

Improving the Bacteriological Diagnosis of Tuberculous Meningitis

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We made a bacteriological diagnosis of tuberculous meningitis in 107 of 132 (81%) adults with clinical tuberculous meningitis; acid-fast bacilli were seen in 77 of 132 (58%) and cultured from 94 of 132 (71%). Volume of cerebrospinal fluid, duration of symptoms, cerebrospinal fluid neutrophils, lactate, and glucose were all independently associated with bacteriological confirmation.

Death from tuberculous meningitis (TBM) is strongly associated with delayed diagnosis and treatment, and there is an urgent need to improve diagnostic methods for this disease (1). The demonstration of acid-fast bacilli (AFB) in the cerebrospinal fluid (CSF) remains the best and the most widely available method, but the sensitivity varies significantly. Many authors report finding AFB in fewer than 20% of TBM patients (1), but the older literature suggests that much better results can be achieved (2, 4). In 1953 Stewart found AFB in the CSF of 91 of 100 consecutive TBM patients; all were subsequently confirmed by culture (4). Similar results were reported in 1979 by Kennedy and Fallon, who found AFB in the CSF of 45 of 52 (87%) patients treated for TBM (2). The reasons why many laboratories fail to replicate these results are uncertain. Anecdotal evidence suggests that sensitivity may be dependent upon the volume of CSF examined, the speed and duration of centrifugation, and the time taken over microscopy, but there are few data to support these assertions. We performed a prospective study that aimed to describe the variables affecting the sensitivity of CSF stain and culture and to identify the key factors in clinical practice that could improve the bacteriological diagnosis of TBM.

We coordinated a diagnostic service for TBM between May 2000 and May 2003 at the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. The hospital admits adults with suspected central nervous system infections from the local community and acts as a tertiary referral center for southern Vietnam. The volume of CSF taken from each adult with suspected meningitis was recorded, the sample was centrifuged at $3,000 \times g$ for 15 min, and 2 drops of the deposit was stained by the standard Ziehl-Neelsen method. First, areas of high cellular density were examined under high power ($\times 100$) with an oil-immersion lens. If AFB were not seen, the rest of the slide was examined. The slide was termed positive if two or more AFB were identified, and the time to see two bacilli was recorded. The time taken to examine negative slides was also recorded. The remaining deposit was cultured in liquid mycobacterial

growth indicator tubes (Becton Dickinson) and on Lowenstein-Jensen medium for 12 weeks.

The clinical and laboratory features and final diagnosis of each patient admitted were recorded prospectively in individual study notes. The diagnosis of TBM was confirmed if AFB were seen or cultured from the CSF, was probable if AFB were found from another site or there was evidence of active extraneural tuberculosis, and was possible if the history was longer than 5 days and the CSF abnormalities included a raised white cell count, predominantly lymphocytes, and low CSF/blood glucose ratio (<0.5). The diagnosis of TBM was excluded if another pathogen was seen or cultured from the CSF or if the patient recovered without antituberculosis chemotherapy (ATC). All patients with TBM were tested for antibodies to human immunodeficiency virus (HIV). Forward logistic regression was used to model admission variables independently associated with the bacteriological diagnosis of TBM. The following variables were selected a priori to enter the model: age, sex, HIV status, duration of symptoms, disease grade, volume of CSF, CSF opening pressure, CSF total white cell count with neutrophil and lymphocyte percentages, CSF total protein, lactate, chloride, and CSF/blood ratio. The analysis was performed using SPSS software version 10.0 (Microsoft). The Hospital Scientific and Ethical Committee approved the study.

Three hundred thirty adults entered the study. The median volume of CSF examined was 4.0 ml (range, 0.2 to 12.0 ml), and the median time to examine each slide was 20 min (range, 1 to 50 min). One hundred ninety-eight adults (241 specimens) did not have TBM: AFB were neither seen nor cultured from these specimens.

One hundred thirty-two adults had clinical TBM: 107 (81%) had bacteriological confirmation of the diagnosis, 15 (11%) had probable TBM, and 10 (8%) had possible TBM. Fourteen of 132 (11%) were infected with HIV. Over the same period approximately 4% of adults with pulmonary tuberculosis treated in Ho Chi Minh City were infected with HIV (3).

AFB were found in the CSF of 73 of 141 specimens taken from 132 patients before ATC (sensitivity, 52%) and 77 of 132 (58%) patients in the first week of ATC. *Mycobacterium tuberculosis* was cultured from 90 of 141 specimens before treatment (sensitivity, 64%) and from 94 of 132 (71%) patients in total. AFB were seen but not cultured from 13 patients, four after the start of treatment. All these specimens came from patients

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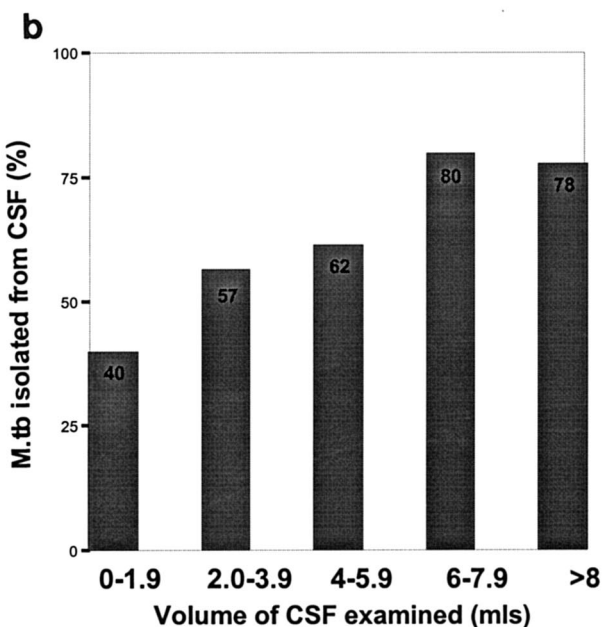
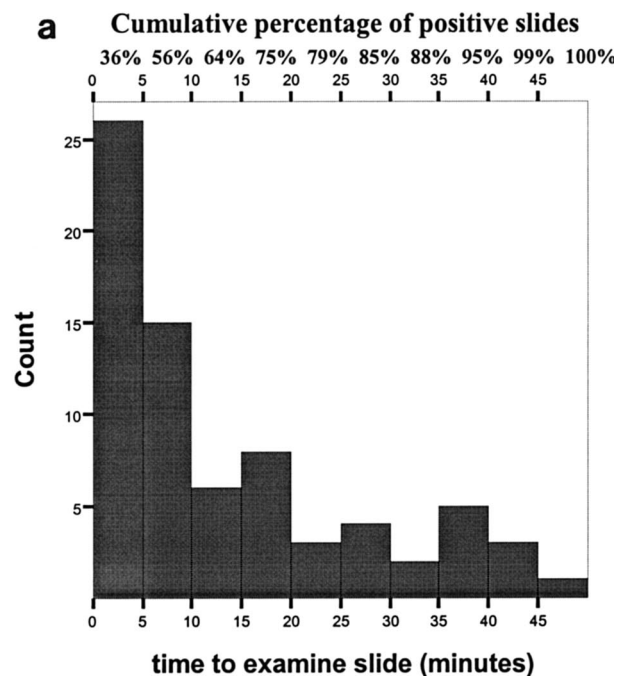


FIG. 1. (a) Time to see two AFB in the CSF of 73 positive slides before starting ATC. (b) Relationship between volume of CSF taken and percentage of specimens with *M. tuberculosis* cultured from 118 HIV-negative patients with TBM.

with clinical features of probable TBM who responded appropriately to ATC and were not considered false positive.

The median time to see bacilli in the CSF before the start of treatment was 10 min (range, 1 to 50 min), and 75% of AFB

TABLE 1. Factors associated with bacteriological confirmation of TBM by forward logistic regression

Factor	Odds ratio	95% confidence interval	P
Duration of symptoms (days)	1.05	1.00–1.10	0.050
Volume of CSF (ml)	1.36	1.06–1.75	0.017
CSF neutrophils (% of white cell count)	1.03	1.01–1.05	0.008
CSF lactate (mmol/liter)	1.42	1.16–1.73	0.001
CSF/blood glucose ratio	0.03	0.02–0.62	0.023

were seen within 20 min (Fig. 1a). Multivariate analysis showed that increasing the volume of CSF increased the likelihood of confirming TBM (Table 1). Figure 1b shows the effect of increasing volume upon culture rates in HIV-negative individuals. *M. tuberculosis* was isolated from smaller CSF volumes from HIV-infected individuals (median, 1.5 versus 4.0 ml; $P = 0.001$), which suggests that these adults may have greater CSF bacterial loads. Variables independently associated with bacteriological diagnosis (Table 1) may also reflect bacillary concentration. We and others have recently shown that the same variables (except duration of symptoms) were associated with death from TBM in the same population (5).

This study confirms the importance of CSF volume and duration of microscopy in the bacteriological diagnosis of TBM and suggests that simple measures can improve diagnostic performance. At least 6 ml of CSF should be taken and examined for at least 30 min. However, this approach may be too time-consuming for many laboratories. Instead, CSF from patients most likely to contain *M. tuberculosis* could be identified, either by diagnostic algorithm (6) or by the clinical variables described by this study (Table 1), and these specimens could be examined accordingly. Initial microscopy of areas of high cellular density, which commonly contain organisms, may reduce the time to see AFB.

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