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Value of single acid-fast bacilli sputum smears in the diagnosis of tuberculosis in HIV-positive subjects

Tuberculosis (TB) is a major public health concern, particularly in third-world nations, where the prevalence is reportedly as high as 40 % (Bhargava *et al.*, 2001). Being the most common opportunistic infection among HIV-positive subjects, TB has re-emerged as a global emergency especially in the Indian subcontinent (Kumarasamy *et al.*, 1995). India reportedly has more TB cases than any other country in the world (Kumarasamy *et al.*, 2003). HIV has a substantial influence on the incidence, clinical manifestations, treatment and disease outcome of TB. In addition, the dual epidemic is a cause for concern in countries where these two infections are prevalent in epidemic proportions (Ramachandran *et al.*, 2003).

Prompt diagnosis of TB is crucial for the success of ensuing treatment in any community setting. Conceptually, most of the available standard laboratory and mycobacteriology guideline texts advocate at least three consecutive sputum specimens to detect acid-fast bacilli (AFB) and performing sputum culture on patients suspected to have the disease (Nelson *et al.*, 1998). Considering the huge economic burden imposed on the HIV/AIDS community to avail of expensive antiretroviral drugs and other diagnostic tests, it may be unmanageable for the patients to provide three consecutive specimens. In such a scenario, the usefulness of a single AFB smear in TB diagnosis needs further evaluation. Therefore, we compared the value of microscopy of a single AFB smear in TB diagnosis with a radiometric culture method as the 'gold standard'.

We compared the results of a single AFB sputum smear with those from AFB culture from 121 HIV-positive patients suspected to have TB during a 4-year period from June 2002 to May 2006. Sputum specimens were collected and processed following the standard procedure recommended by the BACTEC 460TB (Becton Dickinson) operations manual. Briefly, after inoculating the treated specimens in BACTEC 12B vials (Becton Dickinson), an initial smear was prepared, stained by the Ziehl–Neelsen method and observed under oil immersion for AFB. The inoculated vials were incubated at 37 °C in the presence of 5–10 % CO_2 and read in the BACTEC 460TB following the manufacturer's instructions. Following primary isolation, the *M. tuberculosis* complex was identified by the *p*-nitro-α-acetylamino-β-hydroxy-propiophenone differentiation test. Analysis of data was carried out using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 13.0. Particulars on the diagnostic test procedures used, viz. sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were calculated to compare the AFB smear microscopy with culture.

Table 1. Analysis of single AFB sputum smears using the BACTEC 460 TB as the gold standard

Variable	Estimate (%)	95 % confidence interval
Sensitivity	53.3	38–68 %
Specificity	89.5	79.7–95 %
Positive predictive value	68	–
Negative predictive value	82	–

The single AFB smear method showed a sensitivity of 53.3 %, specificity of 89.5 %, PPV of 68 % and NPV of 82 % (Table 1).

The increasing number of reports of mycobacteria other than tubercle (MOTT) as opportunistic pathogens in HIV disease and most reportedly culture-positive and smear-negative (Miguez-Burbano *et al.*, 2006) suggested that we should investigate the influence of MOTT on the sensitivity and specificity of the single AFB smear method. When the MOTT-positive cases were excluded from the study population, interestingly, the sensitivity increased to 61.1 %. Furthermore, when the MOTT cases alone were considered and TB culture positives were excluded, the sensitivity decreased to 22.2 % (Table 2). Nonetheless, the specificity remained the same in both groups.

Rapid diagnosis of TB currently relies on AFB smear examination. As this cannot distinguish tubercle bacilli from MOTT, culture examination is therefore warranted. Furthermore, direct smear reportedly detects AFB only at concentrations of ~10 000 bacilli ml^{-1} of the specimen. Conversely, as few as 100 bacilli ml^{-1} may be required for a positive

Table 2. Influence of mycobacteria other than tubercle (MOTT) on the sensitivity and specificity of single AFB sputum smears as compared with the BACTEC TB 460

Variable	Excluding MOTT cases (n=112)		Excluding TB cases (n=85)	
	Estimate (%)	95 % confidence interval	Estimate (%)	95 % confidence interval
Sensitivity	61.1	43.5–76.3 %	22.2	3.9–59.8 %
Specificity	89.5	79.8–95 %	89.5	79.8–95 %

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culture (Kant, 2001). It is widely known that TB transmission occurs before the level of bacilli reaches 10^4 ml^{-1} in the sputum, and during this period, the person continues to transmit the bacilli. The diagnostic value of sputum microscopy is reportedly eroded owing to the increasing number of HIV-related smear-negative pulmonary TB cases (Kant, 2001; Valadas *et al.*, 2003) and the influence of MOTT, which substantially reduces the sensitivity of the single AFB smear method. Our study has shown that the AFB smear method has relatively lower sensitivity and specificity than the gold standard – radiometric culture. However, three consecutive specimens may be required in prospective studies to improve the validity of the method. Hence we conclude that it may be necessary to choose AFB culture to diagnose TB in HIV-positive patients despite the high cost and time involved.

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