Immunoglobulin A (IgA) and IgG Immune Responses against P-90 Antigen for Diagnosis of Pulmonary Tuberculosis and Screening for 
Mycobacterium tuberculosis Infection

Marcus B. Conde,1,* Philip Suffys,2 Jose Roberto Lapa e Silva,1 Afranio L. Kritski,1 and Susan E. Dorman3

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The purpose of the present study was to evaluate the usefulness of detection of serum immunoglobulin A (IgA) and IgG antibodies directed against the mycobacterial P-90 antigen for the diagnosis of active pulmonary tuberculosis (PTB) among symptomatic individuals and for the detection of Mycobacterium tuberculosis infections among close contacts of PTB patients. Two commercially available enzyme immunoassay (EIA) kits (IgA EIA-TB [EIA-IgA] and IgG EIA-TB [EIA-IgG]; Kreatech Diagnostics) were evaluated in a blinded fashion by using stored serum samples from 268 individuals, including 69 patients with PTB, 41 patients with diseases other than tuberculosis (TB), 12 subjects with healed PTB, 39 close contacts of PTB patients, and 107 healthy volunteers. For the EIA-IgA, the sensitivity was 74% and the specificity was 68% when a cutoff determined by a receiver operator characteristic curve was used. For the EIA-IgG, the sensitivity was 69% and the specificity was 64%. The EIA-IgA was positive for 54% of healthy close contacts of PTB patients but only 8% of healthy controls without contact with a PTB patient or a prior personal history of TB (P < 0.001). The relatively low sensitivities and specificities of these serologic tests make them poor tools for the diagnosis of PTB among patients with suspected PTB. However, the relatively high prevalence of positive EIA-IgA results among healthy close contacts of PTB patients warrants further evaluation of this test with close contacts and other populations at risk for recent M. tuberculosis exposure and development of disease.

Pulmonary tuberculosis (PTB) remains one of the leading causes of morbidity and mortality worldwide, with approximately 8 million new cases and nearly 2 million deaths occurring each year (5). Although the examination of sputum smears for acid-fast bacilli is a rapid diagnostic method with a good sensitivity for the detection of mycobacteria among tuberculous (TB) patients with cavitary pulmonary disease, it has a low sensitivity among patients with noncavitary pulmonary TB, those with extrapulmonary TB, and those who are unable to expectorate spontaneously (8). Because only 40 to 60% of patients with PTB are positive for acid-fast bacilli by sputum smear, culture of Mycobacterium tuberculosis is considered the “gold standard” methodology for the laboratory diagnosis of TB. However, culture may require several weeks before results are available.

There is an urgent need for rapid, cost-effective, and accurate methods for the diagnosis of TB. A serologic test is attractive because it would be relatively rapid and would not require sputum expectoration. Challenges for the development of effective serologic tests include the need to discriminate active disease from latent infection, to avoid cross-reactivity with M. bovis BCG or mycobacteria other than M. tuberculosis, and to perform consistently with genetically and immunologically diverse populations. Although most serologic tests studied to date have evaluated the immunoglobulin G (IgG)-mediated humoral immune response against mycobacterial antigens, other immunoglobulin classes may have more specificity for the diagnosis of TB, depending on the type of antigen used (4).

The purpose of the study described here was to evaluate the usefulness of detection of IgA and IgG antibodies directed against the mycobacterial P-90 antigen for the diagnosis of PTB and for the screening of close contacts of PTB patients in Rio de Janeiro, Brazil, a setting with a high prevalence of TB.

MATERIALS AND METHODS

Subject enrollment. The study setting was the Hospital Universitario Clementino Fraga Filho of the Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil. Between 1 June 1997 and 31 March 1999, subjects (age, 18 years and older) with respiratory symptoms and radiographic findings consistent with PTB (TB suspects) were prospectively enrolled. Leprosy patients, asymptomatic subjects without a history of active PTB or contact with PTB patients, and asymptomatic household contacts of PTB patients were also included in the study. The study was approved by the Ethics Committee of the Hospital Universitario Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, and informed consent was obtained from all study participants.

Clinical and laboratory evaluations. A medical history was obtained from the study subjects, including information about prior BCG vaccination. For TB suspects, a physical examination (including evaluation for the presence or absence of a BCG vaccination scar), chest radiograph, and testing for human
immunodeficiency virus infection were performed. Two sputum samples from each TB suspect were stained with the hematoylin-eosin, Papanicolaou, Ziehl-Neelsen, and Grocott's methamine silver stains and cultured on Löwenstein-Jensen and Sabouraud media. TB suspects unable to expectorate spontaneously underwent sputum induction and/or fiberoptic bronchoscopy with washout and/or transbronchial biopsy. Sputum samples were transported to the laboratory under aerobic conditions. Sputum specimens were inoculated onto Lowenstein-Jensen medium, and 21% of the patients were positive by culture of a respiratory tract specimen.

RESULTS

Two hundred sixty-eight serum samples were evaluated, including 112 from PTB suspects, 10 from patients with leprosy, 39 from close contacts of PTB patients, and 107 from healthy volunteers. Among the 112 TB suspects, 69 were diagnosed with active PTB (63 with bacteriologic diagnosis, 6 with clinical diagnosis), 21 had nontuberculous mycobacterial infections, 10 had lung cancer, and 12 had healed TB. PTB patients had a mean ± standard deviation age of 39.1 ± 14 years. Compared with the PTB patients, TB suspects without PTB were older (mean age, 49 ± 14 years [P < 0.001]), and healthy controls were younger (mean age, 22 ± 4 years [P < 0.001]).

Table 1 shows the OD values, as well as the sensitivities and the specificities, of the EIA-IgA and EIA-IgG by using the manufacturer-recommended OD ratio cutoff values and, separately, by using the OD ratio cutoff values determined by ROC analysis. The OD values of EIA-IgA and EIA-IgG were higher for patients with PTB than for healthy controls (P < 0.001 for EIA-IgA, P < 0.001 for EIA-IgG), close contacts (P = 0.003 for EIA-IgA, P < 0.001 for EIA-IgG), and patients with nontuberculous mycobacterial lung diseases (P < 0.001 for EIA-IgA, P < 0.001 for EIA-IgG). There were no differences between the OD values for PTB patients and individuals with healed TB. By ROC analysis, an OD ratio cutoff value of 1.4 (rather than the OD ratio of 1.15 recommended by the manufacturer) was determined for each test. By using a cutoff value of 1.4, both tests had better specificities for healthy controls, but the rate of false-positive results remained high among TB suspects without PTB. However, the proportion of subjects with a positive EIA-IgA result was significantly higher among close contacts of PTB patients than among healthy controls with no history of PTB or contact with a PTB patient, even after stratification by tuberculin skin test result and BCG vaccination (P < 0.001 for all comparisons).

To improve the diagnostic yields of the assays, we evaluated the sensitivity and specificity of the combination of EIA-IgA and EIA-IgG results, using an OD ratio cutoff value of 1.4. In a scenario in which a subject was considered to have a positive result if the results of both tests were positive, the sensitivity was 51% (95% confidence interval [CI], 45 to 57%) and the specificity was 89% (95% CI, 85 to 93%). In a scenario in which a subject was considered to have a negative result if the results of both tests were negative, the sensitivity was 92% (95% CI, 89 to 95%) and the specificity was 43% (95% CI, 37 to 49%).

By use of an OD ratio cutoff value of 1.4, the NPV calculated for TB prevalence rates of 0.054% (the mean rate for Brazil) and 0.16% (the mean rate for the city of Rio de Janeiro) were greater than 99% for both EIA-IgA and EIA-IgG. By use of TB prevalence rates for Brazil and Rio de Janeiro, the PPVs of EIA-IgA were 0.12 and 0.31%, respectively, while the PPVs of EIA-IgG were 0.10 and 0.30%, respectively.

DISCUSSION

In the present study the best results were obtained by EIA-IgA, which was more sensitive and specific than EIA-IgG. However, the performances of both tests varied according to the cutoff point used for the diagnosis of TB. By using the cutoff point suggested by the manufacturer, the sensitivities were 82 to 83% for EIA-IgA and 67 to 75% for EIA-IgG, but there were many false-positive results for non-PTB subjects. Therefore, we used a ROC curve analysis to determine a new cutoff point of 1.4 for each test (3). By use of the new experimentally derived cutoff point, the overall specificities of both tests were higher, with >90% specificity of the EIA-IgA for healthy controls. Although serum IgA directed against P-90 has been reported to occur only in individuals with active TB (11), we found many false-positive EIA-IgA results for individuals with non-TB respiratory diseases, healed TB, leprosy, and recent close contacts of TB patients. When only smear-negative PTB patients were considered, the sensitivity of EIA-
IgA was only 41%. The poor sensitivity for this important patient subgroup and the poor specificity for non-PTB patients with respiratory symptoms limit the clinical usefulness of EIA-IgA for the diagnosis of PTB among PTB suspects. Overall, our results were generally similar to those previously reported for EIA-IgA P-90-based serological tests and two different IgG-based immunochromatographic serology tests evaluated in our hospital (1, 2, 6, 7, 9, 10).

Interestingly, using a cutoff ratio of 1.4, we found that the EIA-IgA was positive for 21 of 39 (54%) healthy close contacts of PTB patients but just 9 of 107 (8%) healthy individuals without such contact (P < 0.001). The EIA-IgA therefore may be useful for detection of individuals recently infected with M. tuberculosis. To our knowledge, this is the first time that the EIA-IgA has been evaluated with close contacts of PTB patients. Our finding raises the possibility that the low specificity of some serologic tests performed in regions where TB is endemic may be the consequence of a high rate of recent M. tuberculosis infection not evident by tuberculin skin testing.

The present study has several limitations. The serologic tests described here were performed retrospectively with serum that had been stored frozen and thawed once. Although no serum had been thawed more than once and none of the samples had been frozen for more than 28 months, we cannot formally exclude the possibility that the serum storage conditions adversely affected the test performance, although we believe that this is unlikely. The use of fresh serum could have some impact on the sensitivities of the tests. In addition, we did not include sera from individuals with respiratory infections due to mycobacteria other than M. tuberculosis.

We conclude that neither EIA-IgA nor EIA-IgG is useful for the diagnosis of PTB among PTB suspects with respiratory symptoms. Additional studies are warranted to assess the utility of EIA-IgA as a screening test for M. tuberculosis infection among healthy close contacts of PTB patients.

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REFERENCES


