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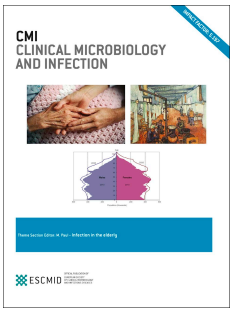

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Improving the diagnosis of tuberculous meningitis: good, but not good enough.

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Tuberculosis (TB) is the leading infectious cause of death globally. Meningitis accounts for 1-2% of TB cases (more in HIV-endemic settings), but kills or disables up to 50% or more of those affected [1]. Tuberculous meningitis (TBM) is notoriously difficult to diagnose with conventional microbiological techniques due to the scarcity of bacilli and *Mycobacterium tuberculosis* DNA.

In the absence of the detection of acid-fast bacilli in the cerebro-spinal fluid and with *M. tuberculosis* cultures pending, specific bacterial DNA can rapidly be detected by nucleic-amplification techniques (NAT). Polymerase chain reaction (PCR), the most common NAT, identifies nucleic acids by amplifying a specific nucleic acid molecule with the enzyme DNA polymerase. Digital PCR (dPCR) is a refinement of conventional PCR methods that can be used to identify and clonally amplify nucleic acids strands (DNA, cDNA or RNA). Instead of performing one reaction per well, dPCR partitions the PCR solution into tens of thousands of nanolitre sized droplets, where a separate PCR reaction takes place in each one [2]. This separation allows a more reliable collection and sensitive measurement of nucleic acids. Digital PCR has proved to be a useful tool in many applications including basic sciences, clinical diagnostics and testing for environmental contamination. When dPCR was first pioneered in the 1990s it was labour intensive, complicated and difficult to perform at scale. However, recently biotech companies have developed commercial dPCR systems that automatically partition samples and digitally count nucleic acid targets [2]. The first dPCR system for routine clinical use was CE-marked in 2017 for the diagnosis of chronic myeloid leukaemia. The full potential of this technology remains to be explored in other infectious and malignant conditions.

In this edition of CMI, *Li et al.* present a clinical evaluation of dPCR for the diagnosis of TBM [3]. The authors examined CSF from 101 HIV-negative patients in Beijing who had presented with meningitis over a 5-year period, including 26 definite TBM cases and 34 probable TBM cases. *IS6110*-dPCR was more sensitive (73%, 95% CI 52.2-88.4%) than *gyrB*-dPCR (39%, 95% CI 20.2-59.4%) against a reference standard of definite TBM, and both assays demonstrated high specificity (97% and 100% respectively). *IS6110*-dPCR performed better than Xpert MTB/Rif (sensitivity 70% versus 30%). Whilst this study was small and with limitations it does highlight that dPCR could be an important technology for improving the diagnosis of TBM and should be explored in larger studies and other populations.

Currently the Xpert MTB/Rif "Ultra" assay (Cepheid), the fully-automated cartridge-based test, is endorsed by the WHO as the best initial test for TBM and roll-out of Ultra is underway in many countries. The re-engineered Ultra assay uses the same platform as the Xpert MTB/Rif assay (Xpert) but has two additional probes (IS6110 and IS1081) and allows double the volume of sample to reach the PCR reaction. As a result, the limit of detection of Ultra is 8-fold lower than Xpert and Ultra proved to be significantly more sensitive

that both Xpert and MGIT culture (90% versus 45% and 45% respectively) against a reference standard of definite TBM[4].

As an alternative to a molecular test specific for TB, unbiased meta-genomic next generation sequencing (mNGS) has the potential to diagnose any organism or multiple pathogens with a single assay [5]. Next generation sequencing technology is advancing rapidly, but it will only have the potential to make an impact on clinical diagnostics and clinical care if the technology gets closer to the bed-side and the turnaround time is shortened. Unbiased mNGS has recently proven to be useful in diagnosing rare central nervous system infections in the USA, some of which had been missed with conventional microbiology techniques [5]. Further, adding an enrichment step for *M. tuberculosis* sequences has allowed DNA to be detected in CSF at such low abundances that it was missed by Ultra, Xpert and MGIT culture [6].

When *M. tuberculosis*-specific DNA cannot be detected from the CSF a rapid and moderately accurate diagnosis of TBM is still possible by demonstrating recruitment of *M. tuberculosis*-specific lymphocytes to the CSF with a conventional ELISpot interferon-γ release assay (T-Spot.TB Test, Oxfordimmunotec, Abingdon, UK) [7]. However, failure of the positive control often leads to indeterminate test results [8] and this technology is not widely available in resource-limited settings and the evaluation requires several millilitres of CSF to obtain enough viable cells for an analysis.

A major consideration regarding the public health impact of novel diagnostic technologies for TB, especially mass sequencing, is their transferability to low and middle-income countries, where the greatest burden of disease and morbidity and mortality co-exist. The capital cost of the equipment, per sample cost, infrastructure requirements, laboratory expertise, the need for batching and turnaround time are important considerations. Digital PCR still has some way to go to overcome these challenges but is moving in the right direction.

Importantly, so far no molecular or other laboratory test can exclude TBM, and patients should be started on treatment based on a presumptive clinical diagnosis even if microscopy and PCR are negative. But making a (presumptive or confirmed) diagnosis of TB is only a single (but important) step in a TB cascade of care. The cascade is a model for evaluating patient retention across sequential stages of care required to achieve a successful treatment outcome. The cascade identifies concerning attrition in every step of the cascade, which need to be addressed with multifaceted interventions and collaboration from all partners and stakeholders in order to meet the WHO End TB strategy targets. With regard to TBM, early initiation of TB treatment before

the onset of coma is crucial in order to avoid long-term disability and death. In addition, the importance of good supportive care for TBM patients cannot be understated [9].

The notion of 'intensified treatment' for TBM is receiving considerable interest in light of the fact that two of the four first-line anti-TB drugs, rifampicin and ethambutol, do not readily cross the blood brain barrier and thus are only found in low concentrations at the site of disease. Intensification options include adding additional anti-TB drugs (e.g. fluoroquinolones or linezolid) or using higher doses of first-line drugs (i.e. rifampicin or isoniazid) [10]. A small phase II trial in Indonesia showed a significant reduction of mortality with a higher dose intravenous rifampicin for the first two weeks [11], a much larger phase III trial with a modest increase of oral rifampicin and additional levofloxacin did not result in a reduction in mortality except for patients with isoniazid resistant MTB [10]. However, the rifampicin dose increase in that trial was modest and several large trials will test a much higher dose of oral rifampicin, including one that is soon to begin in Indonesia, Uganda and South Africa (ISRCTN 15668391).

Better strategies are also needed to control the damaging inflammation associated with TBM [3]. Tuberculous meningitis typically presents with a thick exudate at the base of the brain that can lead to hydrocephalus, brain infarction and cerebral palsy, and can also cause inflammatory mass lesions. Therefore, patients routinely receive adjuvant corticosteroids, but although corticosteroids improve survival, they do not reduce neurological sequelae, and seem least effective in patients with advanced disease. Alternative host-directed strategies are therefore needed as well, with aspirin showing promising results in a phase II randomised controlled trial [12]. Another recent study combining CSF metabolomics and genome-wide SNP-typing identified tryptophan metabolism as a strong predictor for patient mortality, and a potential target for therapy [13].

Still, besides optimal microbiological diagnosis, optimal antimicrobial and supportive treatment, and personalized host-directed therapies, the most important predictor of outcome of patients with TBM or other CNS infections, especially in low-resource settings, will be the social and health-service context. Patient need access or referral to specialised services, with well-trained staff who can provide an appropriate diagnostic work-up and treatment for presumed CNS infections. Major gaps have been identified in the care for patients with CNS infections in high-burden settings, such as the inability to do lumbar punctures, perform indicated diagnostic tests or start appropriate antimicrobial treatment [14].

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