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# Detection of Lipoarabinomannan Antibodies in Patients with Newly Acquired Tuberculosis and Patients with Relapse Tuberculosis

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**A commercially available dot immunoassay that employs the lipoarabinomannan antigen was evaluated for the serologic diagnosis of tuberculosis. The test showed a high specificity (100%); however, its sensitivity was low (18.5%). Antibodies to lipoarabinomannan were detected in the sera of 7 of 71 patients with newly acquired tuberculosis and in sera of 10 of 21 patients with relapse tuberculosis. It has been shown by others that sera from patients with relapse tuberculosis had a higher concentration of antibodies and reacted with a greater variety of antigens (native culture filtrates of *Mycobacterium tuberculosis* H37Rv) than did sera from patients with newly acquired tuberculosis. Our data confirm the results of these previous studies as far as lipoarabinomannan is concerned. We conclude that the differences in the production of antibodies shown by the two groups of tuberculous patients (new and relapse) must be taken into account when assessing the usefulness of serologic tests for the diagnosis of tuberculosis.**

More people died from tuberculosis (TB) in 1995 than in any other year in history, according to a recent report released by the World Health Organization (5). Nearly 3 million people died from TB in 1995, a rate surpassing that in the worst years of the epidemic, around 1900, when an estimated 2.1 million people died annually (5). The quick establishment of a short course of chemotherapy administered to infectious individuals, and direct observation that this treatment is being carried out, is the strategy (known as directly observed treatment, short course [DOTS]) endorsed by the World Health Organization to stop this disease (6). Fast and inexpensive methods to diagnose TB would hasten the identification of patients with communicable TB and contribute to the success of DOTS programs. The ideal test should be cheap, reliable, and easy to read. A serologic test could comply with these requisites. In recent years new reagents, both purified antigens and monoclonal antibodies, have been developed to increase the sensitivity and the specificity of the serologic tests (2).

Lipoarabinomannan (LAM) is a lipopolysaccharide which constitutes one of the dominant antigens of the mycobacterial cell wall. The particular characteristics of this antigen, which presents repetitions of D-arabinofuranose residuals, induce strong and extremely pure immune reactions. A specificity of 91% and a sensitivity of 72% were reported for this antigen in a study performed in the Republic of Mexico (4).

The study described here was conducted to evaluate the MycoDot test (Genelabs, Geneva, Switzerland). The MycoDot test is a commercially available serologic assay designed to aid in the diagnosis of active TB (pulmonary and extrapulmonary) and other active mycobacterial diseases. It detects specific immunoglobulin G antibodies against the LAM antigen, which is bound to the plastic combs used in the test. The test is carried out in only 20 min. The reading can be made with the naked eye, and a positive result consists of the appearance of a red spot on the plastic combs. Serum, heparin-derived plasma, or whole blood can be used in the MycoDot test. Serum samples from 92 patients with active TB, 41 of whom were human immunodeficiency virus (HIV) positive (TB-HIV group) (Table 1), and serum samples from 14 patients with mycobacterial disease produced by nontuberculous mycobacteria (NTM), 12 of whom were HIV positive, were tested in this study. These patients were admitted to the Hospital Universitari Germans Trias i Pujol in Barcelona, Spain, for diagnosis and treatment of mycobacterial infection. In all cases TB was confirmed by isolation and identification of *Mycobacterium tuberculosis*. TB was classified as new TB if a patient had never had documented or treated TB before. Relapse TB was the classification used for patients who, some time after having finished a suitable but short treatment, had again developed bacteriologically active TB. Patients in whom therapeutic failure had occurred, due to resistance to drugs or prior poor compliance with the prescribed treatment, were also included in the relapse TB group. Of the 14 patients with mycobacterial disease produced by NTM, 5 had disease due to *Mycobacterium kansasii*, 4 had disease due to *Mycobacterium xenopi*, and 5 had disease due to the *Mycobacterium avium* complex. All serum samples were obtained before chemotherapy was carried out. The control population included 35 healthy subjects (13 purified protein derivative test positive), who were employees of the Hospital Universitari Germans Trias i Pujol; 36 patients with lung infections other than TB (*Streptococcus pneumoniae* [9 patients], *Coxiella burnetii* [3 patients], *Chlamydia* spp. [8 patients], *Mycoplasma pneumoniae* [10 patients], and *Legionella pneumophila* [6 patients]); and 14 asymptomatic HIV-infected patients. All sera were stored at −40°C before testing. The test was performed according to the manufacturer's recommended procedure. The specificity shown by the evaluated test was 100%. All healthy controls, the patients with lung diseases different from TB, and the HIV-positive asymptomatic patients yielded negative results with the MycoDot test. LAM antibodies were not detected in any of the 14 patients

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TABLE 1. Classification of patients with active TB

| Type of TB | No. of patients in group: | | |
|---|---|---|---|
| | TB-HIV | | TB |
| | CD4 cell count $\leq$200/mm$^3$ | CD4 cell count $>$200/mm$^3$ | |
| Disseminated | 30 | 2 | 5 |
| Pulmonary | 4 | 4 | 36 |
| Pleural | 1 | 0 | 4 |
| Lymphatic | 0 | 0 | 4 |
| Other | 0 | 0 | 2 |
| Total | 35 | 6 | 51 |

with mycobacterial diseases produced by NTM. The results obtained with tuberculous patients are summarized in Table 2.

Among the 51 tuberculous HIV-negative patients (TB group), 11 (21.5%) were MycoDot positive, while 6 (14.6%) of the 41 patients in the TB-HIV group were MycoDot positive. Thus the sensitivity of the test seemed to be somewhat lower for the TB-HIV group than for the TB group. Recently, Boggian et al. (1) reported a very low sensitivity (10.6%) of the MycoDot test with tuberculous HIV-positive patients. For both groups of patients, TB and TB-HIV, the result of the MycoDot test seemed to be somewhat influenced by the smear microscopic examination. We have, however, found a very clear positive correlation between the result of the test and the type of TB, new or relapse. In the TB group 100% of the patients with relapse TB were MycoDot positive, compared with only 11.1% of the patients with new TB. In the TB-HIV group, 26.6% of the patients with relapse TB were MycoDot positive, compared with only 7.7% of the patients with new TB. In all, 9.8% of the patients with new TB were MycoDot positive, while the percentage of positive results amounted to 47.6% among the relapse TB patients.

TABLE 2. Results of MycoDot test for patients with active TB

| Source of serum samples | New TB patients | | Relapse TB patients | |
|---|---|---|---|---|
| | No. tested | No. (%) positive | No. tested | No. (%) positive |
| Tuberculous HIV-negative patients | | | | |
| Smear positive | 17 | 4 (23.5) | 4 | 4 (100) |
| Smear negative | 28 | 1 (3.5) | 2 | 2 (100) |
| Tuberculous HIV-positive patients | | | | |
| Smear positive | 12 | 2 (16.6) | 9 | 2 (22.2) |
| Smear negative | 14 | 0 (0) | 6 | 2 (33.3) |
| Total | 71 | 7 (9.8) | 21 | 10 (47.6) |

Kaplan et al. (3), using mycobacterial culture filtrates as the antigen (native culture filtrates of *M. tuberculosis* H37Rv), detected an antibody-positive response in 46% of the new TB patients. The percentage of responders rose to 66% among relapse TB patients, whose sera reacted with more antigens than did sera from patients with new TB (3). Our data back up the results obtained by Kaplan and Chase (3) and suggest that, in the population studied, new TB patients have a sparse antibody response to LAM and that this response is considerably higher in relapse TB patients.

In the present study the MycoDot test has proved to be a very specific test, and LAM seems to be a good antigen for studying the significance of antibodies in tuberculous illness; however, the low degree of sensitivity shown by the test in this study does not support its use in the diagnosis of TB in Spain.

We believe that the differences in antibody production shown by the two groups of tuberculous patients (new and relapse) should be taken into account when evaluating the usefulness of serologic tests for the diagnosis of TB.

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