False negativity in AFB Smear microscopy: An insight into the caveats of the most widely used screening tool for tuberculosis

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Abstract

Objective: To study the ratio of false negativity in sputum samples in diagnostic smears received for acid fast bacilli smear microscopy.

Methods: The retrospective cross-sectional study was conducted at the Ojha Institute of Chest Diseases, Karachi, and comprised specimens for microscopy and culture from presumptive tuberculosis patients for 38 months starting from November 2010. All laboratory investigations had been done as per the National Tuberculosis Control Programme guidelines. Statistical analyses were performed on MedCalc and Social statistics calculators, and Open Epi software.

Results: Of the 2,158 specimens, 1,316 (60.98%) were of men and 842 (39.02%) of women (male-to-female ratio: 1.56:1). Besides, 843 (39.06%) were smear-negative, of which 99 (11.74%) were false negative. Of the 1,275 (96.88%) men whose age was reported, 808 (63.37%) were aged 19-45 years, whereas out of the 792 (94.06) women whose age was known, 517 (65.28%) were in that age group. Microscopic outcomes were significantly related to gender (p<0.001).

Conclusion: Smear microscopy cannot be solely relied upon for diagnosis and its results must be correlated with additional clinical information and other diagnostics due to considerable amount of false negativity, especially in female population.

Keywords: Tuberculosis, Microscopy, Culture. (JPMA 66:1116; 2016)

Introduction

Pulmonary tuberculosis (TB) is a serious public health problem in the whole of South Asia.1 Due to risk of transmission in the community, it requires early diagnosis, proper treatment and continuous monitoring.2 After clinical examination, presumptive TB patients are referred for smear microscopy which remains the most important tool for screening and diagnosis3 as it is the best available resource in high-burden settings of developing countries. Unfortunately, microscopy may not yield positive results for all cases of TB due to false negativity in smear negative patients.3 In addition, microscopy cannot differentiate between viable and non-viable bacteria and is unable to distinguish the different types of bacilli, due to which mycobacterial culture remains superior to the microscopy as it can even detect cases with low bacterial load.4 However, culture can be an expensive and time-consuming process and requirements of proper infrastructure, expert handling and cost can be limiting factors.5-6 Despite these facts, culture remains the gold standard for TB diagnosis as it is the most reliable tool to detect bacilli in smear negative patients which are critical to diagnose and treat.7

The current study was planned to find the ratio of false negativity in sputum samples in diagnostic smears received for acid fast bacilli (AFB) smear microscopy using culture as a standard.

Materials and Methods

The 38-month retrospective cross-sectional study, started in November 2010, was conducted at the Ojha Institute of Chest Diseases (OICD) of the Dow University of Health Sciences (DUHS), Karachi, and comprised specimens for microscopy and culture from presumptive TB patients. OICD receives patients from various parts of the Sindh province, which has a population of approximately 42.4 million distributed over an area of 140,914 sq km.8 The OICD is designed to diagnose, treat and monitor the progress of conventional and multi-drug-resistant (MDR)/extensively drug-resistant (XDR), smear-negative and extra-pulmonary TB cases.
Ethical approval was obtained from the institutional committee. Data was de-identified and tabulated without recording patients’ particulars.

Sputum was routinely collected under expert supervision. Slides were prepared from sputum directly by coiling method and stained with Ziehl-Neelsen stain using standard protocol and observed under simple microscope. Cultures were grown on Ogawa medium, according to modified Kudoh method. Media quality for mycobacterial growth was confirmed using American Type Culture Collection (ATCC) strains of H37Rv. All cultures were kept under observation for the first 48 hours after inoculation and were checked weekly till eight weeks to observe growth.

Data from all diagnostics smears was included. All data from follow-up cases was excluded. For stratification according to age, records of all patients whose ages were not reported were also excluded.

Data was stratified and descriptively analysed for demographics using Microsoft Excel. Further statistical analysis was performed using online resources. Sensitivity, specificity and predictive values were calculated by online MedCalc calculator using values for 'true' and 'false', 'positive' and 'negative' samples for 95% confidence interval (CI). P=0.05 was considered significant. Z-test for proportions was performed on Social statistics calculator using proportions of false negativity among all negative samples in both genders. Age-wise stratified data for both genders was analysed using chi-square test with Open Epi software.

Results

Of the 2,158 specimens, 1,316(60.98%) were of men and 842(39.02%) of women (a ratio of 1.56:1). Besides, 843(39.06%) were smear negative (Tables-1 and 2).

Culture showed that the sensitivity of microscopy remained 92.38% (95%CI 90.81%-93.77%), while specificity was 88.26% (95%CI 85.89-90.35). Among the negative cases, 99(11.74%) were false negatives. Positive predictive value (PPV) for smear microscopy remained 91.33% (95% CI 85.89 % to 90.35).

The proportion of false negativity among negative cases was (24.1%) in women (42/174) compared to (8.5%) in men (57/669) (p=0). Chi-square showed that the microscopic outcomes were significantly related to the gender of patients (p<0.001)

Age was not reported for 41(3.11%) men and 50(5.94%)

Table 1: Gender-wise distribution of smear and culture analysis of presumptive TB patients.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>(X^2)</th>
<th>df</th>
<th>p</th>
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<tbody>
<tr>
<td>True Positive</td>
<td>587</td>
<td>614</td>
<td>1201</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True Negative</td>
<td>612</td>
<td>132</td>
<td>744</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>False Positive</td>
<td>60</td>
<td>54</td>
<td>114</td>
<td>219.3</td>
<td>3</td>
<td>&lt;0.000</td>
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<tr>
<td>False Negative</td>
<td>57</td>
<td>42</td>
<td>99</td>
<td></td>
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</table>

True Positive= Smear + Culture +, False Negative= Smear - Culture -, True Negative = Smear - Culture -
Chi square test performed using Open Epi *, p= 0.05 taken as significant
TB: tuberculosis
df: degrees of freedom.

Table 2: Age wise stratified data of patients with different microscopic results.

<table>
<thead>
<tr>
<th></th>
<th>&lt;18</th>
<th>19-45</th>
<th>&gt;46</th>
<th>Total</th>
<th>(X^2)</th>
<th>df</th>
<th>p</th>
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<tbody>
<tr>
<td>True Positive</td>
<td>53</td>
<td>384</td>
<td>129</td>
<td>566</td>
<td>127</td>
<td>392</td>
<td>69</td>
</tr>
<tr>
<td>False Negative</td>
<td>8</td>
<td>35</td>
<td>13</td>
<td>56</td>
<td>7</td>
<td>23</td>
<td>8</td>
</tr>
<tr>
<td>False Positive</td>
<td>6</td>
<td>34</td>
<td>20</td>
<td>60</td>
<td>17.6</td>
<td>6</td>
<td>0.007</td>
</tr>
<tr>
<td>True Negative</td>
<td>45</td>
<td>355</td>
<td>193</td>
<td>593</td>
<td>19</td>
<td>77</td>
<td>22</td>
</tr>
</tbody>
</table>

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<th>&lt;18</th>
<th>19-45</th>
<th>&gt;46</th>
<th>Total</th>
<th>(X^2)</th>
<th>df</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>112</td>
<td>808</td>
<td>355</td>
<td>1275</td>
<td>166</td>
<td>517</td>
<td>109</td>
</tr>
</tbody>
</table>

True Positive= Smear + Culture +, False Negative= Smear - Culture -, True Negative = Smear - Culture -
Chi square test performed using Open Epi *, p= 0.05 taken as significant
df: degrees of freedom.
women.

Of the 1,275 (96.88%) men whose age was reported, 808 (63.37%) were aged 19-45 years, 355 (27.84%) 46 years or above and 112 (8.78%) 18 years or below. Of the 792 (94.06%) women whose age was reported, 517 (65.28%) were in the age group of 19-45 years, 109 (13.76%) were 46 or above and 166 (20.96%) were 18 or below.

Age-wise stratified data showed significant relationship between age groups and microscopic findings in males (p=0.007), but these were independent of each other in females (p=0.089).

**Discussion**

Our study showed significant differences among men and women at different stages of diagnosis of TB. The referral for smear microscopy had a male-to-female ratio of 1.56:1. However, the ratio of true positivity became almost equal on smear microscopy. This was also consistent with our previous results which showed that there were significant differences in proportion of both the genders availing TB screening services and getting diagnosed with TB.15

Our analysis of three-year data showed that approximately 12 out of every 100 smear negative patients were suffering from TB. This false negativity is alarming as such patients have the potential to spread TB in the general population.7,15-16 This shows that though smear microscopy remains the backbone of diagnostic services in any TB screening programme, its limitations must be kept in mind as it is not very efficient tool in case of patients with low mycobacterial load. Analysis performed on a number of studies by Van Deun showed that smear results can be extremely variable in TB patients.5 Smear-negativity is especially common in human immunodeficiency virus (HIV) patients suffering from TB, but the presence of high number of smear-negative patients in a population with low HIV load is an important finding.17 However, unless a better screening tool is found, smear microscopy remains the best available option with a sensitivity of 92.38% (95%CI 90.81%-93.77%) and specificity of 88.26% (95%CI 85.89-90.35).

Another important finding was the significantly higher false negativity of TB in females. These findings are somewhat in contrast to the international trends reported by the World Health Organisation (WHO) which showed that globally, men suffer and die more than women due to TB.18

This implies that in a high-burden country like Pakistan, special care must be taken in smear-negative suspects before declaring them TB free. The best way is to refer all suspicious smear-negative suspects for culture, which can detect latent cases and provide diagnostic accuracy. It not only prevents unnecessary treatment of the patients suffering from diseases other than TB, but also helps in the correct and timely treatment of the smear negative TB patients.

**Conclusion**

Smear microscopy cannot be solely relied upon for diagnosis and its results must be correlated with additional clinical information and other diagnostics due to considerable amount of false negativity, especially in female population. However, it would remain an important screening tool due to its low cost and relatively high sensitivity and specificity. Moreover, addition of fluorescence microscopes can help improve the efficiency of diagnostic services.

**Acknowledgement**

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**Conflict of Interest:** No

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**References**


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