Tuberculous meningitis (TBM) is a devastating condition with high mortality and survivors are often left with severe neurological sequelae. HIV-associated TBM can have mortality rates exceeding 60% (1). Early diagnosis and initiation of treatment is critical for survival. Unfortunately the diagnosis of TBM can be more of an art than a science, as a recent report in the Lancet by Bahr and colleagues highlights. There is currently no diagnostic test with high sensitivity and specificity. Culture of the slow growing Mycobacterium tuberculosis from cerebrospinal fluid (CSF) takes 10–21 days, even in the faster commercial liquid culture systems; smear microscopy on CSF is fast but insensitive. Molecular techniques offer faster testing and the recent scale-up of the cartridge based molecular Xpert MTB/RIF test has greatly reduced the skill level required to perform molecular tests. However sensitivity at only around 60% of clinically diagnosed cases, needs improvement. Consequently these tests cannot be used to ‘rule-out’ TBM, leaving clinicians to use formal or informal algorithms for diagnosis of a disease that requires rapidly administered appropriate treatment for optimal outcome.

In the absence of a robust gold standard against which to evaluate the novel diagnostic test (in this case GeneXpert Ultra), the investigators have used the standardized clinical case definition, which is accepted practice (2,3). This classifies cases evaluated into ‘definite’, ‘probable’, ‘possible’ or ‘not TBM’, based on a range of microbiological, biochemical and imaging test results. However, in this study reported in the Lancet, the authors also include a positive result for the test under investigation, Xpert Ultra, in their definition of ‘definite TBM’. They justify this by claiming the novel test is unlikely to give false positive results. The obvious flaw in this approach is the assumption that the novel diagnostic is accurate, precluding impartial evaluation. Yet evaluating only against the gold standard, which is known to be imperfect also precludes any advances in the diagnosis of the disease. This conundrum is not unique to TBM which is one of many diseases where no perfect gold standard exists and the evaluation of novel diagnostic tests are therefore difficult to interpret. Cryptococcal meningitis, which the patients in this study were also being evaluated for suffers from the same issue, as does pulmonary TB to a lesser degree.

How then should we interpret the findings by Bahr and colleagues in deciding how to reform policy recommendations for a disease where an improved diagnostic test is urgently needed?

The study evaluated 129 HIV-infected individuals for TB, who were being screened for inclusion in a trial of cryptococcal meningitis treatment in Mbarra, Uganda. CSF samples were tested by M. tuberculosis culture, Xpert and also underwent routine biochemical evaluations and tests for other pathogens. A frozen aliquot of CSF was later tested by the new Xpert Ultra test.

Xpert Ultra is a new version of the molecular GeneXpert MTB/RIF test (Cepheid, USA). The Ultra cartridge incorporates a number of modifications aimed at lowering the limit of detection for M. tuberculosis bacilli thereby
increasing the test sensitivity. These include increasing the sample volume entering the test chamber (50 µL for Ultra vs. 25 µL for MTB/RIF) and adding an additional amplification target (IS6110 and IS1081). GeneXpert Ultra gives a semi-quantitative categorical result for positive samples of ‘trace/very low/low/medium/high. The limit of detection for Xpert Ultra is reported to be 16 colony forming units (cfu/mL) compared to 114 cfu/mL for Xpert MTB/RIF. For pulmonary TB diagnosis, WHO has endorsed the Xpert Ultra test but recognized that the increased sensitivity has also decreased specificity (4).

A large multi-country evaluation of Xpert Ultra for pulmonary TB gave a sensitivity of 88% and a specificity of 96%, compared 83% to 98% respectively for Xpert MTB/RIF (5). This reduced specificity compared to the Xpert MTB/Rif cartridge is thought to be attributable to previous TB, with nonviable bacilli giving a positive Xpert Ultra result in some individuals without current active disease. ‘Trace’ calls, indicating the lowest bacillary numbers, must be further evaluated in the clinical context according to a WHO algorithm, complicating diagnostic interpretation in routine settings and non-specialist centres. For pediatric TB, where previous TB episodes are rare, the Xpert Ultra increases sensitivity without reduced specificity and should therefore be used as the test of choice, where available, for pediatric specimens. The test is an obvious candidate for improving the diagnosis of TBM where few bacilli are present in the CSF, and detection by culture takes several weeks (6). Additionally, non-viable bacilli in the CSF are unlikely and will cause immunopathogenesis. WHO currently recommends Xpert MTB/RIF as the test of choice for TBM because it returns a result in 2 hours with high specificity, although it has a sensitivity of only approximately 60% (7,8).

In the current study of HIV-infected adults, 107/129 patients were negative by culture, Xpert and Xpert Ultra tests. Of the remaining 22, 10 were culture positive, 10 were positive by Xpert and 21 were positive by Xpert Ultra. If we assume, as the authors do, that Xpert Ultra positives are ‘true positives’ this appears to offer a greatly increased sensitivity, of 95%. If we assume the Ultra positive results in individuals negative by the other microbiological tests are ‘false positives’ then we will dismiss the Xpert Ultra test as having poor specificity. We know that other tests suffer from low sensitivity due to clinical experience of many patients who recover on TBM treatment despite testing negative, and post mortem studies which confirm TBM in patients who do not survive. Historically, evaluations of novel diagnostics for TBM have resorted to various clinical case definitions to try and provide an appropriate evaluation, but each study used a slight variation of the classification of cases into ‘definite/probable/possible/not TBM’. The standardized clinical case definition published in 2010 was an attempt by the TBM research consortium to address this issue by ensuring at least that studies were comparable, and to allow robust meta-analysis which is extremely valuable for rare diseases like TBM (3). However, although the case definition includes ‘commercial PCR positive’ in the definite TBM case criteria, to avoid incorporation bias this should not include the test under evaluation.

In a second analysis the authors excluded Xpert Ultra results from the case definition, reporting a less impressive, but still improved, sensitivity of 70% and a specificity of 95%. We concur with the authors that it is likely the majority of those with Xpert ultra positive test in the CSF are in fact TBM, however a study in 129 individuals, in one country setting, and including only HIV-associated TBM is too small to recommend global policy revisions. Further reports from other centres are imminent and will help to clarify the true potential of Xpert Ultra in TBM diagnosis.

The attempt to confirm positive results by sequencing the residual Xpert fluid post-amplification does not confirm diagnostic accuracy, in that it does not uncover whether cross-contamination has occurred during sample processing and is responsible for the putative ‘false positives’. Although the self-contained Xpert cartridge is much less susceptible to cross-contamination than ‘old fashioned’ PCR techniques, it cannot be ruled out in these early evaluations and a valid method to exclude this should be incorporated into subsequent, larger studies. HIV-associated TBM has higher CSF bacillary loads than TBM in HIV negative individuals (a scenario reversed in pulmonary TB, where HIV-infected individuals have lower bacillary counts in sputum). It should be expected that the sensitivity of Xpert Ultra will be considerably lower in HIV-negative TBM, as has been seen for previous molecular evaluations. However, some improvement in sensitivity over Xpert MTB/ RIF is likely in HIV-negative TBM.

Bahr and colleagues confirm previous reports that concentrating larger volumes of CSF by centrifugation results in higher yields for TBM diagnosis, with positive yields of 26% for volumes of 6 mL or greater, compared to 7% with lower volumes. Importantly, this study also tested a lower volume of centrifuged CSF in the Xpert Ultra cartridge (0.5 mL) than in the MTB/RIF cartridge (1 mL). Given the known correlation between CSF volume
tested and likelihood of a positive result, this should have considerably disadvantaged the Ultra test.

Overall, the data presented in this evaluation suggests that Xpert Ultra has the potential to substantially improve diagnostic confirmation of TBM, particularly if the volume of CSF tested by Ultra is maximized rather than splitting the CSF between multiple tests. Both the “optimistic” analysis, which suffers from incorporation bias, and the more rigorous analysis strictly applying the standardized case definition, show improved sensitivity: the question is the degree to which Ultra can improve sensitivity of TBM diagnosis. A much larger, multi-country study including both HIV infected and uninfected individuals is required to establish if this promising early report can be confirmed. Evidence that Xpert Ultra is able to deliver not only improved rapid confirmation of TBM but also avoid the pitfall of a high false positive rate that all too often accompanies increased sensitivity, is needed. We know that early diagnosis and treatment initiation is crucial in recovery from TBM. If Xpert Ultra can show a sensitivity of >70% in HIV-associated TBM it will be a significant advance that has the potential to save many lives. We eagerly await further data to confirm these findings.

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Footnote

Conflicts of Interest: SJ Dunstan and M Caws are members of the International Research Consortium on tuberculous meningitis, as are Bahr and Boulware (authors on the linked Article). However, they have never worked on any collaborative research projects.

References


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