Western blot is an efficient tool in differential diagnosis between paracoccidioidomycosis and pulmonary tuberculosis

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Running title: differential diagnosis between PCM and TB

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ABSTRACT

Sputum and sera from 134 patients screened for tuberculosis (TB) were analyzed to investigate TB and paracoccidioidomycosis (PCM). Of these patients, 11 (8.2%) were confirmed to have TB, but six (4.5%) were positive only for PCM. All patients with PCM presented anti 43KDa component antibodies in Western Blot (WB), while in the TB-positive patients it did not appear. This preliminary study suggests WB as a potential tool for the differential laboratory diagnosis between TB and PCM.

Key-words: Paracoccidioidomycosis. Tuberculosis. Differential diagnosis. Serology
Paracoccidioidomycosis (PCM) is an endemic Latin American mycosis caused by *Paracoccidioides brasiliensis* and also by the recently described *P. lutzii*. It is an important systemic mycosis, which presents with a wide range of clinical signs and symptoms. Although PCM has been described for more than one hundred years and is considered endemic in many countries, until today there are serious problems in relation to differential diagnosis of this important systemic mycosis (11, 13). The lungs are affected in about 75% of cases, and the initial pulmonary lesions are similar to the tuberculosis (TB) (7). Furthermore, the association between PCM and TB is not uncommon, it occurs in a frequency varying between 5.5 and 15.8% (10, 13) so the differential diagnosis between these two diseases as well as to detect co-infection with TB and PCM would be very important.

A characterization based only on clinical and radiological data, can be difficult, especially in endemic areas, since both diseases may occur simultaneously or sequentially. Diagnostic error can occur, especially in basic health units, as a consequence of the fact that the clinical history and radiological findings do not always allow a clear distinction between the two diseases (13). This is a serious public health problem since incorrect treatment increases the risk of pulmonary sequel such as fibrosis, bronchiectasis, and chronic respiratory insufficiency.

The definitive diagnosis of PCM has been established by the finding of budding yeast cells of *P. brasiliensis* through direct mycological examination (DME) of fresh biological material such as sputum, or by histopathological techniques; or alternatively by isolation and identification of the fungus in culture (15). Similarly, the diagnosis of TB is established by bacilloscopy, a direct investigation of the acid-fast bacilli (AFB), and by isolation and identification of *Mycobacterium tuberculosis*. However, these techniques have some important limitations that are inherent to the nature of each one:
the low sensitivity of the direct techniques (DME and bacilloscopy) and long time necessary for development and identification of the agents and are the most common problems. Furthermore, the difficulty in obtaining the most appropriate samples of biological material means that sputum is routinely used to investigate AFB and P. brasiliensis. However, spontaneous or induced sputum is highly contaminated and may carry only a small number of pathogenic microorganisms, insufficient to provide a positive result on direct examination. Due to these problems, patients have often received prior empirical treatment for TB, which makes it even more difficult to demonstrate the agents and makes the microbiological diagnosis less discriminatory.

Therefore, indirect diagnostic methods that provide more rapid and reliable results would be of great relevance. The appropriate serology for the diagnosis of PCM would be useful for managing the patients’ treatment (15). Serological tests provide results more rapidly than those from culture and histopathology, and can certainly be used to diagnose (1). However, they still require validation for routine use in clinical laboratories. The double immunodiffusion test (DID) is the classical serological method which is standardized; however for diagnosing PCM its utility is limited because of its low sensitivity (2). On the other hand, more sensitive tests such as enzyme linked immunosorbent assay (ELISA) have shown cross-reactivity (9, 17) including with tuberculosis (10, 14). Recently we showed that Western blot (WB) could contribute with diagnosis of PCM, delivering safe, reliable and fast results (12). Now our objective was to apply WB to patients suspected of TB, in order to investigate also PCM.

Samples of sputum and serum from 134 human patients, with pulmonary symptoms, who were selected from a research project that investigates PCM and TB simultaneously were analyzed to perform the complete laboratory battery of the tests including: bacilloscopy and AFB culture, DME, ELISA (8) DID and WB (12). Sera
from patient with TB or PCM exclusively (confirmed by clinical and laboratory criteria) were used as control. All patients had symptoms of respiratory disease and they had been clinically screened for TB. The antigen used in WB test was the same employed by Perenha-Viana et al., 2012, i.e. a crude soluble exoantigen, obtained from a seven day culture filtrate, strain Pb339 in YPD, which is rich in 43KDa component. Sera were diluted serially from 1:50 to 1:800 and the cutoff point for ELISA was OD greater than 1.0.

In this population there was no concomitance of the two diseases. Of the 134 patients, 11 (8.2%) were confirmed to have pulmonary TB, and six (4.5%) were positive only for PCM by DME, giving an approximate ratio of 2:1 TBxPCM, results are summarizing in table 1. These data are cause for concern, since that the ill persons were suspected to have TB, with no indication of PCM, suggesting that the incidence of this mycosis is being underestimated.

Despite the small number of patients our results allow us to think that the laboratory aspects of the PCM need to be revised. DID actually has no diagnostic value for PCM, because this test would have detected only one of the six individuals who were positive for PCM. ELISA had higher sensitivity than DID, but still requires adjustments in the standardization, because with the cutoff value used it would have detected only four of these six patients. Perhaps this technique was really most suitable as an epidemiological tool using serological investigations (8). On the other hand, WB allowed showing antibodies against the specific antigen (gp43) in sera of 100% of positives PCM patients. These antibodies have been considerate important markers for the diagnosis of PCM (3, 6, 12, 16).

The major contribution of this preliminary study was, therefore, to show that the WB technique can be used as a tool to confirm the differential diagnosis between TB
and PCM, and to reinforce our previous publication (12) and it proved to be particularly useful tool PCM patients with negative DID test. The use of this technique resulted in referrals for appropriate treatment of at least six patients who were triaged and referred for AFB investigation. This study prevented that six patients (4.5% of study population) had received empirical treatment for TB because of their clinical and radiological screening and certainly would have died, as they actually suffered from PCM. Thus it has contributed to the valorization of WB as reliable, rapid serological methods that may be useful in making a differential diagnosis between TB and PCM. The greater advantage of the WB assay is be a serological technique with more sensibility than DID, which can to be offered by specialized centers, since serum is more easy to transport to reference labs.

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Table 1 Summary of laboratorial results on paracoccidioidomycosis and some characteristics of six patients supposedly with tuberculosis§

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>Genre</th>
<th>Age</th>
<th>DME*</th>
<th>DID†</th>
<th>ELISA*</th>
<th>43KDa antibodies in WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>75</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>-</td>
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<td>+</td>
</tr>
</tbody>
</table>

§ All of six patients were negative for TB by culture and microscopy
* Paracoccidioides brasiliensis cells visualized in a direct mycological exam
† Immunodiffusion tests
♪ Optical density

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