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Utilization of bacteriological culture for increased diagnostic performance at a tuberculosis reference center hospital

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ABSTRACT

The purpose of this study was to assess the increase in positive results of bacteriological diagnostic tests for tuberculosis with the utilization of culture at a referral hospital for tuberculosis (TB). A retrospective analysis was conducted based on the positive bacteriological results obtained at the Júlia Kubistchek Hospital. The number of bacteriological diagnoses was increased by 24.6 % with the utilization of culture of sputum samples and by 56.1 % of bronchoalveolar lavage samples. With regard to pleural fluid, all six positive cultures were negative for bacilloscopy. Mycobacterium tuberculosis was isolated in 59.6 % of positive cultures. Since mycobacterial culture was not undertaken for all clinical samples, this procedure is an important laboratory routine at the Júlia Kubistchek Hospital in order to learn the real TB prevalence.

Key words: tuberculosis, diagnostic, bacilloscopy, mycobacterial culture

RESUMEN

Uso del cultivo para un mejor rendimiento diagnóstico en un hospital de referencia para tuberculosis.

El objetivo de este estudio fue evaluar el aumento de la positividad en el diagnóstico bacteriológico de la tuberculosis mediante el uso del cultivo en un hospital de referencia para tuberculosis. Se realizó un análisis retrospectivo de los resultados bacteriológicos positivos en el Hospital Júlia Kubitschek de Belo Horizonte, Brasil. Hubo un incremento en el diagnóstico bacteriológico con cultivo del 24.6 % en las muestras de esputo y del 56.1 % en las muestras de lavado broncoalveolar. En líquido pleural, de 6 cultivos positivos, todos fueron negativos por baciloscopia. En 59.6 % de los cultivos positivos se aisló Mycobacterium tuberculosis. Se concluye que el cultivo para micobacterias es importante en la rutina de laboratorio del Hospital Júlia Kubitschek para el conocimiento de la verdadera prevalencia de tuberculosis, ya que este procedimiento no se realiza en todas las muestras clínicas.

Palabras clave: tuberculosis, diagnóstico, baciloscopia, cultivo de micobacterias

Tuberculosis (TB) continues to be one of the main public health issues in most countries. According to the WHO – World Health Organization, in 2009 alone the number of new TB cases was 9.4 million. Brazil currently ranks 19th regarding TB incidence and is among the 22 countries responsible for 80 % of all TB cases (14).

Although curative treatment is available, a significant number of TB patients are still treated in hospitals and intrahospital mortality remains high, with estimates ranging from 2 % to 12 %. Co-morbidities, including HIV, and late initiation of treatments have been considered the causes for the high intrahospital mortality (12). The situation is even more severe due to the emergence of Mycobacterium tuberculosis, which is resistant to the main drugs being used and by its high dissemination capacity (13).

The Júlia Kubistchek Hospital (HJK), part of the FHEMIG (Fundação Hospitalar de Minas Gerais) healthcare system, is a state referral center for TB treatment that treats nearly 600 patients every year, with a monthly average of 60 inpatients, and an average of around 220 TB cases reported per year.

For a bacteriological diagnosis of pulmonary TB, the first test requested is a sputum bacilloscopy, which has an elevated positive predictive value in our field (> 95 %) but low sensitivity (40 - 60 %). Cultures allow for the identification of the mycobacterium species and for a sensitivity test, in addition to an increase in the diagnostic performance,
as it is a more specific test that provides greater sensitivity (3).

The Brazilian Society of Pneumology and Tisiology defined and publicized criteria for conducting the culture test. However, in high complexity hospitals and TB referral centers, this test should be undertaken routinely, mainly in extrapulmonary samples that are paucibacillary, with the purpose of providing a response to local demands, increasing the detection of mycobacteria and assessing and improving the performance of the TB control program (3, 8).

The objective of this study was to assess the increase in tuberculosis diagnostic performance with the use of mycobacteriological culture in a specific medium, with respect to the bacilloscopy in samples of pleural and pulmonary origins. The present study was undertaken in the Microbiology Sector of the HJK Laboratory. A retrospective analysis was conducted based on positive bacteriological results (bacilloscopy and/or mycobacterial culture) recorded in the institution’s books from July, 2009 through December, 2010. This study was approved by the FHEMIG Research Ethics Committee.

The Ziehl-Neelsen technique was adopted for the bacilloscopy procedure applied to the in natura sputum samples (direct sampling). The bronchoalveolar lavage (BAL) and pleural fluid samples were centrifuged and the bacilloscopy was performed on the sediment thereby obtained. The culture was done in a Lowenstein-Jensen medium, following decontamination using the sodium lauryl sulfate method and the identification of mycobacterial species via biochemical methods (9, 10).

A bacilloscopy diagnostic test was adopted to assess the laboratory performance of the culture. Pleural and respiratory samples with positive bacteriological diagnoses were included (bacilloscopy and/or culture). To calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), the result of the culture was adopted as the gold standard (6). All clinical samples of any other origin were excluded. Clinical assessments of patients were not made.

Within the period studied, the laboratory received 965 clinical samples (respiratory and pleural) for the isolation of mycobacteria, of which 412 were sputum samples, 476 were bronchoalveolar lavages and 77 were pleural fluid samples. Twenty-nine percent (279/965) showed positive bacilloscopies or cultures (Table 1). Of the 279 clinical samples registered as positive, 191 were sputum samples, 82 were bronchoalveolar lavages and 6 were pleural fluid samples, which corresponded to 76 patients. In the samples with positive cultures, the positive results increased by 20.4 % for sputum samples and by 54.8 % for bronchoalveolar lavages when compared to bacilloscopy results. In the sputum samples, sensitivity, specificity, PPV, and NPV for the bacilloscopy were 74.3 %, 98.1 %, 94.4 % and 89.8 %, respectively. For the BAL samples, these values were 43.2 %, 99.8 %, 97.2 % and 91.2 % for

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Positive Culture</th>
<th>Negative Culture</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive bacilloscopy</td>
<td>136</td>
<td>8</td>
<td>144 (75.4)</td>
</tr>
<tr>
<td>Negative bacilloscopy</td>
<td>47</td>
<td>0</td>
<td>47 (24.6)</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>8</td>
<td>191 (100)</td>
</tr>
<tr>
<td>BAL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive bacilloscopy</td>
<td>35</td>
<td>1</td>
<td>36 (43.9)</td>
</tr>
<tr>
<td>Negative bacilloscopy</td>
<td>46</td>
<td>0</td>
<td>46 (56.1)</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>1</td>
<td>82 (100)</td>
</tr>
<tr>
<td>Pleural Fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive bacilloscopy</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Negative bacilloscopy</td>
<td>6</td>
<td>0</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

HJK: Júlia Kubischek Hospital; BAL: bronchoalveolar lavage
sensitivity, specificity, PPV and NPV. Values were not calculated for pleural fluids as there were no samples with positive bacilloscopy.

Of the 191 positive sputum samples, in 47/191 (24.6 %) the diagnoses were obtained exclusively via culture, as demonstrated in Table 1. In 4.2 % (8/191) of the samples, the culture was negative, despite the positive bacilloscopy.

Of the 82 positive BAL samples, 56.1 % (46/82) of the bacteriological diagnoses were obtained solely via culture. Only 1.2 % (1/82) of the samples did not increase in the culture, despite the positive bacilloscopy (Table 1).

The overall performance of sputum and BAL materials was: positive bacilloscopy 180/279 (64.5 %); negative bacilloscopy 99/279 (35.5 %); positive culture 270/279 (96.7 %); negative culture 9/279 (3.3 %), respectively. A total of six pleural fluid samples were positive in culture and negative in bacilloscopy.

Among the positive cultures presenting negative bacilloscopy, M. tuberculosis was isolated in 59/99 (59.6 %). In 36.4 % (36/99), it was not possible to identify the mycobacterial species. Four out of the 99 positive cultures (4.0 %) were identified as nontuberculous mycobacteria (NTM).

It was observed that the sensitivity obtained with sputum bacilloscopy (74.3 %) was higher than that in the data reported in the literature, which present values on the order of 40 - 60 % (3). The positive predictive value was 94.4 %. The same authors report a positive predictive value greater than 95 % in our field. In BAL samples, when the bacilloscopy sensitivity is assessed, 43.2 % are in accordance with the values found by Conde et al. (2).

With respect to sputum samples, positive results increased by 20.4 % in the bacteriological test with culture, and are in accordance with the data in the literature, as they demonstrate an increase of 20 % to 40 % in mycobacteria diagnoses (2-4). It is important to note the remarkable increase in positivity in BAL (54.8 %) in relation to bacilloscopy. This increase may be due to the low sensitivity of bacilloscopy in direct samples. As described by other authors, the increase in positivity of bacteriological diagnostic tests due to the introduction of culture in BAL may range from 56.6 % to 87 % (1, 5).

The negativity of all pleural fluid samples analyzed via bacilloscopy may be due to possible technical issues during sample processing, even though some authors have reported that in pleural fluid, both bacilloscopy and culture have performances lower than 5 % and 40 %, respectively (12). However, the culture played an important role in diagnosing pleural TB in six of the cases.

When analyzing the overall performance of the TB diagnosis using bacilloscopy and culture, a satisfactory result (positive 64.5 % and 96.7 %, respectively) was observed.

As regards the result of the negative cultures presenting positive bacilloscopy, this may be due to treatment control patients, when dead bacilli are still being eliminated. However, values above 3 % may also indicate technical problems. This might happen due to drastic decontamination procedures, oscillating temperatures in the incubator, improper storage of clinical samples or even false positive bacilloscopy results (10). Therefore, better quality control during laboratory processes might aid in decreasing the number of flaws.

In Brazil, an increase in the disease by NTM is observed following the AIDS epidemic. However, the data on the frequency of species and the prevalence of the disease are not yet truly known (15). In the present study, it is not possible to relate the 4 % of NTM to the disease as there is no clinical data on the patients.

Since HJK is a referral hospital for TB and resistant TB, the utilization of new and quicker techniques, in addition to molecular tests that may come to reduce the time required for diagnosing, should be assessed, including the identification of species and the detection of mycobacterial resistance to drugs. Accuracy studies assessing new methods are being tested in Brazil for validation of the diagnosis. However, the incorporation of these technological innovations into clinical routines still requires cost-effectiveness studies to assess the impact on the healthcare system in which it is to be adopted (3). Even though culture in a solid medium has a slow growth time, it is what is available in the laboratory. Thus, both identification and supervised treatment will be enabled, as well as the real prevalence of species and the resistance of M. tuberculosis to drugs may be understood, since HJK receives patients from several different regions of the state of Minas Gerais, Brazil, with antibiotic failure diagnoses, relapses and suspected resistance.

One of the limitations of this study was the lack of data on the patients’ clinical conditions and the fact that the culture test was not run for all clinical samples, which might even further increase the number of positive bacteriological diagnoses, in addition to
phenotypic and genotypic testing. Furthermore, the progress of the patients diagnosed with *Mycobacterium* sp. (species not identified due to insufficient number of colonies) was not assessed, which does not eliminate the possibility of it being *M. tuberculosis*, along with those grouped as NTM, meaning that it was not possible to determine whether it was a disease, contamination or simply colonization (7).

The present study allowed for the assessment of culture, since this procedure would only be conducted when requested by a physician, and was not a routine procedure at the HJK. With these data, coordinators of the TB Control Plan at the HJK and at the Laboratory are already assessing the implementation of culture for all incoming samples. As a result, an increase in the number of TB diagnoses, as well as a reduction in unconfirmed in-hospital treatments to less than 15% are expected, along with the possible identification of species and assessment of sensitivity to drugs.

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