Western Blotting Is an Efficient Tool for Differential Diagnosis of Paracoccidioidomycosis and Pulmonary Tuberculosis

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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-43-kDa-component antibodies in Western blotting (WB) assays, while in the TB-positive patients these antibodies did not appear. This preliminary study suggests WB as a potential tool for differential laboratory diagnosis between TB and PCM.

Paracoccidioidomycosis (PCM) is a mycosis endemic to Latin America caused by *Paracoccidioides brasiliensis* and also by the recently described *Paracoccidioides 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 100 years and is considered endemic in many countries, until now there have been 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 those of tuberculosis (TB) (7). Furthermore, the association between PCM and TB is not uncommon; it occurs at a frequency varying between 5.5 and 15.8% (10, 13), and so differential diagnosis between these two diseases as well as detection of coinfection with TB and PCM is very important.

A characterization based only on clinical and radiological data can be difficult, especially in areas of endemicity, since the two 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 sequelae 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, 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 in the nature of each one: the low sensitivity of the direct techniques (DME and bacilloscopy) and the long time necessary for development and identification of the agents 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 do culture and histopathology and can certainly be used for diagnosis (1). However, they still require validation for routine use in clinical laboratories. The double immunodiffusion (DID) test is the classical serological method which is standardized; however, for diagnosing PCM, its utility is limited because of its low sensitivity (2, 4, 5). On the other hand, more-sensitive tests such as enzyme-linked immunosorbent assay (ELISA) have shown cross-reactivity (9, 17), including that with tuberculosis (10, 14). Recently, we showed that Western blotting (WB) could contribute to the 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 PCM also.

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, and ELISA (8) and DID testing and WB (12). Sera from patients with TB or PCM exclusively (confirmed by clinical and laboratory criteria) were used as controls. All patients had symptoms of respiratory disease, and they had been clinically screened for TB. The antigen used in the WB test was the same one employed by Perenha-Viana et al. (12), i.e., a crude soluble exoantigen, obtained from a 7-day culture filtrate of strain Pb339 in yeast extract-peptone-dextrose (YPD), which is
TABLE 1 Summary of laboratory results on paracoccidioidomycosis and some characteristics of six patients believed to have tuberculosisa

| Patient | Genderb | Age (yr) | DMEc | DIDd | ELISAe | Anti-43-kDa-
|---------|---------|----------|------|------|--------| component
|         |         |          |      |      |        | antibodies in
|         |         |          |      |      |        | WB
| 1       | M       | 75       | +    | +    | 1.34   | +
| 2       | F       | 48       | +    | -    | 1.00   | +
| 3       | M       | 41       | +    | -    | 1.77   | +
| 4       | M       | 35       | +    | -    | 1.00   | +
| 5       | M       | 65       | +    | -    | 0.49   | +
| 6       | M       | 40       | +    | -    | 0.58   | +

a All six patients were negative for TB by culture and microscopy.
b M, male; F, female.
c Paracoccidioides brasiliensis cells visualized in a direct mycological exam.
d Immunodiffusion tests.
e Optical density.

rich in the 43-kDa component. Sera were diluted serially from 1:50 to 1:800, and the cutoff point for ELISA was an optical density (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 TB to PCM cases; results are summarized in Table 1. These data are cause for concern, since 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 PCM need to be revised. DID testing 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 DID testing but still requires adjustments in 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 in serological investigations (8). On the other hand, WB allowed demonstration of antibodies to the specific antigen (gp43) in sera of 100% of patients positive for PCM. These antibodies have been considered 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 WB proved to be a particularly useful tool for PCM patients with a 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 six patients (4.5% of the study population) from receiving empirical treatment for TB because of their clinical and radiological screening; they would certainly have died, as they actually suffered from PCM. Thus, it has contributed to the valorization of WB as a reliable, rapid serological method that may be useful in making a differential diagnosis between TB and PCM. The greater advantage of the WB assay is that it is a serological technique with more sensitivity than that of DID testing, which can be offered by specialized centers, since serum is easier to transport to reference labs.

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