 has been reported to occur only in patients with TB, in contrast to patients with leprosy. However, in the above-mentioned study by Das and coworkers, only a small number of patients with TB were analyzed; to our knowledge, no other studies are available in the literature about this topic.

In the present work, we studied a larger group of patients with TB; they were divided according to the results of searching for acid-fast bacilli on sputum.
smears. Subjects with healed TB and skin-positive healthy volunteers were also evaluated.

Subjects with pTB showed significantly higher titers of IgA against P-90 antigen compared with control groups; mean levels observed in patients with positive sputum smears were higher than in patients with negative sputum smears; however, this difference did not reach statistical significance. With a cutoff of 0.221 OD, the test was positive in 24 of 32 (75.0%) and in 38 of 56 (67.8%) patients with positive and negative results of Ziehl-Neelsen staining, respectively. Similarly, Verbon et al27 reported a sensitivity of 55% and 32% in tuberculous patients with positive or negative sputum smears, respectively, by using an enzyme-linked immunosorbent assay test based on the mycobacterial antigen of molecular weight of 16,000. Likewise, higher sensitivity of serodiagnosis with A60 as antigen was found in patients with cavitary lesions as compared to patients with productive forms,18 and it is well known that positive results of searching for acid-fast bacilli by sputum microscopy are much more frequent in patients with cavitary lesions than in patients with noncavitary forms.10-12 The reasons for better sensitivity of serologic tests in patients with positive sputum smears are not well known; the exposure to a higher bacillary load has been proposed.27

The above-mentioned sensitivity increases with the lowering of the cutoff line (Table 2); however, also in these conditions, in a few tuberculous patients the test result is persistently negative. We can hypothesize that, at least in some cases, immune complex formation and/or inhibition of specific lymphocytic clones might be responsible for this phenomenon, as suggested by others to explain the false-negative results of A60 antigen-based serology.21 It must be stressed that the above-mentioned results were obtained from measurements performed on blood samples kept 10 to 24 days after the onset of clinical symptoms; it has been reported that titers of IgG against cord factor are significantly higher (with a corresponding increase in sensitivity of the test) in patients evaluated 2 months after the onset of symptoms, compared to subjects with a history of 1-month duration;17 so it is also likely that the sensitivity of the P-90-based test might increase by repeating the test some weeks later.

In subjects with healed pTB, titers of anti-P-90 IgA were significantly lower than in patients with active TB and not significantly different from the other control groups. It has been reported that both anti-A6028 and antigord factor17 antibody levels decrease subsequent to therapy; however, the percentage of subjects showing persistence of high titers following treatment is variable, according to different authors;28,30 in our experience, in 1 of 12 subjects with healed pTB, the test result was slightly positive at the chosen cutoff of 0.221 OD.

We also observed the production of anti-P-90 IgA in some healthy subjects, both PPD− and PPD+, leading to false-positive results in a different percentage according to the cutoff chosen (2/47 at the cutoff of 0.221 OD). It is possible to hypothesize, as proposed for the A60-based test,18,30 that clinically silent infection with environmental mycobacteria may cause this kind of result, considering that P-90 is not a species-specific antigen. Patients with nontuberculous lung disease showed a more frequent incidence of false-positive results (4/28 cases); this is in agreement with other reports about the immune response against the A60 antigen18,30 and confirms that some nontuberculous diseases (especially acute pneumonia or lung cancer)40 may cause interferences, possibly owing to disorders in immune response occurring in the course of these diseases.

However, the overall specificity of the P-90 test may be increased up to 97.7% (with a corresponding sensitivity of 45.4%) if the cutoff is established at a higher value (0.356 OD). Likewise, it has been reported that different values of sensitivity and spec-

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>pTB Smear−</th>
<th>pTB Smear+</th>
<th>All pTB Patients</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.356</td>
<td>42.9</td>
<td>50.0</td>
<td>45.4</td>
<td>97.7</td>
</tr>
<tr>
<td>0.281</td>
<td>53.6</td>
<td>62.5</td>
<td>56.8</td>
<td>96.5</td>
</tr>
<tr>
<td>0.228</td>
<td>66.1</td>
<td>62.5</td>
<td>64.7</td>
<td>94.2</td>
</tr>
<tr>
<td>0.221</td>
<td>67.8</td>
<td>75.0</td>
<td>70.4</td>
<td>91.9</td>
</tr>
<tr>
<td>0.191</td>
<td>75.0</td>
<td>81.2</td>
<td>77.2</td>
<td>83.9</td>
</tr>
<tr>
<td>0.180</td>
<td>78.6</td>
<td>87.5</td>
<td>81.8</td>
<td>66.6</td>
</tr>
<tr>
<td>0.126</td>
<td>85.7</td>
<td>87.5</td>
<td>86.3</td>
<td>62.0</td>
</tr>
<tr>
<td>0.123</td>
<td>85.7</td>
<td>90.6</td>
<td>87.5</td>
<td>58.6</td>
</tr>
<tr>
<td>0.100</td>
<td>87.5</td>
<td>90.6</td>
<td>88.6</td>
<td>47.1</td>
</tr>
</tbody>
</table>

*See Table 1 for explanation of abbreviations.

![Figure 2. ROC curve of P-90 EIA test. Each point on the curve indicates the true-positive rate (sensitivity) and the false-positive rate (100% minus specificity) for a particular cutoff value (range 0.356 to 0.1 OD).](http://publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21745/ on 06/17/2017)
ificity of A60-based test are obtained according to the cutoff chosen. For comparison, Gevaudan et al. reported sensitivities of 95.5%, 94%, and 82.5% when the cutoff line was placed at 150, 200, and 300 international serounits, respectively; the corresponding specificity values were 41%, 64%, and 80.5%.

The choice of cutoff line is an important step in the clinical decision-making processes, so a positive result at a cutoff level allowing a high specificity (in our study 97.7% with a cutoff of 0.356 OD) in a patient suspected of having the disease may justify the starting of therapy while awaiting the results of cultural examination; however, a positive result at a lower cutoff may represent one of the elements leading to a presumptive diagnosis of TB. In conclusion, the measurement of anti-P-90 IgA may constitute a useful tool in rapid serodiagnosis of PTB; results of the test should be carefully interpreted taking into account the finality envisaged.

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