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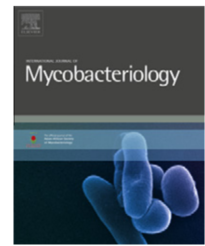

Short communication

Performance indicators of fluorescence microscopy for sputum samples in pulmonary tuberculosis

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ABSTRACT

To get insight into the sensitivity of fluorescence microscopy for diagnostic and follow-up sputum samples from pulmonary tuberculosis patients in clinical trials, the yield of smear positivity – among culture positive sputum samples – encountered in diagnostic and follow-up samples was retrospectively analyzed from the data available in a mycobacteriology laboratory in India. The sensitivity of fluorescence microscopy for diagnostic and follow-up samples respectively was found to be 94.3% and 60.7%. With these values as guidelines, the performance of fluorescence microscopy in the treatment of multi-drug resistant tuberculosis under DOTS plus program remains to be monitored and studied.

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Introduction

Sputum acid-fast bacilli (AFB) smear microscopy is the most employed diagnostic tool used in the diagnosis of pulmonary tuberculosis (PTB) all over the world [1]. It is performed either using light microscopes or fluorescent microscopes. With the rapid expansion of culture and drug susceptibility testing (DST) laboratories across the globe for rapid diagnosis of multi-drug resistant tuberculosis (MDR-TB), the performance indicators for microscopy and culture and DST procedures in mycobacteriology laboratories are to be monitored and the quality of work has to be ascertained [2]. Though there are internationally accepted consensus guidelines for ensuring the quality of Ziehl Neelsen (ZN) sputum AFB microscopy [3], no such guidelines have been framed for fluorescence microscopy (FM). Recently, McCarthy et al. [2] suggested laboratory performance indicators for assessing the quality of smear and solid (Lowenstein–Jensen) culture methods in mycobacteriology laboratories. They considered only diagnostic samples to define performance indicators for microscopy and culture and DST methods, and pointed out that performance indicators for different types of samples (diagnostic, follow-up, morning, spot, direct samples, concentrated samples, preserved samples, transported samples, and different samples from different extra-pulmonary TB) could vary and are to be studied. Sufficient information is available on the laboratory indicators of ZN microscopy for diagnostic samples [4]. However, the information for follow-up samples, collected from PTB patients on treatment and follow-up, is not well documented. It is also essential to segregate the diagnostic and follow-up samples and analyze the various performance indicators for ZN/FM microscopy. In the present study, an attempt was made to precisely gauge the performance of FM in a mycobacteriology laboratory in

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culture positive diagnostic and follow-up samples collected from PTB patients enrolled in controlled clinical trials.

Methods

From each of the patients admitted and followed in controlled clinical trials at the National Institute for Research in Tuberculosis, Chennai, India, four samples (two morning and two spot samples), collected before initiating treatment, three samples (one spot and two morning) collected at each month during treatment, and two samples (one spot and one morning) collected during the 24-month follow-up period after completing treatment were subjected to bacteriological examination. A direct smear from each of the samples was prepared and stained by auramine phenol staining method. The smears were examined by FM (mercury vapor lamp) as per standard operating procedures followed in the laboratory [5]. All AFB positive smears and 20% of the negative smears were checked by a senior technologist before reporting the smear results. All the samples were cultured on solid LJ medium for growth of *Mycobacterium tuberculosis*, after NaOH decontamination and concentration by centrifugation [5]. Of the cultures isolated from each of the patients, two among diagnostic and one among follow-up samples, if available, were selected for DST. The smear and culture grade of these selected cultures were recorded and maintained in a register in the mycobacteriology laboratory to ensure timely reporting of DST results to the patients. The smear results for 562 diagnostic and 352 follow-up culture positive samples from 281 patients were available for analysis. The controlled clinical trial was approved by the Institute's ethics committee, and individual patient's consent was obtained. The patients, treated with short-course regimens ranging from 3 to 6 months, were followed for 24 months. The data (name of the patient, unique ID number, laboratory number, and smear and culture grade of the culture) were entered using Microsoft Excel software, and the sensitivity of FM was calculated against the culture as the gold standard.

Results

Table 1 shows the distribution of smear grades among diagnostic and follow-up culture positive samples. Of the 562 diagnostic culture positive samples, 530 (94.3%) were smear positive. Of the 562, 503 and 59, respectively, were high (2+ and 3+) and low (cols and 1+) grade culture positive. Of 503 high-grade and 59 low-grade culture positive samples, 484 (96.2%) and 46 (77.9%) respectively were smear positive. Of the 352 follow-up culture positive samples, 214 (60.7%) were smear positive. Of the 352, 86 and 266 were, respectively, high- and low-grade culture positive samples. Of the 86 high and 266 low-grade culture positive samples, 80 (93%) and 134 (50.3%) were positive by smear respectively. Among all culture positive and in the low-grade culture positive samples, the difference in smear positivity between diagnostic and follow-up samples (94.3% vs. 60.7%) and (77.9% vs. 50.3%) attained statistical significance as shown in Table 1.

Table 1 – Distribution of smear results against the corresponding culture results.

Smear[a]	Culture[b]				Total
	1+	2+	3+	Col	
a. *Diagnostic samples*					
1+	34	138	71	3	246
2+	9	81	135	0	225
3+	0	12	47	0	59
NEG	11	17	2	2	32
Total	54	248	255	5	562
b. *Follow-up samples*					
1+	63	37	9	59	168
2+	6	9	18	6	39
3+	0	1	6	0	7
NEG	67	5	1	65	138
Total	136	52	34	130	352

[a] 3+: More than 100 AFB per field (200×) in at least 20 fields; 2+: 5–100 AFB per field in at least 50 fields; 1+: minimum 4 AFB/less than 5 AFB per field in at least 50 fields.
[b] 3+: confluent growth; 2+: innumerable number of colonies; 1+: 20–100 colonies; Cols: 1–19 colonies.

Discussion

The sensitivity of FM for diagnostic and follow-up samples was 94.6% and 60.7% respectively. The very high sensitivity (94.6%) in diagnostic samples, achieved in this study, could be the result of the selective referral of PTB suspects from the diagnostic centers in the study area. The low sensitivity for follow-up samples (60.7%), especially in low-grade culture positive samples (50.4%; 134/266) could be attributed to the paucibacillary nature of samples often with damaged and difficult to stain bacilli [6]. This is evident with the observation that 266 of 352 follow-up samples yielded low positives (less than 20 colonies and 1+) during the follow-up period. This provides foresight into the performance indicator for FM in follow-up samples. However, it should also be kept in mind that the sensitivity of FM for follow-up samples might vary depending upon the regimen used to treat patients and in the follow-up period; it needs further analysis of data from different sites having different treatment regimens.

The data in the present study was collected from a specially designed culture card for each patient where only laboratory numbers of presumptively culture positive samples were recorded and maintained in the laboratory. It should be pointed out that the Revised National Tuberculosis Control Programme (RNTCP) recommended culture and DST register contains information on diagnostic and follow-up samples, and separate analysis of performance indicators for diagnostic and follow-up samples is feasible [7]. In the present study, for the first time, the smear results of culture positive diagnostic and follow-up samples obtained in a controlled clinical trial were analyzed exclusively to get an insight into the

sensitivity of FM. Though all the culture results which became culture positive for *M. tuberculosis* during the follow-up period were not available for analysis, two positive cultures among the diagnostic samples and one culture, isolated from each month during treatment and the follow-up period, when available, were included in the analysis.

Conclusion

The sensitivity of FM as a performance indicator for diagnostic and follow-up samples in PTB was found to be 94.3% and 60.7% in controlled clinical trial settings, and these values can be a guide to monitoring the quality of FM in the DOTS plus program in different settings.

Conflict of interest

No conflict of interest is declared for the article titled “Performance indicators of fluorescence microscopy for sputum samples in pulmonary tuberculosis” submitted for publication in your journal.

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