C.5 Articles

Articles en què es fonamenta aquest treball. Els articles següents descriuen i discuteixen els resultats en els quals està basada aquesta tesi doctoral:


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 α-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 kansasi, 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...
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 and MycoDot positive, while the percentage of positive results amounted to 47.6% among the relapse TB patients.

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

<table>
<thead>
<tr>
<th>Source of serum samples</th>
<th>New TB patients</th>
<th>Relapse TB patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. (%) positive</td>
</tr>
<tr>
<td>Tuberculous HIV-negative patients</td>
<td>Smear positive</td>
<td>17 4 (23.5)</td>
</tr>
<tr>
<td></td>
<td>Smear negative</td>
<td>28 1 (3.5)</td>
</tr>
<tr>
<td>Tuberculous HIV-positive patients</td>
<td>Smear positive</td>
<td>12 2 (16.6)</td>
</tr>
<tr>
<td></td>
<td>Smear negative</td>
<td>14 0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>71 7 (9.8)</td>
<td>21 10 (47.6)</td>
</tr>
</tbody>
</table>

REFERENCES


Evaluation of a new serodiagnostic tuberculosis test based on immunoglobulin A detection against Kp-90 antigen

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SUMMARY

We evaluated a new serological test for tuberculosis (TB) diagnosis, an enzyme-linked immunosorbent assay which detects the presence of immunoglobulin A (IgA) antibodies in human serum, the Kreatech TB IgA EIA test. The study was performed using 166 serum samples collected in the city of Barcelona, Spain. Fifty-six serum samples were from TB patients and 111 from controls, 40 of which were non-TB pneumonia patients. The test sensitivity was 70.58% in the adult group with pulmonary TB and 59% in the group with extra-pulmonary TB. These sensitivities were similar to those previously reported by others. However, we found that the test had a low specificity of only 68.68%, while specificities of 90% and 95% have been reported. This discrepancy may be attributable to differences in the respective study populations. In our control group we included 40 non-TB pneumonia patients (36% of the entire control group), in whom test specificity dropped to 47.22%. In those studies in which specificities of 90% and above were achieved, very few serum samples from non-TB pneumonia patients were tested (6-12% of the entire control group).

KEY WORDS: enzyme-linked immunosorbent assay; IgA; Kp 90 antigen; tuberculosis

TUBERCULOSIS (TB) INCIDENCE in Spain is 40 new cases for every 100,000 inhabitants.1 In the city of Barcelona, the incidence in 1991 was 67.1/100,000. Since then, a slight but constant decrease has been observed, and in 1998 the incidence was 43.8/100,000.2 For 2000 the objective is to achieve an incidence of 33/100,000.

The key to controlling TB in our environment depends on the rapid detection of new cases, and on following through with treatment. In spite of the availability of optimal methods for microbiological diagnosis of tuberculosis (traditional and molecular techniques), data from 19982 show that in 20-30% of adult tuberculosis cases it was necessary to wait for culture results to obtain a bacteriological diagnosis, and in 10% of the cases this diagnosis was not obtained.2 These percentages are higher in child TB. We therefore believe it to be of interest to investigate other diagnostic alternatives such as those based on the detection of a specific humoral response. A serological test would be useful in the rapid diagnosis of pulmonary TB, extra-pulmonary disease and child tuberculosis when smear or molecular techniques prove negative. In the case of Barcelona, a good serological test would also be useful for case finding in those groups most affected, such as prisoners, drug addicts, the poor, and neighbourhoods with a high incidence of the disease. In the present study, we evaluate the Kreatech TB IgA enzyme-linked assay (EIA) test for its utilization in our city.

STUDY POPULATION AND METHODS

A total of 166 serum samples taken from human immunodeficiency virus (HIV) negative persons were studied. Their characteristics are listed in the Table.

**Tuberculous patients**
Between June 1995 and February 1998, serum samples were collected from patients who had been admitted to the Universitari Germans Trias i Pujol Hospital (HUGTIP) in Barcelona, with clinical suspicion of pulmonary and/or extra-pulmonary TB. The only serum samples included in the study were from patients in whom the disease had been confirmed by isolation of the tubercle bacillus on culture. Löwenstein-Jensen and the non-radiometric MItBact system (Organon Teknika, Durham, NC). Forty-seven serum samples were from adult patients, 36 of whom had pulmonary TB, and 11 extra-pulmonary TB. The...
extra-pulmonary sites comprised disseminated TB (four), lymphatic (two), puncture (two), pus abscess (one), bone (one) and cutaneous (one). Patients had not yet started antituberculosis treatment when the serum samples were taken.

Nine serum samples from tuberculous children were also included in the study; the illness was diagnosed by clinical parameters, and in five it was confirmed by isolation of Mycobacterium tuberculosis in cultures of samples obtained from gastric lavage or respiratory specimens.

Control subjects
In addition, 110 HIV-seronegative serum samples were included in the study as negative controls (Table). Forty of the samples were from non-TB pneumonia patients. The causes of their disease were Streptococcus pneumoniae (seven samples), Coxiella burnetii (six), Chlamydia spp. (nine), Mycoplasma pneumoniae (nine) and Legionella pneumophila (nine). All were purified protein derivative (PPD) negative. A further two samples were included in the study from patients with mycobacterial diseases, one due to M. kansasi and the other to M. xenopi.

Nine samples belonging to PPD-positive children were included, as well as three samples from persons who had suffered from TB more than 5 years previously and who had completed treatment for TB with isoniazid, pyrazinamide and rifampicin for 2 months, followed by isoniazid and rifampicin for a further 4 months. All the serum samples were obtained from the Microbiology Service seroteca at the HUGTIP.

Finally, 56 serum samples were obtained from healthy controls, who were employees of the HUGTIP and PhD students at the Autonomous University of Barcelona (the HUGTIP is attached to the Autonomous University). Eight of these had BCG-vaccinated in the past, eight were PPD-positive, and 40 were PPD-negative at the time the serum samples were taken. Samples were collected and stored at -40°C until use.

Serological test
The new test uses a compound, the Kp-90 antigen, which is obtained from the M. bovis Calmette-Guérin bacillus (BCG). The Kp-90 antigen is the pellet obtained by centrifuging sonicated, French-pressed, broken bacilli at 90000g for 2 hours at 4°C.

The test was performed according to the manufacturer’s instructions (Kreatech Diagnostics, Amsterdam, The Netherlands). The serum samples and positive and negative controls included in the kits were tested in duplicate, and the cut-off control was tested in triplicate. The ratio value of the samples was calculated in function of the cut-off level and the blank in each trial. The result was considered negative when the ratio was <0.85, positive when it was >1.15, and equivocal when it was between 0.85 and 1.15. Samples for which the results were equivocal were not considered in the statistical analysis.

RESULTS
The assay was simple and performed in only 3 hours. Using the cut-off recommended by the manufacturer, the overall sensitivity of the test was 57.69%. The sensitivity increased to 70.58% in the group of adults with pulmonary tuberculosis, but decreased considerably in the group of patients with extra-pulmonary TB and in child TB, with sensitivities of 50% and 12.5%, respectively (Table). In the pulmonary TB group, the sensitivity increased when only smear-positive patients were considered (80%), while it only reached 44.44% in smear-negative patients. Our specificity in the healthy PPD-negative group was 86.11%, but the overall specificity decreased to 68.68%, mainly due to the low specificity (only 47.22%) obtained in the group of patients with non-
TB pneumonia (Table). The positive and negative predictive values of the test were respectively 49.18% and 75.55%. In 15 samples the results were equivocal. These samples were re-tested and the same results were obtained. They were therefore not considered in the statistical analysis (Table).

**DISCUSSION AND CONCLUSIONS**

The data show that the Kreatech test is not useful for TB diagnosis. Sensitivities are too low, especially in the group in which serology is particularly useful, such as smear-negative pulmonary TB (44.44%), extrapulmonary disease (50%) and child TB (12.5%). Nor do the overall sensitivity and specificity recommend the test for case finding in Barcelona.

On comparing these results with data reported by other authors, we found a coincidence in sensitivity but not in specificity. Sensitivity was similar in the previous assays, between 67% and 84% in adult pulmonary TB,4 6 and between 50% and 55% in adult extrapulmonary TB.3

We also obtained a low specificity (68.68%) that contrasts with the very high specificities (90% and 95%) reported by others. When discrepancies exist between the results of a commercially available serological test, they can be attributed, in the majority of cases, to differences in the population studied. In the case of the group of 40 non-TB pneumonia patients (36% of the control group) in our study. The specificity in this group was only 47.22%, which considerably lowers the overall specificity of our study.

The antigen determines the specificity of the test; Kp-90 is not a species-specific antigen,4 nor even a semipurified antigen. It is a complex mixture of compounds which probably contains common epitopes present in other infectious agents that also produce diseases in the respiratory tract. On the other hand, this new test detects IgA antibodies in serum. An increase in circulating IgA was found, not only in tuberculosis patients,2,8 but also in the serum of patients with other infectious respiratory diseases,6,9 as well as in patients with rheumatic and auto-immune diseases and persistent infections.10,11 Thus, the detection of IgA may increase sensitivity in the detection of pulmonary TB, but it may also be the cause of the false-positive results obtained in the group of non-TB pneumonia patients. In spite of the fact that, in earlier studies, IgA detection against purified antigens showed a high specificity,12 in this case the combination of a complex non-specific antigen and a serum with high IgA concentration is likely to be responsible for the large number of positive reactions obtained in the non-TB pneumonia patients.

In the reports where high specificities (90% and 95%) were obtained, very few serum samples belonging to non-TB pneumonia patients were studied (6.8% and 12.6%, respectively).6,13 Bearing in mind the characteristics of the test (non-purified antigen and the detection of IgA), in order to achieve a satisfactory appraisal it is necessary to include in the control group a sufficient number of patients with infectious pathologies generating antibodies which may give rise to false-positive results. One of the most widespread shortcomings in the clinical appraisal of serodiagnostic tests is the evaluation of a large number of healthy people in contrast to a small number of patients with pathologies produced by other infectious agents.

We can therefore conclude that the Kreatech test is not useful for TB diagnosis or case finding. Perhaps more than ever before, the world needs procedures for the rapid detection of all forms of TB. It is therefore essential to continue investigating both serological and other techniques in order to achieve this objective.

**Acknowledgements**

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**References**

Nous avons évalué un nouveau test sérologique ELISA de diagnostic de la tuberculose (TB) qui détecte la présence dans le sérum humain d’anticorps du type immunoglobuline A (IgA), en l’occurrence le test Kreatech TB IgA EIA. L’étude a été conduite sur 166 échantillons de sérum prélevés dans la ville de Barcelone en Espagne. Les échantillons de sérum provenaient de 56 patients TB et de 111 sujets-contrôle, dont 40 étaient atteints de pneumonie non-tuberculeuse. La spécificité du test fut de 70,58% dans le groupe adulte atteint de TB pulmonaire et de 59% dans le groupe de TB extrapulmonaire. Ces spécificités sont du même ordre que celles décrites antérieurement par d’autres auteurs.

Toutefois, nous avons observé que la spécificité du test était faible (68,68% seulement), alors que l’on avait signalé antérieurement des spécificités de 90% et de 95%. Cette discordance pourrait être attribuable à des différences dans les populations étudiées. Dans notre groupe-contrôle, nous avons inclus 40 patients atteints de pneumonie non-TB, représentant donc 36% de l’ensemble du groupe-contrôle. La spécificité du test baisse à 47,22% chez ces patients. Dans les études dans lesquelles des spécificités de 90% ou davantage avaient été signalées, très peu de patients atteints de pneumonies non-TB avaient été testés (6 à 12% de l’ensemble du groupe-contrôle).

Hemos evaluado un nuevo test serológico para el diagnóstico de la tuberculosis (TB), el test Kreatech TB IgA EIA, un enzimoinmunoensayo que detecta la presencia de anticuerpos inmunoglobulina A (IgA) en el suero humano. El estudio fue realizado utilizando 166 muestras de suero recogidas en la ciudad de Barcelona, España. De éstas, 56 pertenecían a enfermos con TB y 111 a controles, 40 de los cuales eran enfermos con neumonía no-TB. La sensibilidad del test fue del 70,58% en el grupo de adultos con TB pulmonar, y del 50% en el grupo con TB extrapulmonar. Las sensibilidades fueron similares a las publicadas previamente por otros autores.

Sin embargo, encontramos que el test tenía una baja especificidad, sólo el 68,68%, mientras que se habían publicado especificidades del 90% y 95%. Esta discrepancia puede ser atribuida a diferencias en las respectivas poblaciones estudiadas. En nuestro grupo control incluimos 40 enfermos con neumonía no-TB (36% del total del grupo control). La especificidad del test disminuye al 47,22% en estos enfermos. En los estudios previos con especificidades del 90% y superiores, fueron incluidos muy pocos sueros pertenecientes a enfermos con neumonías no-TB (6-12% del total del grupo control).