Immunoglobulin G response to mammalian cell entry 1A (Mce1A) protein as biomarker of active tuberculosis

Iukary Takenami a, *, 1, Carolina C. de Oliveira a, 1, Filipe R. Lima a, b, Jéssica Soares a, b, Almério Machado Jr. b, c, Lee W. Riley d, Sérgio Arruda a, b

a Laboratório Avançado de Saúde Pública, Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz, P.O. Box 121, Salvador, BA 40296 710, Brazil
b Escola Bahiana de Medicina e Saúde Pública, P.O. Box 3386, Salvador, BA 41150 100, Brazil
c Hospital Especializado Octávio Mangabeira, Secretaria da Saúde do Estado da Bahia, Salvador, BA 40320-350, Brazil
d Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, 530E Li Ka Shing Bldg, Berkeley, CA 94720, USA

* These authors contributed equally.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Tuberculosis (TB) is a chronic bacterial infection, caused primarily by the obligate human pathogen Mycobacterium tuberculosis. Although nearly one-third of the human population is infected with M. tuberculosis, only 10% of these individuals develop active disease during their lifetime [1]. Early diagnosis and effective treatment of TB cases is the most effective tool available to control the disease.

The identification of the bacillus by microscopic examination of sputum smear or by culture has several limitations. Approximately 40% of TB patients test negative by microscopy, and culture requires a long time for the growth of M. tuberculosis, which delays diagnosis [2,3]. Additionally, other pulmonary non-TB diseases such as cancer, pneumonia, pulmonary abscess, bronchitis and bronchiectasis may present with similar clinical symptoms and radiographic patterns [4,5].

Therefore, a rapid diagnostic test with both high sensitivity and specificity is still needed. The most significant advance in last few years was the development of real time PCR assay (Xpert® MTB/RIF) for detection of M. tuberculosis DNA and mutations associated with resistance to rifampicin. However, the higher cost and sophisticated infrastructure requirements have remained major barriers for their large-scale implementation for routine use. Further, the test does not eliminate the need for conventional tests, which are required to monitor treatment success and detect resistance to drugs other than rifampicin [6]. Currently the standard method to monitor treatment response is still sputum smear microscopy conversion after two months of treatment. In a meta-analysis, Horne et al.
were considered TB patients when they had clinical and Ziehl-Neelsen staining and the results were grouped as negative, tuberculosis drugs. Sputum smear microscopy was performed by (AFB) on microscopy, 3) individuals who responded to anti-TB opacities, 2) sputum samples that contained acid-fast bacilli following characteristics: 1) chest radiography (CXR) suggestive of participate in this study. The diagnosis of pulmonary TB was categorized into

2.1. Setting

This prospective study was conducted at Centro de Saúde Rodrigo Argolo (CSRA) in Salvador, Brazil. A total of 104 eligible participants were recruited using convenience sampling from January 2012 to October 2013.

2.2. Study population

The study was previously approved by the Human Subject Ethics Committee of Oswaldo Cruz Foundation in Salvador, Brazil. All subjects provided informed consent to participate in this study according to national guidelines. Then, the participants were categorized into five groups, as follows.

2.2.1. Pulmonary TB patients (n = 39)

All cases of pulmonary TB attending in the CSRA were invited to participate in this study. The diagnosis of pulmonary TB was established by symptoms consistent with TB and one or more of the following characteristics: 1) chest radiography (CXR) suggestive of TB opacities, 2) sputum samples that contained acid-fast bacilli (AFB) on microscopy, 3) individuals who responded to anti-tuberculosis drugs. Sputum smear microscopy was performed by Ziehl-Neelsen staining and the results were grouped as negative, 1+, 2+, or 3+. Patients whose sputum smears were AFB negative were considered TB patients when they had clinical and radiological characteristics suggestive of pulmonary TB and when they showed clinical improvement after the anti-TB treatment.

2.2.2. Household contacts (n = 34)

At the time of the TB case identification, their household contacts (HHC) were enrolled into the study. HHC included all those who lived in the same household as the TB patient, or who have reported at least 100 h of contact with the patient. The tuberculin skin test (TST) was performed on all HHC. TST was done by the Mantoux procedure with 5TU of RT23 purified protein derivate (RT23 PPD) (Statens Serum Institute, Copenhagen, Denmark). Reading was performed after 72 h by a trained nurse. The TST response was categorized as: 0–5 mm, negative; ≥5 mm, positive and indicative of infection by M. tuberculosis. To confirm M. tuberculosis latent infection (LTBI), interferon-gamma release assay (IGRA) was also performed. We used Quantiferon-TB Gold In Tube (QFT-IT; Cellestis Limited, Carnegie, Victoria, Australia). The test was performed according to the manufacturer’s instructions [14]. The cut-off value for a positive response was 0.35 IU/ml. Samples that gave indeterminate or discordant TST and IGRA results were excluded. Blood was drawn for the baseline IGRA before the TST was administered; both were conducted on the same day. The household contacts were stratified into two groups: 1) TST-negative and IGRA-negative [healthy household contact; HHC(−)] or 2) TST-positive and IGRA-positive [latently infected household contacts; HHC(+)]. Those with TB-like symptoms were further evaluated, including by sputum examination and/or chest radiography to exclude disease.

2.2.3. Non-TB patients (n = 13)

This category included subjects with symptoms of TB (cough, fever, loss of appetite) but with other lung diseases. TB was ruled out in this group by sputum smear and mycobacterial cultivation. Among patients who presented with other lung diseases, five had bacterial pneumonia, two had lung cancer, one had bronchial asthma and the remaining five subjects had other pulmonary infections.

2.2.4. Leprosy patients (n = 18)

An additional control group included cases of leprosy. These cases were confirmed bacteriologically using smear microscopy and histological examination. The sera from these leprosy patients were obtained from Hospital Couto Maia. Exclusion criteria were volunteers who tested positive for human immunodeficiency virus (HIV) and patients taking immunosuppressive drugs.

2.3. Serum specimens and ELISA

Serum specimens were obtained upon recruitment and stored at −80 °C until tested. Patients who had a confirmed diagnosis of TB received anti-TB treatment with isoniazid, rifampin, pyrazinamide and ethambutol for two months followed by isoniazid and rifampin for four months. Serum samples were prospectively collected at baseline, two months and six months after starting the treatment. Measurement of total IgG, IgM, and IgA against Mce1A protein was performed with an indirect enzyme-linked immunosorbent assay (ELISA) [15]. The recombinant protein used in this study was provided by Dr. L.W. Riley (University of California, Berkeley, CA, USA), which has been described previously [16]. Mce1A protein (10 μg/ml) was diluted to 1:1000 in ethanol and 50 μl of the solutions were dried overnight in polystyrene ELISA well plates (Greiner bio-one). ELISA plates were blocked with 100 μl of 3% low fatty-acid bovine serum albumin (BSA) (US Biologicals) and washed with phosphate buffered saline (PBS, pH 7.4) (GIBCO, Invitrogen), according to washing protocol. Frozen serum samples were thawed and
twice and diluted 1:100 in 3% BSA. One hundred microliter of diluted sample was added to the plate and incubated for 1 h at room temperature (RT), followed by three washes with 1× PBS. Then, 100 µl of 1:10,000, 1:50,000, 1:10,000 goat-derived anti-human IgM, total IgG and IgA, respectively, labeled with horseradish peroxidase (HRP) (Sigma–Aldrich) diluted in 3% BSA/PBS was added, followed by incubation at RT for 1 h and washed again with 1× PBS. The secondary antibodies were tested using a titration to determine the optimum working dilution. Finally, 100 µl of tetramethylbenzidine substrate (TMB) (Invitrogen Life Technologies) was added and the plate was incubated for 1 h. Then, the reaction was stopped with 100 µl of 2 N sulfuric acid.

Reactions were read within 10 min at 450 nm in a spectrophotometer (Thermo Scientific). The results were read out as the average of optical density (OD) of triplicate samples and were re-run if >10% coefficient of variance was observed.

2.4. Statistical analysis

Data were analyzed by GraphPad Prism v.5.0 (GraphPad Inc., San Diego, CA). The immunoglobulin levels were expressed as median and interquartile interval (IQR). Statistical variations were analyzed by Mann–Whitney U test or Kruskal–Wallis test followed by the Dunn test. For longitudinal analysis of immunoglobulin levels on anti-TB treatment, differences between time points were first assessed by Friedman tests. Spearman’s correlation test was used to assess correlation between immunoglobulin levels and clinical data. The ability of immunoglobulin levels to discriminate active TB from non-TB disease was performed with receiver operating characteristic (ROC) curve and the area under curve (AUC) was also analyzed. The significance of association for categorical variables was estimated by Chi-squared test or Chi-square test for linear trend. The level of statistical significance was set at \( p < 0.05 \).

3. Results

3.1. Characteristics of study population

The active TB group included 39 pulmonary TB patients. Of these, 26 (67%) had a positive sputum smear result. From these TB patients’ households, 57 HHC were enrolled into the study. Of these, 23 (40%) were excluded due to the following reasons: six (10%) did not return for TST reading; three (5%) had indeterminate IGRA and fourteen (25%) had discordant results between TST and IGRA. Of the 34 eligible HHC, 19 (56%) were latently infected [HHC(+)] and 15 (44%) were healthy controls [HHC(−)]. Besides the HHC group, we included non-TB (\( n = 13 \)) and leprosy (\( n = 18 \)) patients. The demographic and clinical characteristics of these study subjects are listed in Table 1. Chi-squared tests demonstrated no significant differences (\( p > 0.05 \)) in these demographic characteristics among the study groups (Table 1).

3.2. IgA, IgM and total IgG response against Mce1A protein

The anti-Mce1A IgA, IgM and total IgG ELISA results are shown in Figure 1. All immunoglobulin patterns were significantly higher in untreated TB patients than in the control groups (\( p < 0.0001 \)). The median values of IgA against Mce1A protein were significantly higher in pulmonary TB patients (median: 0.601 [IQR: 0.393–1.199]) than in HHC (median: 0.279 [IQR: 0.137–0.464]), HHC(+) (median: 0.152 [IQR: 0.109–0.249]), and leprosy patients (median: 0.307 [IQR: 0.208–0.514]; Figure 1A). IgM levels were also able to discriminate TB patients (median: 0.591 [IQR: 0.370–0.871]) from leprosy patients (median: 0.039 [IQR: 0.022–0.110]; Figure 1B).

Further, TB patients were found to have higher levels of total IgG against Mce1A (median: 0.774 [IQR: 0.570–1.098]) than those in the control groups [HHC, HHC(−), HHC(+), non-TB and leprosy patients (Figure 1C)]. The median (IQR) for the control groups was 0.426 (0.351–0.558), 0.414 (0.346–0.503), 0.431 (0.353–0.765), 0.481 (0.301–0.541) and 0.350 (0.322–0.378), respectively. Interestingly, seven (37%) of 19 HHC with LTBI had median IgG levels similar to those of the TB patients.

3.3. Correlation between IgA, IgM and total IgG response against Mce1A protein and clinical data

When TB patients were categorized by chest X-ray status, total IgG anti-Mce1A levels were higher in pulmonary TB patients with cavitary lesions than in those without cavitary lesions (\( p = 0.046 \)) (Figure 2). On the other hand, no significant differences were observed for other immunoglobulins (IgA and IgM, \( p = 0.438 \) and \( p = 0.674 \), respectively). Furthermore, no difference was found in immunoglobulin levels when compared with sputum smear

### Table 1
Demographic and clinical characteristics of pulmonary TB patients, their household contacts, non-TB and leprosy patients.

<table>
<thead>
<tr>
<th></th>
<th>TB patients (( n = 39 ))</th>
<th>HHC (( n = 34 ))</th>
<th>HHC infection status</th>
<th>Non-TB patients (( n = 13 ))</th>
<th>Leprosy patients (( n = 18 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>40.8 ± 15.5</td>
<td>30.4 ± 20.8</td>
<td>29.9 ± 18.3</td>
<td>30.7 ± 22.8</td>
<td>44.6 ± 13.6</td>
</tr>
<tr>
<td>Male, (%)</td>
<td>25 (64.1)</td>
<td>15 (44.1)</td>
<td>7 (46.7)</td>
<td>8 (42.1)</td>
<td>5 (38.5)</td>
</tr>
<tr>
<td>BCG scar, (%)</td>
<td>26 (66.7)</td>
<td>26 (76.5)</td>
<td>11 (73.3)</td>
<td>15 (78.9)</td>
<td>11 (84.6)</td>
</tr>
<tr>
<td>History of tuberculosis, n (%)</td>
<td>2 (5.1)</td>
<td>1 (2.9)</td>
<td></td>
<td>1 (6.2)</td>
<td>1 (7.7)</td>
</tr>
<tr>
<td>Sputum density of index case, n (%)</td>
<td>Negative 13 (33.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>13 (100)</td>
</tr>
<tr>
<td></td>
<td>1+ 11 (28.2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>2+ 8 (20.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3+ 7 (18)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CXR, (%)</td>
<td>Cavities 16 (57.1)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>No cavities 12 (42.9)</td>
<td>34 (100)</td>
<td>15 (100)</td>
<td>19 (100)</td>
<td>–</td>
</tr>
<tr>
<td>Antibody anti-Mce1A, median (IQR)</td>
<td>IgA 0.601 (0.393–1.199)</td>
<td>0.279 (0.137–0.464)</td>
<td>0.454 (0.322–0.543)</td>
<td>0.152 (0.109–0.249)</td>
<td>0.293 (0.258–0.400)</td>
</tr>
<tr>
<td></td>
<td>IgM 0.591 (0.370–0.871)</td>
<td>0.433 (0.363–0.581)</td>
<td>0.412 (0.363–0.449)</td>
<td>0.447 (0.323–0.635)</td>
<td>0.435 (0.372–0.599)</td>
</tr>
<tr>
<td></td>
<td>IgG 0.774 (0.576–1.098)</td>
<td>0.426 (0.351–0.558)</td>
<td>0.414 (0.346–0.503)</td>
<td>0.431 (0.353–0.765)</td>
<td>0.481 (0.301–0.541)</td>
</tr>
</tbody>
</table>

\* CXR not available for eleven TB patients.

\* BCG = bacille Calmette-Guérin; CXR = chest radiograph; HHC = household contacts; SD = standard deviation; TB = tuberculosis.
status, sex, race, age, BCG, use of alcohol, tobacco and drugs in the TB patients group (p > 0.05).

Additionally, immunoglobulin’s levels were also evaluated in HHC according to their TST and IGRA results. There was no correlation between TST induration diameter and IgM (r = 0.173, p = 0.329) or IgG levels (r = 0.226, p = 0.206). Similarly, there was no relationship between IFN-γ levels measured by IGRA and the IgM (r = 0.144, p = 0.415) or IgG levels (r = 0.235, p = 0.187). However, negative correlation was observed between IgA levels and TST diameter (r = −0.642, p < 0.0001) or IFN-γ (r = −0.609, p < 0.0001), respectively.

3.4. Qualitative ELISA results and diagnostic values for pulmonary TB

Based on the significant difference found in the quantitative analysis of serological tests, we performed an ROC analysis. For this purpose, we defined pulmonary TB group as the “diseased group” and their HHC, non-TB and leprosy patients as the “control group”. AUC analysis results were obtained for all, independent of the immunoglobulin used. The positive test results for all immunoglobulins in each group are shown in Table 2. With the cut off value of 0.482 OD, established according to the ROC method, the IgA test was positive in 24 (62%) of 39 TB patients. Overall sensitivity and specificity of the test was 59% and 77.3%, respectively (Figure 3A,B). With the cut off value of 0.585 OD, the IgM test was positive in 21 (54%) of 39 TB patients. The sensitivity and specificity was 51.3 and 83.3%, respectively (Figure 3C,D). The ROC curve determined that the optimal index cut off value for the anti-Mce1A total IgG ELISA was 0.546 OD, with a corresponding sensitivity of 79.5% and a specificity of 84.4% (Figure 3E,F). The total IgG anti-Mce1A was positive in 32 (82%) of 39 TB patients. Further, when we considered the combination of immunoglobulins (IgG + IgA, IgG + IgM or IgG + IgA + IgM) results, the sensitivity was not improved (data not shown). Interestingly, seven latently infected HHC with positive IgG test results showed a negative correlation with the IFN-γ production (r = −0.857, p = 0.024). All of them had a TST reaction of ≥15 mm of induration.

3.5. Time-course changes in IgG, IgM and IgA antibody titers after initiation of anti-TB treatment

The median levels of IgG, IgM and IgA antibodies against Mce1A protein in TB patients decreased during treatment. The median of all immunoglobulins decreased significantly between the first and third (six months) serum samples or between the second and third serum samples (p = 0.036 and p = 0.019, respectively; Figure 4A and B). Similarly, the median IgG levels

<p>| Table 2: Positivity to Mce1A protein in different groups of study. |
|---------------------|---------------------|---------------------|---------------------|
| Study groups       | No. of cases | No. of seropositive patients (%) |</p>
<table>
<thead>
<tr>
<th></th>
<th>IgA</th>
<th>IgM</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB patients</td>
<td>39</td>
<td>24  (62)</td>
<td>21  (54)</td>
</tr>
<tr>
<td>Healthy HHC</td>
<td>15</td>
<td>7   (47)</td>
<td>2   (13)</td>
</tr>
<tr>
<td>Latently infected HHC</td>
<td>19</td>
<td>1   (5)</td>
<td>6   (32)</td>
</tr>
<tr>
<td>Non-TB patients</td>
<td>13</td>
<td>3   (23)</td>
<td>3   (23)</td>
</tr>
<tr>
<td>Leprosy patients</td>
<td>18</td>
<td>4   (22)</td>
<td>—</td>
</tr>
</tbody>
</table>

Chi-squared test, p < 0.0001 for all immunoglobulins.
TB = tuberculosis; HHC = household contacts.

Figure 1. IgA (A), IgM (B) and IgG (C) antibody levels against Mce1A protein in different groups of sera tested. Statistical significance was determined by Kruskal–Wallis test followed by the Dunn test; significance was considered at p < 0.05, p < 0.01 or p < 0.001 as represented by *, ** and *** respectively. TB = tuberculosis patients (n = 39); HHC = household contacts of pulmonary tuberculosis patients (n = 34); HHC(−) = healthy household contacts (n = 15); HHC(+) = latently infected household contacts (n = 19); Non-TB = patients without tuberculosis (n = 13); LP = leprosy patients (n = 18); OD = optical density. The height of the line within each bar represents the median OD value.
decreased significantly between the first, second (two months) and third (six months) serum samples \((p < 0.0001, \text{Figure 4C})\). Furthermore, after the completion of treatment, no statistical difference was observed between median IgM and total IgG levels in TB patients and HHC(−) or HHC(+) \((p = 0.290 \text{ and } p = 0.185, \text{respectively; Figure 4B and C})\).

The frequency of positive test results for IgA, IgM and total IgG is shown in Table 3. There was also a significant negative linear trend in the positive test results among the TB group before the treatment, at the end of two months and six months of treatment (IgA, \(p = 0.023\); IgM, \(p = 0.001\) and IgG, \(p < 0.0001\)).

4. Discussion

Here we assessed the humoral response to Mce1A as a potential biomarker for the diagnosis of TB and response to treatment. We found that immunoglobulin levels were significantly higher in new pulmonary TB patients than those of the control groups. In this study, a cut-off value of 0.546 for the total IgG ELISA provided the best result, detecting 82% of the TB patients compared with IgA and IgM results. However, the combination of immunoglobulin tests did not increase the positive test results. In the control groups, very few individuals had a positive serology; 23% \((15/65)\) were positive for IgA, 17% \((11/65)\) for IgM or total IgG. Other studies that examined humoral response against other \(M. \text{tuberculosis}\) proteins in the control population found responses ranging from 4.1 to 23.4% for IgA, \(7\)−\(19.5\)% for IgM and \(12\)−\(24.7\)% for IgG\(\cite{17}−\cite{19}\). In the present study, routine sputum smear examination diagnosed 26 \((67\%)\) of 39 TB cases. The IgG ELISA identified 10 \((77\%)\) additional cases among

![Figure 2](image)

**Figure 2.** Total IgG anti-Mce1A levels in cavitary and non-cavitary groups. Horizontal line represents the median value of each group. Statistical significance was determined by performing Mann−Whitney U test \((p < 0.05)\). OD = optical density.

![Figure 3](image)

**Figure 3.** Receiver operating characteristic analysis for comparison of IgA anti-Mce1A \((A, B)\), IgM anti-Mce1A \((C, D)\) and total IgG anti-Mce1A \((E, F)\) between active pulmonary TB patients \(n = 39\) and control group \(n = 65\). AUC = area under the curve; CI = confidence interval; Se = sensitivity; Sp = specificity; LR+ = positive likelihood ratio; TB = tuberculosis.
those who tested sputum-smear negative. ROC-curve analysis showed high sensitivity and specificity of the IgG ELISA. These results are better than those previously reported in other studies using different \(M.\) \(tuberculosis\) protein antigens for TB serodiagnosis [17–19]. The total IgG in response to \(Mce1A\) is associated with advanced cavitary TB. Mizusawa et al. reported that titers of IgG to glycolipid were higher in pulmonary TB patients with cavitary lesions than in those without cavitary lesions [20]. This increase of total IgG in cavitary TB patients may reflect liquefied caseum containing high numbers of the tubercle bacilli. Here, we hypothesize that the \(mce1\) gene is upregulated during growth and multiplication of the bacilli in the host, leading to higher expression of \(Mce1A\) protein and thereby promoting antibody response to the protein. Hence, IgG antibody could play a role as an inflammatory marker in pulmonary TB.

Interestingly, a group of seven HHC with LTBI with TST induration diameter > 15 were found to be positive by the IgG ELISA. It is known that latently infected individuals produce high levels of IFN-\(\gamma\), and that this cytokine is a key determinant in protection against TB [21]. We wonder if low-level IFN-\(\gamma\) production associated with high-level anti-\(Mce1A\) IgG response may indicate a predictive biomarker for progression from LTBI to active disease? We will follow these seven HHC and expand our study to identify more such HHC to see if any of them develop TB over time.

\(Mycobacterium\) \(leprae\) has a homolog of the \(mce1A\) gene, as do nontuberculous mycobacteria (NTM) [22–24]. Das et al. demonstrated that an immunodominant linear epitope KRRITPKD (residues 131–138 in \(Mce1A\)) is highly conserved in \(M.\) \(tuberculosis\). This may explain the difference in response we observed between TB and leprosy patients. The \(Mce1A\) protein is also present in cell wall of \(Mycobacterium\) \(bovis\) BCG [25–27]. However, we did not observe any statistical difference in the anti-\(Mce1A\) ELISA response between BCG-vaccinated and non-vaccinated patients. Since BCG is given at birth in Brazil, such a difference is unlikely to be observed in adults. The high anti-\(Mce1A\) IgG response indicates active disease but perhaps also recent infection in those with LTBI (as observed among HHC with LTBI).

Alternative ways to monitor TB treatment response are needed. We found that changes in serum IgG anti-\(Mce1A\) antibodies appear to be a useful marker to monitor treatment response. The decline in total IgG may reflect decrease in bacterial burden and healing of lung lesions in these patients. However, despite the decrease in the immunoglobulin levels, 69% of TB patients had IgG test that remained positive after two months of anti-tuberculosis treatment. In addition, only five of the 32 (16%) patients had conversion from seropositivity to seronegativity for IgG test. On the other hand, 20 of the 26 (77%) patients had sputum conversion at the first two months. Taken together, these considerations suggest that sputum

---

**Figure 4.** Levels of serum IgA (A), IgM (B) and total IgG (C) against the \(Mce1A\) protein in active TB patients during the anti-TB treatment. Sampling time (0) just before treatment, (2) after two months of treatment, (6) after six months of treatment. Statistical significance was determined by Friedman tests (between TB patients) or Kruskal–Wallis test (between groups); significance was considered at \(p < 0.05, p < 0.01\) or \(p < 0.001\) as represented by *, ** and *** respectively. The height of the line within each bar represents the mean OD value. TB = tuberculosis; HHC = household contacts; Mo = months; OD = optical density.

**Table 3**

<table>
<thead>
<tr>
<th>TB patients</th>
<th>No. of seropositive patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>At baseline</td>
<td>24 (62)</td>
</tr>
<tr>
<td>After 2 months of treatment</td>
<td>23 (59)</td>
</tr>
<tr>
<td>At the end of treatment</td>
<td>18 (46)</td>
</tr>
</tbody>
</table>

Chi-squared test for trend: IgA: \(p > 0.023\); IgM: \(p = 0.002\); IgG: \(p = 0.0001\).

TB = tuberculosis.
smear is more sensitive than IgG test in monitoring treatment response among smear-positive TB patients.

Serologic tests based on *M. tuberculosis* are not recommended by WHO for the diagnosis of TB. However, our test based on response to Mce1A may be usable as an initial screening test, especially in those who are sputum-smear test negative. It may also have utility as a superior to smear tests for monitoring treatment response. The sensitivity of serologic tests in acquired immune deficiency syndrome (AIDS) patients is unacceptably low in tests based on most *M. tuberculosis* proteins. A larger study including HIV-infected patients is necessary to further evaluate the validity of this new test.

Acknowledgments

The authors would like to thank the Dra Teresinha Siquara and the nurses Eurides dos Santos and Ana Lúcia by support in examining patients. We wish to offer our thanks to the following students who participated in this project by technical support: Ana Gomes, Carolina Cunha, Lara Andrade, Nana Guimarães, Raquel Cavalcante and Tomás Viana.

Funding: This work was supported by Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), Brazil.

Competing interests: None declared.

Ethical approval: Ethical approval was provided by the Human Subject Ethics Committee of Oswaldo Cruz Foundation in Salvador, Brazil (number 256/2012).

References

2. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. Am J Respir Crit Care Med 2000;161:1376–95. http://dx.doi.org/10.1164/ajrccm.161.4.16141.