Diagnosing tuberculous meningitis - a testing process

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Managing tuberculous meningitis (TBM), considered the most severe form of tuberculosis, remains a challenge even for experienced clinicians. Disease presentation may mimic other meningitides, diagnostic tests are less sensitive than for other forms of TB, and numerous hard-to-predict complications can result in substantial mortality and morbidity.[1–4]

Bacterial loads seen in cerebrospinal fluid (CSF) are lower than those usually encountered in sputum, and diagnostic tests designed for more common forms of TB haven't always served this paucibacillary disease as well. Indeed, even assessing new diagnostic platforms in TBM has been a challenge as the relative insensitivity of even bacterial culture has left the field devoid of a gold standard.

The inherent problem with insensitive reference tests is that they select for a sub-group with higher *M. tuberculosis* loads. Understanding the meaning of an apparent false positive result from a new and more sensitive diagnostic test is thus challenging. The Marias criteria[5], which categorise TBM as definite, probable, possible, and not TBM, have been used as an attempt to rectify this issue. These standardised TBM diagnostic criteria are increasingly being used as reference standards for diagnostic tests in TBM.[6–11] Yet this approach too is problematic. Which combination of definite, probable, and possible TBM includes all true TBM cases, without including non-TBM cases? The answer may vary between different patient populations, with HIV co-infection a particularly important factor. As such, the performance of diagnostic tests is often presented in different ways, against definite TBM; definite plus probable TBM; and definite plus probable plus possible TBM, resulting in multiple different results for 'sensitivity' and 'specificity'. Adding in mycobacterial culture as a reference standard, and stratification by HIV co-infection, the clinician is left with a bewildering range of diagnostic performance values, and a muddied message regarding test performance.

This challenging landscape for evaluating diagnostic tests for TBM is the backdrop to an encouraging increase in TBM diagnostic research in recent times. Given ZN smear microscopy has low sensitivity at most centres, and mycobacterial culture is too slow to influence early drug management, PCR testing has dominated the last 10 years of TBM diagnostic research – particularly the game changing GeneXpert MTB/RIF. Its successor GeneXpert MTB/RIF Ultra (Xpert Ultra) is now recommended as a first line test for extrapulmonary TB in all settings,[12] with diagnostic capabilities in TBM recently described in large studies.[7,9] Further data describing how newer diagnostics perform in TBM would nevertheless be hugely valuable and welcome to the field.

In this issue of the International Journal of Tuberculosis and Lung Disease, Sharma *et. al.* present a comparative analysis of Truenat MTB Plus (TruPlus) and Xpert Ultra for the diagnosis of TBM.[13] TruPlus (Molbio Diagnostics, Goa, India) is a portable battery-operated semi-automated chip-based PCR assay that can function in tropical climates, detect *M. tuberculosis* in ~1 hour, and test for rifampicin resistance within a further 1 hour.[13,14] TruPlus has comparable accuracy to Xpert Ultra for

pulmonary TB diagnosis, and Truenat MTB RIF (TruRif) comparable accuracy to Xpert Ultra for rifampicin resistance detection.[15] Until now, TruPlus had not been studied for the diagnosis of TBM.

Sharma *et. al.* prospectively collected CSF samples from 148 individuals, who were then assigned to one of three groups; definite TBM, probable TBM, or non-TBM. At least 2-3 ml of CSF was sampled, and the centrifuged CSF pellet was used for TruPlus (0.5ml) and for Xpert Ultra (1ml). Diagnostic performances of TruPlus and Xpert Ultra were evaluated against Marais criteria[5] (definite TBM, and definite plus probable TBM), and also against mycobacterial culture. As Xpert Ultra was included in the reference, there could be no false positives for Xpert Ultra and specificity was fixed at 100%.

Against a reference standard of definite plus probable TBM, there was no significant difference in the sensitivities of TruPlus and Xpert Ultra. As expected, diagnostic sensitivities increased for both assays when the reference standard was definite TBM alone, and also where positive mycobacterial culture was used as the reference. There was no statistically significant difference between TruPlus and Xpert Ultra and for any of these diagnostic sensitivity comparisons.

As important as the confirmation of TBM is the identification of drug resistance, in particular multi-drug resistance. Sharma and colleagues therefore assessed the performance of TruRif for the detection of rifampicin resistance. Although the numbers were small, it was encouraging to find that TruRif incorrectly reported rifampicin sensitivity in just one case.

How should these results be interpreted? Firstly, the data showing TruPlus to be comparable with Xpert Ultra for the diagnosis of TBM are promising. Increasing competition in the field of TB diagnostics may serve to improve standards and lower prices. TruPlus requires technical expertise – samples must be pipetted from an initial device used for sample preparation and DNA extraction, to test chips for *M. tuberculosis* identification, and for rifampicin susceptibility testing.[16] Yet the ability to operate equipment at tropical temperatures ($\leq 40^0$C)[17] is hugely attractive given how tropical climates carry a large proportion of the global TB burden.

Where next for TruPlus and TBM diagnosis? Certainly, more studies are needed to evaluate how TruPlus performs in different settings where population age, host genetics, *M. tuberculosis* strain, HIV co-infection and CSF processing may each be different. But these data represent a promising start for TruPlus in the field of TBM. In the long term, TBM diagnosis may need different approaches to those seen to date. These may include tests specifically designed with TBM in mind, rather than tests designed for pulmonary TB that are then 'borrowed' for TBM. Tests that do not require CSF sampling, which is not always rapid, straightforward, or indeed safe, would be most welcome. Until then, TruPlus appears to add to the TBM test options, and will hopefully contribute to improving patient outcomes from this catastrophic disease.

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