Tuberculosis (TB) is still a serious public health problem in the world, with about 8.9 to 9.9 million new cases and 1.3 million deaths occurring worldwide annually (45); it has been estimated that one-third of the world’s population is infected with Mycobacterium tuberculosis (MT) (41). One of the principles of TB control is rapid and accurate diagnosis of infected patients in order to allow prompt initiation of antibiotic therapy and to prevent transmission (2). Microscopic examination of sputum is a rapid, technically simple, and inexpensive test available for the routine diagnosis of TB in most developing countries. However, sputum smear microscopy with Ziehl-Neelsen staining is only 60 to 70% sensitive for the diagnosis of pulmonary TB (pTB) compared with the sensitivity of sputum culture (27, 29). Mycobacterial cultures are more sensitive but take at least 2 weeks, or longer if solid media are used, and culture facilities are not available in many countries. On the other hand, the lack of radiological appearances specific to TB makes chest radiography a relatively subjective and error-prone practice for the diagnosis of pTB (28). In addition, coinfection with HIV may change the clinical presentation of TB and reduce the sensitivity of classical microbiology methods (12, 20, 31). Therefore, in developing countries, it is especially important to have an inexpensive and rapid test for TB identification so that infected individuals can be isolated and treated immediately (21).

There is an urgent need for rapid, cost-effective, and accurate methods for the diagnosis of active TB. A serological test may be attractive because it would be relatively rapid and would not require sputum expectoration. Challenges for the development of effective serological tests include the need to discriminate active TB disease from other pulmonary non-TB diseases, such as bronchitis, cancer, and pneumonia, that can mimic both the clinical and the radiographic symptoms of pTB patients (35) and the need for consistent performance with genetically and immunologically diverse populations.

Several purified mycobacterial antigens, such as 85A antigen, lipoarabinomannan, plasma membrane antigen, and antigen 60 (A60), have been used for the serodiagnosis of TB, as we and other have previously reported (5, 6, 16, 22, 47). The 38-kDa protein is an immunodominant lipoprotein antigen isolated as a component of antigen 5 by affinity chromatography and is specific only for the M. tuberculosis complex (11, 39, 43). It is one of the most important immunogenic antigens of M. tuberculosis (23, 25), inducing B- and T-cell responses with high specificity for TB, and is considered a prime candidate for the development of new diagnostic reagents for diagnosis of active TB (8). The 38-kDa antigen is also a core component in various commercial serological tests (Pathozyme TB complex kit [Omega Diagnostics, Alloa, Scotland], Pathozyme Myco kits for IgG, IgM, and IgA [Omega Diagnostics], Rapid TB test [Quorum Diagnostics, Vancouver British, Columbia, Canada], and ICT Tuberculosis AMRAD-ICT [Amrad, Sydney, Australia]). Antibodies to the 38-kDa antigen occur in a high percentage of TB patients and provide the serodiagnostic test with the most favorable characteristics described to date (23, 36, 43).

The 16-kDa antigen is an immunodominant antigen, frequently called 14-kDa antigen, related to the family of low-molecular-weight heat shock proteins (HSP). This antigen contains B-cell epitopes specific for the M. tuberculosis complex (19, 33). Furthermore, it has been suggested that the 16-kDa antigen is immunogenic in the early stages of infection with M. tuberculosis.
tuberculosis and in primary TB (9). This antigen has shown considerable promise as a serodiagnostic target in assay protocols based on monoclonal antibody (Mab) competition and direct enzyme-linked immunosorbent assay (ELISA) formats (10, 26).

The 6-kDa antigen, characterized and purified in 1995 by Sorensen et al. (40), was subsequently used in different studies as an antigen with diagnostic potential, since it was found to be specific for M. tuberculosis and absent in Mycobacterium bovis BCG and in most environmental mycobacteria. The 6-kDa antigen stimulates T cells from patients with active TB, leading to an increase in gamma interferon production (4).

Many serological methods for the diagnosis of active TB have been designed and commercialized. Recently, we have evaluated commercial ELISAs for detection of antibody responses raised against mycobacterial antigens such the 38-kDa antigen, the 16-kDa antigen, lipoarabinomannan (6), and A60 (5). In this study, we evaluated a rapid immunochromatographic test to detect antibody directed against any one of the three 38-kDa, 16-kDa, and 6-kDa recombinant antigens in TB patients in Sousse, Tunisia, a region characterized by a moderate TB prevalence (9.5 new cases per 100,000 population) and incidence (21 cases/100,000/year) and a predominant M. tuberculosis strain (46).

MATERIALS AND METHODS

Setting. The present study was conducted from July 2007 to December 2010 in the Laboratory of Microbiology and Immunology, Farhat Hached University Hospital, Sousse, Tunisia, a setting with a moderate incidence (21 cases/100,000/year) of TB (46) and a predominant M. tuberculosis strain. Informed written consent was obtained from all individuals prior to blood sampling, and this study was approved by the ethics committee of the Farhat Hached University Hospital.

A total of 246 total serum samples were obtained: 171 from patients with active TB, 73 from patients without evidence of TB, and 2 from subjects with leprosy. All subjects had previously been BCG vaccinated. None of the individuals, including patients with active TB and controls, had a history of severe pathologies, including HIV infection and cardiovascular disease. Demographic data, including sex, age, and clinical types of TB, are summarized in Table 1. Serum specimens were obtained upon admission before any therapy and stored at −80°C until immunochromatographic testing.

Study population. Serum specimens were obtained from the following three groups.

(i) Active tuberculosis group. Inclusion criteria for patients in the active tuberculosis group were determined according to the recommendation defined by the American Thoracic Society (2). The diagnostic criterion for the presence of TB disease was defined as the presence of at least one of the following: (i) clinical and radiological findings consistent with TB disease and positive sputum smears for acid-fast bacilli; (ii) culture positivity of sputum, bronchial lavage, or pleural fluid, as well as samples from organ systems other than the lung, for M. tuberculosis; and (iii) pathological evidence of TB disease in biopsy materials by histological examination. The sera were obtained from 171 patients in two distinct groups: 128 patients with a diagnosis of pulmonary TB and 43 patients with a diagnosis of an extrapulmonary form of TB (epTB) (Table 1).

(ii) Nontuberculous lung disease group. The control population consisted of 73 patients without clinical evidence of TB who were being treated for underlying disorders other than mycobacteriosis (Table 1).

(iii) Leprosy group. An additional control group was established for underlying disorders other than mycobacteriosis (Table 1).

Immunochromatographic test. The SD Bioline TB IgG/IgM immunochromatographic test is based on the detection of IgG and IgM raised against one or more of the three 38-kDa, 16-kDa, and 6-kDa recombinant mycobacterial antigens. The test device has a conjugate pad containing C (control), M (TB IgM test line), and G (TB IgG test line) on the surface of the case. The inner test strip is composed of (i) a conjugate pad containing recombinant TB-specific antigens (38 kDa, 16 kDa, and 6 kDa) conjugated with colloidal gold and (ii) a nitrocellulose membrane containing C (control), M (TB IgM test), and G (TB IgG test) lines. The M line is precoated with monoclonal anti-human IgM, the G line is precoated with monoclonal anti-human IgG, and the C line is precoated with goat anti-TB antibody. The control line is used for procedural control, and it should always appear if the test procedure is performed properly and the test reagents of the control line are working. During the test, TB IgG and/or TB IgM, if present in the specimen, migrates through the conjugate pad, where it binds to the conjugate antibodies. The antibody conjugates are then captured by anti-human IgM immobilized on the M line or the specific reagents to TB IgG immobilized on the G line of the membrane, forming a colored band on the test region (M and/or G), indicating a positive result.

Immunochromatographic testing was performed according to the manufacturer’s instructions. Briefly, 10 μl of serum was added to the square sample well, and then 4 drops (90 to 120 μl) of assay diluents provided in the kit was added to the round diluent well. Interpretation of the test results was completed in 15 min after sample application. Each sample was tested in duplicate by two investigators without prior knowledge of the diagnosis status of each sample. Specimens were labeled with sequential numbers and processed blindly. This immunochromatographic test does not require special equipment and technical skill.

Statistical analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with Epi Info 6.0 software. All statistical tests were conducted using SPSS for Windows statistical software (version 15.0; SPSS, Chicago, IL). Differences were accepted as significant when the P value was <0.05.

RESULTS

In this study, we characterized the antibody response during active pTB and epTB by detecting IgG and IgM antibodies raised against the mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens in 171 patients with active TB (128 with pTB and 43 with epTB), 73 patients with pulmonary diseases other than TB, and 2 leprosy patients. The results obtained with the immunochromatographic test kit are shown in Tables 2 to 5.

IgG test. The results for detection of IgG against the mycobacterial 38-, 16-, and 6-kDa antigens are displayed in Table 2. The positivity rate of this test was around 68.4% in the active TB group (pTB plus epTB). When this group was subdivided into pTB and epTB subgroups, the positivity rate increased...
Rapid diagnostic tests for TB are required to facilitate early treatment of TB and prevention of *M. tuberculosis* transmission. Incorporation of multiple antigens in a diagnostic test may increase the sensitivity of an antibody-based test. This study evaluated the sensitivities and specificities of the multiantigen SD Bioline TB IgG/IgM test for the detection of active tuberculosis. We report, for the first time, that the IgG antibody response raised against a panel of the most important mycobacterial antigens, the 38-kDa, 16-kDa, and 6-kDa antigens (8, 9, 23, 36, 42), could be a significant marker for active pTB and could complement conventional bacteriological tests for the rapid diagnosis and discrimination of this clinical TB form from other pulmonary diseases having similar symptomatology. This test is rapid and easy to perform and could be a significant adjunct in diagnosis, particularly in resource-poor areas. This multiantigen immunochromatographic test is feasible in laboratories, with minimal technical requirements for testing.

The protective immune response in TB is cell mediated, but an antibody response is common and may be correlated with the lack of an effective cell-mediated response (7, 14, 17). Compared to assays of cell-mediated response, detection of antibodies is considerably simpler and less expensive (13), so serodiagnosis of TB has been extensively investigated (13, 15). However, after almost a century of effort, serological tests still have suboptimal sensitivity and specificity (15). The poor sensitivity may be due to substantial and unexplained heterogeneity of antibody responses to different antigens of *M. tuberculosis* among patients with active TB (32) and provides the rationale for development of multiantigen tests (1, 3). Although the poor specificity limits the utility of serological tests for diagnosis, the occurrence of false positives may provide insight into the immunopathogenesis of TB.

In this study, sensitivities of the IgG test were very high compared to those of the IgM test. These results were in agreement with previously reported data (18, 24, 43). Thus, several studies have been focused on the detection of IgG, IgM, and IgA against the 38-kDa antigen (18, 24, 43). IgA and IgM test sensitivities were significantly lower than that of IgG assays (18, 24, 43). Sensitivities ranged widely (from 16% to 94%) and specificities ranged slightly (from 93% to 100%), depending upon the smear status of TB patients and selection of patient populations in each study (18, 36, 38, 43). However, in our study, all pTB cases were smear negative and culture positive for *M. tuberculosis*. Smear microscopy status did not appear to be a determining factor for the heterogeneity of IgA, IgM, and IgG responses to the 38-kDa antigen in this study.

We suggest that the heterogeneity of the rates of positivity of immunochromatographic IgG and IgM antibodies to mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens depends on the phase of TB. Thus, in primary pulmonary TB, IgM titers were initially high but IgG titers were very low, which results in low positivity of IgG. In postprimary pulmonary TB, low titers of IgM and high titers of IgG could explain high rates of positivity of IgG. In our study, the high positive rate for IgG could be due to one or both of the following factors. (i) It could be related to delays in consulting a pneumologist for diagnosis of a highly suspected active TB. According to previously published data (18), the sensitivity of the tests also depends on the phase of the disease and on the presence of mycobacteria in sputum. In chronic and culture-positive cases, antigenic stimulation persists, and if the patient is genetically able to mount an antibody response to the specific antigens, antibody levels are expected to be elevated (18). (ii) It could be related to prolonged exposure to *M. tuberculosis* in our setting, in which TB was moderately prevalent (46).

### TABLE 2. Sensitivities and specificities of IgG in active tuberculosis cases

<table>
<thead>
<tr>
<th>Disease type</th>
<th>No. (%) of positive cases</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTB + epTB</td>
<td>117 (68.4)</td>
<td>68.4 (60.8–75.2)</td>
<td>100 (93.9–100)</td>
<td>10⁻⁸</td>
</tr>
<tr>
<td>pTB</td>
<td>100 (78.1)</td>
<td>78.1 (69.8–84.7)</td>
<td>100 (93.9–100)</td>
<td>10⁻⁸</td>
</tr>
<tr>
<td>epTB</td>
<td>17 (39.5)</td>
<td>39.5 (25.4–55.6)</td>
<td>100 (93.9–100)</td>
<td>10⁻⁸</td>
</tr>
<tr>
<td>Non-TB</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Non-TB, nontuberculosis disease; epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.

### TABLE 3. Positive and negative predictive values of IgG in active tuberculosis cases

<table>
<thead>
<tr>
<th>Disease type</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTB + epTB</td>
<td>100 (96–100)</td>
<td>58.1 (49.1–66.7)</td>
</tr>
<tr>
<td>pTB</td>
<td>100 (95.4–100)</td>
<td>72.8 (63–80.9)</td>
</tr>
<tr>
<td>epTB</td>
<td>100 (77.1–100)</td>
<td>74.3 (64.4–82.2)</td>
</tr>
</tbody>
</table>

* epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.
in agreement with WHO recommendations for the clinical utility of the single use of this new immunochromatographic test because of its specificity and tendency (in term of sensitivity) for rapid diagnosis of active pTB.

In clinical practice, delayed diagnosis of active TB is an important problem in many general hospitals in industrialized countries because it results in greater patient morbidity and mortality and intracranial spread of TB (30, 34, 37). Thus, it is very important to rapidly and correctly diagnose TB before the onset of antituberculosis therapy. In this setting, the elevated values of sensitivity, and especially positive predictive value, of detecting IgG raised against the 38-kDa, 16-kDa, and 6-kDa antigens in the pTB group compared to the group with non-TB pulmonary disease argue for the utility of clinical use of this test.

There were a number of limitations to the current study. (i) Serology testing was done retrospectively, using stored frozen sera. It is possible that the use of fresh serum may increase sensitivity. (ii) M. tuberculosis culture was used as the reference standard for the diagnosis of pTB. It is possible that some patients suspected of having pTB had culture-negative pTB disease and were improperly assigned to the group considered not to have pTB, thereby reducing the observed sensitivities of the test. However, this is unlikely since all cases included in our study are smear positive. Future studies of this test should include cases that are smear negative and culture positive. (iii) Patients from a single geographic region were included in this study, limiting the certainty that these data apply to other regions. (iv) The size of the studied populations should be expanded, and the question of whether this test is useful in the diagnostic of epTB remains. (v) The serological test has not been studied in cases where other pulmonary infections are implicated.

In summary, the main findings of the current study were as follows. (i) Clinical use of a multiple-antigen immunochromatographic test, specifically the SD Bioline TB IgG/IgM test, allowed detection of a significantly elevated number of patients with active pTB without a loss of specificity. (ii) This test can be used to facilitate and accelerate pTB diagnosis in smear-positive patients long before M. tuberculosis culture results are available. (iii) The specificity of the test was not affected by BCG vaccination of adult patients. (iv) Its elevated sensitivity and NPV argue for the clinical usefulness of this test to discriminate between patients with active pTB and patients with non-TB diseases. (v) Serodiagnosis of active TB with a simple multiantigen immunochromatographic test is feasible in laboratories, with minimal technical requirements for testing. A better understanding of the dynamic repertoire of antibody responses in patients with M. tuberculosis infection and also other mycobacterial infections may facilitate the development of more sensitive and specific antibody-based methods for the diagnosis of active pTB disease. Further studies with large numbers of patients are required to confirm our results.

ACKNOWLEDGMENT

No competing financial interests exist.

REFERENCES


TABLE 4. Sensitivities and specificities of IgM in active tuberculosis cases

<table>
<thead>
<tr>
<th>Disease type</th>
<th>No. (%) of positive cases</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTB + epTB</td>
<td>4 (2.3)</td>
<td>2.3 (0.8–6.3)</td>
<td>100 (93.9–100)</td>
<td>0.23*</td>
</tr>
<tr>
<td>pTB</td>
<td>3 (2.3)</td>
<td>2.3 (0.6–7.2)</td>
<td>100 (93.9–100)</td>
<td>0.24*</td>
</tr>
<tr>
<td>epTB</td>
<td>1 (2.3)</td>
<td>2.3 (0.1–13.8)</td>
<td>100 (93.9–100)</td>
<td>0.36*</td>
</tr>
<tr>
<td>Non-TB</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Non-TB, nontuberculosis disease; epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.

TABLE 5. Positive and negative predictive values of IgM in active tuberculosis cases

<table>
<thead>
<tr>
<th>Disease type</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTB + epTB</td>
<td>100 (39.6–100)</td>
<td>31 (25.3–37.3)</td>
</tr>
<tr>
<td>pTB</td>
<td>100 (31–100)</td>
<td>37.5 (30.8–44.6)</td>
</tr>
<tr>
<td>epTB</td>
<td>100 (5.5–100)</td>
<td>64.1 (54.7–72.6)</td>
</tr>
</tbody>
</table>

* epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.


