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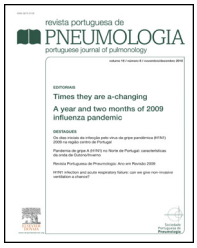

ORIGINAL ARTICLE

Diagnostic yield of sputum microbiological analysis in the diagnosis of pulmonary tuberculosis in a period of 10 years☆

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Abstract
Introduction: Pulmonary tuberculosis (TB) requires an early diagnosis for prompt introduction of treatment and prevention of transmission. Definitive diagnosis is obtained by microbiological culture and identification of *Mycobacterium tuberculosis* in respiratory specimens, mostly sputum samples.
Materials and methods: Retrospective data analysis of all patients suspected of pulmonary TB that submitted three consecutive sputum samples to the Pulmonology Diagnostic Center (PDC) Laboratory between 2004 and 2013. Extrapulmonary TB cases were excluded. Four microbiological analyses were executed on each specimen: two smears with Ziehl–Neelsen staining, direct and concentrate; and two culture examinations, one in liquid and one in solid medium. Statistical analysis was performed by SPSS.
Results: A total of 694 patients were enrolled in this study (65% men, mean age 48.5 ± 18.6 years, 97% Portuguese), most of them exhibiting TB-related complaints. Pulmonary TB was diagnosed in 41% of the patients; 54% had non-specific radiological changes and 34% had pulmonary cavitation. The cumulative sensitivity rates of each of the three smears were 24.6%, 27.7% and 28.8% for concentrated samples and 19.3%, 20.4% and 22.5% for direct samples. The cumulative sensitivities of sputum culture were 33.3%, 37.9% and 41.8% for solid medium, and 43.9%, 51.6% and 55.4% for liquid medium. Pondering all forms of microbiological analysis, the cumulative sensitivities of each sample were 51.2%, 59.6% and 63.2%. There was an incremental yield of 8.4% for the second specimen and 3.5% for the third specimen. All sensitivity rates were higher among patients with pulmonary cavitation.

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Conclusions: This study showed an incremental yield with more than one sputum sample. However, overall sensitivity remained low, suggesting a need for new diagnostic strategies and novel and better diagnostic tools.

Introduction

Tuberculosis (TB) is still associated with a high global burden; there was an estimated 8.6 million new cases and 1.3 million deaths in 2012, most of them occurring in low- and middle-income countries.[1]

Early diagnosis and immediate treatment are essential to cure this airborne infectious disease and prevent transmission in the community. A definite diagnosis can only be established if *Mycobacterium tuberculosis* is isolated and identified from respiratory specimens, most frequently expectorated or induced sputum.

Therefore, when pulmonary TB is suspected, standard guidelines recommend that clinical specimens should be collected and submitted for laboratory testing, such as smear microscopy, culture and nucleic acid amplification to increase the detection rate of *Mycobacterium tuberculosis*.[2–5]

It has been stated that laboratory analysis should be performed on at least three sputum specimens, collected over consecutive days.[6,7] However, there has been some controversy attached to this methodology and some authors find examination of multiple specimens excessive in relation to the yield gain and hard to achieve especially in resource-limited settings.[8–13]

Culture identification is still the gold standard for diagnosis of pulmonary TB despite the fact that *Mycobacterium tuberculosis* is a slow growing organism and solid medium culture may take up to 4–8 weeks to provide a positive result. To overcome this limitation, liquid medium culture has emerged as a more sensitive and speedier technique to detect bacilli growth and simultaneously test for drug susceptibility.[14–16]

In developing countries, sputum smear microscopy is the most commonly used diagnostic test for pulmonary TB. It is a simple technique that quickly identifies acid-fast bacilli (AFB) with a relatively low cost and high positive predictive value, especially when concentrated samples are used. It is important as a tool not only to establish a presumptive diagnosis of pulmonary TB but also to monitor the patient response to anti-TB treatment. In 2009, the WHO recommended that the conventional bright field microscopy using Ziehl–Neelsen (ZN) stain should be replaced by the more sensitive fluorescent light-emitting diode (LED) microscopy, but up to 2012 only two percent of the microscopy centers had this kind of equipment.[1,17] Nowadays, many countries, including Portugal, use fluorescent microscopy for sputum microscopy on a routine basis.

Rapid molecular tests are recent diagnostic instruments that can be used to simultaneously test for pulmonary TB and rifampicin resistance with higher sensitivity than sputum smear microscopy and which could replace conventional culture-based drug susceptibility testing.[18] However, these groundbreaking tests are more expensive, require appropriately equipped laboratory settings which are still unavailable in many high-prevalence countries and demand a change in the diagnosis paradigm.

Thus, at the present time, ZN smear microscopy and solid or liquid medium cultures remain the diagnostic methods most widely used in the diagnosis of TB. The aim of this study was to analyze the sensitivities of these conventional methods in a Portuguese public health department.

Materials and methods

Patient selection

Between 2004 and 2013, data from all patients submitted to sputum analysis at the Pulmonology Diagnostic Centre (PDC) laboratory was retrospectively assessed. Only those that had three adequate samples collected in consecutive mornings were included in this study. Non-pulmonary and/or pleural tuberculosis, latent tuberculosis infection (LTBI) and presumed TB based only on clinical grounds were excluded.

Patients were diagnosed with LTBI when they exhibited no symptoms or signs of the disease except for a positive tuberculin skin test and/or a positive interferon-γ release assays (IGRA) test. A diagnosis of tuberculosis was made if *Mycobacterium tuberculosis* was isolated from respiratory samples or if a histological diagnosis was made. Pulmonary TB diagnosis was excluded when all standard diagnostic procedures, including bacteriologic examination, chest radiograph and occasionally histologic analysis, did not confirm active disease suspicion and also, when during follow-up appointments, there was clinical improvement or establishment of an alternative diagnosis.

Microbiology technique

Sputum collection

All patients received prior oral and written specific instructions on the correct sputum collection technique. Collection was done at home immediately after waking up and washing the mouth with water, avoiding toothpaste or antiseptic solutions. Samples were collected to a sterile container three days in a row, placed inside a thermal bag at low temperature in the fridge, avoiding overgrowth of other bacteria, and delivered to the laboratory in the fourth day.