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ADVANCES IN THE DIAGNOSIS OF HIV-ASSOCIATED TUBERCULOSIS

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ABSTRACT

HIV-associated tuberculosis (HIV-TB) remains a global public health challenge, with the major burden being borne by countries in low-resource settings. If World Health Organization targets to reduce TB deaths by 95% and new cases by 90% are to be met by 2035, major improvements in diagnostic strategies are among the most pressing needs. HIV coinfection presents particular challenges in the diagnosis of TB due, for example, to the relatively low mycobacterial burden in sputum specimens and rapid dissemination beyond the lungs. Low and middle-income countries still typically rely on traditional diagnostics such as chest radiology and sputum microscopy, which lack sufficient accuracy. Desired characteristics for an HIV-TB diagnostic test are well described and include the ability to test a wide variety of clinical samples, diagnose extra-pulmonary TB, have good accuracy to detect low mycobacterial burden disease, and be deployable at the peripheries of healthcare systems. Following a long period of under-investment in TB research, development of TB diagnostics has progressed rapidly over the past decade and the technology landscape looks much more promising. This article will summarise advances in diagnostics that are particularly relevant to HIV-TB. The Xpert® MTB/RIF and Determine™ TB LAM assays have the most evidence assessing their use in HIV-TB. In addition to nucleic-acid amplification tests and antigen detection we will review new diagnostic technologies. Finally, we discuss whether use of empirical TB treatment offsets the potential impact and reduces the need for new diagnostics.

Keywords: Tuberculosis, HIV, AIDS, Mycobacterium tuberculosis, diagnosis, Xpert MTB/RIF, nucleic acid amplification test, lipoarabinomannan, Determine TB LAM, antigen detection, urine.

INTRODUCTION

HIV-associated tuberculosis (HIV-TB) remains a major global public health challenge and is the leading cause of HIV-related mortality with approximately 1.1 million cases and 360,000 deaths in 2013.1 Although Sub-Saharan Africa accounts for the majority of the global caseload (85%), focussed epidemics also occur elsewhere, for example, among intravenous drug users and prisoners in Eastern Europe, where there is a major overlap with high rates of drug-resistant TB and high mortality attributable to TB.1-4 In patients living with HIV infection, the relatively low mycobacterial burden in sputum specimens and rapid dissemination beyond the lungs render diagnosis challenging.5-9 Low and middle-income countries still typically rely on traditional diagnostic tests such as chest radiology and sputum microscopy, which lack sufficient accuracy.10,11 Turnaround times for liquid culture (the most sensitive diagnostic test) are often too long to be clinically useful and the required laboratory infrastructure is very often not available in the highest-burden settings. Clinical suspicion and empirical treatment are widely used but can lead to under or over-treatment.12,13 Data from hospital post-mortem studies show that up to half of HIV-TB remains undiagnosed at the time of death.14-16
Addressing the scale of the TB epidemic and accelerating progress towards elimination is the focus of an ambitious post-2015 Global Health Strategy announced by the World Health Organization (WHO) and partners.\textsuperscript{17} If targets to reduce TB deaths by 95% and new cases by 90% by 2035 are to be met, major improvements in diagnostic strategies are among the most pressing needs.\textsuperscript{17–19} WHO have also recognised the public health importance of HIV-TB coinfection and have recommended a series of 12 collaborative interventions to reduce the burden of HIV-TB and strengthen delivery of care;\textsuperscript{20} better diagnostics are central to many of these interventions.

Desired characteristics for an HIV-TB diagnostic test are well described,\textsuperscript{21} and include the ability to test a wide variety of clinical samples to detect extra-pulmonary TB (EPTB) in addition to pulmonary TB (PTB). Diagnostics need to be suitable for use at peripheral health units to screen those most at risk and intervene early in the disease. Point-of-care (PoC) assays are needed to enable diagnosis and treatment initiation in the same clinical encounter. Assays need to be amenable for use not only in adults but also in children, who are even less likely to be able to produce sputum and to test positive using smear microscopy.\textsuperscript{22} Diagnostic tests also need to differentiate latent from active TB (due to differences in their subsequent management) but still be able to detect low mycobacterial burden disease. Obtaining and testing samples should be safe for patients and healthcare workers, even in resource-limited settings where biosafety equipment is often unavailable. Multi-drug resistant TB (MDR-TB), which is a leading challenge for TB control and for which HIV is a risk factor,\textsuperscript{23,24} remains mostly undiagnosed and inadequately treated due to lack of access to drug-susceptibility testing. Therefore, new diagnostics should ideally be able to screen simultaneously for drug resistance using the initial diagnostic sample.

Following a long period of under-investment in TB research, development of laboratory diagnostics has progressed rapidly over the past decade and the technology landscape looks more promising (Figure 1). Advances include improvements in old diagnostic technologies such as sputum-smear microscopy and culture-based systems.\textsuperscript{25,26} However, much progress has also been made with respect to development of new technologies, including rapid molecular tests.\textsuperscript{27} This article will summarise advances in laboratory diagnostics that are particularly relevant to HIV-TB, focussing especially on recently developed technologies, but excluding radiological imaging techniques and advances in mycobacterial culture-based diagnostics. The enormous diagnostic challenges of TB drug resistance and TB diagnosis in HIV-infected children are important, but are beyond the scope of the present article. Since the vast majority of the HIV-TB burden is in resource-limited settings where TB diagnostic capacity is weakest, we provide considerable focus on solutions for these settings. Finally, we discuss whether use of empirical TB treatment offsets the potential impact and reduces the need for new diagnostics.

\section*{NUCLEIC ACID AMPLIFICATION TESTS}

Nucleic acid amplification tests (NAATs) rely on the amplification and detection of nucleic acid sequences specific to \textit{Mycobacterium tuberculosis} (MTB) complex. Early NAATs were developed for TB over 20 years ago, although limited test performance meant that these were not widely implemented. Development of newer NAATs, however, makes them one of the leading commercially available TB diagnostic technologies at present (Figure 1).

\subsection*{Line Probe Assays}

Line probe assays (LPAs) use polymerase chain reaction (PCR) amplification of DNA for diagnosis directly from clinical samples or by testing culture isolates. Their use was endorsed by WHO in 2008, and they are capable of screening for genotypic drug resistance by detecting known genetic mutations.\textsuperscript{29} A key disadvantage of LPAs is that they function best when testing samples with high bacillary load (smear-positive). The cost and complexity of LPAs also requires laboratory infrastructure and personnel capable of performing DNA extraction and PCR (rarely available in high-burden settings). Further advances in LPA technology now permit genotypic testing for resistance to some second-line TB drugs with moderate sensitivity and high specificity (GenoType MTBDRsl assay, Hain Lifescience, Germany).\textsuperscript{30} Moreover, newer assays have lower limits of detection and can be used to directly test smear-negative sputum samples (MTBDRplus version 2.0, Hain Lifescience).\textsuperscript{31–33} These have the potential to be used at reference laboratory level and screen for resistance to important second-line agents, especially in better-resourced settings and where drug resistance is more common.
The next NAAT endorsed by WHO has been referred to as a ‘game changer’ in the field of TB diagnostics. The Xpert MTB/RIF assay (Xpert, Cepheid Inc., USA) is a self-contained, fully integrated, and semi-automated NAAT developed for relatively unskilled users. Within a single-use cartridge, it uses hemi-nested real-time PCR and ‘molecular beacon’ technology to detect DNA sequences within MTB’s RNA polymerase β subunit gene (rpoB), allowing diagnosis of MTB and genotypic detection of 95% of rifampicin (RIF)-resistant strains. It was endorsed by the WHO in 2010 due to its diagnostic accuracy and several other positive attributes. These included ease of use, a substantially reduced biosafety risk and the ability to provide results within hours.
compared with sputum-smear microscopy, and relatively short processing time (just over 2 h).\textsuperscript{40,41} Initial recommendations were for Xpert’s use as the primary diagnostic test for HIV-TB, or in settings where MDR-TB was suspected.\textsuperscript{41}

The potential limitations of Xpert use in resource-constrained settings include the need for a stable electricity supply and operating temperatures, external quality assurance, regular calibration of instruments, and robust supply chains and storage facilities for Xpert cartridges.\textsuperscript{42} On a health-systems level, challenges to implementation and scale-up include changes to diagnostic pathways, policies and notification systems, increased human resource needs, and increased capacity for investigating and treating suspected drug-resistant TB.\textsuperscript{42} These logistical and health-system factors may mean that use of Xpert outside of the laboratory environment will be challenging, which may in turn reduce potential impacts of Xpert on patient outcomes.\textsuperscript{43}

Several studies assessing the diagnostic accuracy of the Xpert for HIV-TB have been undertaken since the seminal multi-country assessment.\textsuperscript{44} The pooled sensitivity of Xpert for diagnosis of PTB in HIV-infected individuals is 79% (95% confidence interval [CI]: 70-86%), and specificity is 99% (95% CI: 98-100%).\textsuperscript{32} Sensitivity is 61% (95% CI: 40-81%) for sputum smear-negative TB, compared with 97% (95% CI: 90-99%) for sputum smear-positive disease.\textsuperscript{32} Xpert’s sensitivity is related to mycobacterial load, thus it is likely to be lower in patient populations with fewer symptoms and low rates of smear-positive disease, for example, screening asymptomatic HIV-infected patients.\textsuperscript{42,45} This may explain the variability in sensitivity observed between studies.

**Xpert MTB/RIF assay for non-respiratory samples**

The high proportion of patients with HIV-TB who have EPTB provides another important diagnostic opportunity using this assay.\textsuperscript{46} Systematic reviews have reported very high specificity of Xpert when testing a very wide variety of non-respiratory clinical samples,\textsuperscript{47,48} despite the fact that culture is an imperfect reference standard for EPTB and may lead to underestimation of specificity.\textsuperscript{49}

Overall sensitivity was high for smear-positive samples (97.4%, 95% CI: 95.5-99.3%)\textsuperscript{47} and for all culture-positive samples tested (median: 83%, interquartile range [IQR]: 68-94%).\textsuperscript{48} Sensitivity using a culture reference standard varied substantially between sample types, with good sensitivity observed in lymph node tissue (83.1%, 95% CI: 71.4-90.7%) and cerebrospinal fluid (80.5%, 95% CI: 59.0-92.2%), and poor sensitivity in pleural fluid (46.4%, 95% CI: 26.3-67.8%).\textsuperscript{47} It is difficult to directly compare the diagnostic accuracy of Xpert for EPTB in HIV-infected and uninfected individuals due to paucity of data, but accuracy estimates did not differ substantially in studies with high proportions of HIV-infected patients.\textsuperscript{47} WHO has now endorsed the use of Xpert for non-respiratory samples.\textsuperscript{50}

Two studies have also assessed the Xpert’s utility in diagnosing HIV-TB using urine samples. Urine was positive in 44.4% of outpatients with sputum culture-positive TB and CD4 count <50 cells/µl, and 47.8% of inpatients with culture-positive HIV-TB.\textsuperscript{51,52} Diagnostic yield was higher among those with lower CD4 counts (<100 cells/µl) in both studies, suggesting positive urine samples represent disseminated TB in the sickest HIV-infected patients.

**Xpert MTB/RIF assay in resource-rich settings**

Few data exist on the use of Xpert in resource-rich settings, despite recommendations in guidelines and wide availability in European centres.\textsuperscript{53,54} A study conducted in a university hospital TB clinic in Canada (low TB and HIV prevalence) reported only moderate sensitivity compared with liquid-culture reference standard (46% overall, 28% in smear-negative cases).\textsuperscript{55} It was concluded that the potential for impact was minimal due to less extensive TB disease on admission and robust existing diagnostic algorithms. However, other researchers have suggested that using Xpert as the initial investigation for TB could reduce the use of empirical treatment, unnecessary contact investigations, in-hospital respiratory isolation, and length of hospital stay.\textsuperscript{56-58} There is also potential impact when used as a ‘rule-in’ test,\textsuperscript{59} for example in immunosuppressed HIV-infected patients, when TB is a common opportunistic infection and can be difficult to rapidly distinguish from non-tuberculous mycobacterial (NTM) disease and other bacterial infections. There is also potential benefit in settings where HIV-TB and MDR-TB overlap (for example prison populations and injecting drug users), as Xpert has demonstrated accurate detection of MTB and RIF resistance.\textsuperscript{60} However, cost-effectiveness of the Xpert assay has not been proven in resource-rich settings, and this will depend on factors such as prevalence of HIV, MDR-TB, and existing laboratory infrastructure.\textsuperscript{61}
Loop-Mediated Isothermal Amplification

The loop-mediated isothermal amplification (LAMP, Eiken Chemical Co, Japan) is another simplified laboratory-based NAAT that is under development for use in resource-limited settings. Up to 14 specimens can be batch-processed in 2 hours, and the assay detects MTB by rapidly amplifying DNA in sufficient quantities so that results can be read by simple visual determination of fluorescence (compared with positive and negative controls).2762 Although no specific large-scale evaluations have been undertaken for HIV-TB, sensitivity and specificity for PTB were 80% (95% CI: 86-93%) and 96% (95% CI: 95-97%), respectively, compared with culture reference standard.63 Sensitivity is lower when testing smear-negative samples (53.8%), suggesting that it may be less useful in the context of HIV.64 WHO endorsement was declined in 2013 due to ‘inadequate evidence’ and persisting concerns over sub-optimal specificity.65 However, further evaluations are underway.

Emerging Nucleic Acid Amplification Test Technologies

Several ‘fast follower’ technologies to Xpert are emerging, although none have yet been endorsed by WHO (Figure 1).27 These systems have several potential benefits over the Xpert. Many are smaller (often handheld), more robust, lower-cost, have lower power requirements due to isothermal amplification, and can operate from batteries. Genedrive (Epistem, United Kingdom) is a new molecular PoC PCR that can diagnose MTB and detect genotypic RIF resistance.27 A single study reported sensitivity of 90.8% (95% CI: 81.0-96.5%) for MTB and 72.3% (95% CI: 59.8-82.7%) for rpoB mutations, although few smear-negative samples were assessed.66 Truelab MTB (Mobilio, India) is a chip-based NAAT using a battery-powered device with sensitivity of 99.1% in smear-positive pulmonary TB, and 75.9% in smear-negative, culture-positive pulmonary TB, with 100% specificity.2767 If these PoC technologies demonstrate good sensitivity for specimens with low mycobacterial load then there is huge potential for their use in screening HIV-infected patients for TB, and large studies in high-burden settings will be warranted.

ANTIGEN DETECTION

Whereas immune-based assays of the host response to MTB are likely to be undermined in patients with HIV coinfection, direct detection of MTB antigens has long been recognised as having great diagnostic potential in such patients. Several different antigens have been detected in the urine of patients with pulmonary TB.5869 Urine has several advantages over sputum as a diagnostic medium, including relative ease of collection and lower biohazard risk during specimen handling. The most promising antigen to date is lipoarabinomannan (LAM), a major glycolipid component of the MTB cell wall, and commercial enzyme-linked immunosorbsent assays detecting LAM have been available for a decade.7071 LAM is released from metabolically active MTB and is only detectable in those with active TB as opposed to latent infection. The mechanism by which LAM enters the urine of TB patients remains unproven, but possible mechanisms are free LAM entering the urine from the systemic circulation or direct infection of the renal tract with MTB.7273

Urine Lipoarabinomannan Assays

Over the past 3 years, a simple and low-cost lateral-flow assay has become commercially available (Determine TB LAM Ag, Alere, USA). This test can be performed at the bedside by adding 60 µl of unprocessed urine to the test strip - the result is read after 25 minutes by comparing visible bands with the manufacturer’s reference card (Figure 2). In contrast to other diagnostic assays for TB, urine-based LAM detection appears to have greater diagnostic accuracy in patients with HIV coinfection.74 Sensitivity is <25% in HIV-uninfected populations, making it of little diagnostic use in this group.74

Several studies have assessed the diagnostic accuracy of urine-LAM testing for HIV-TB. Early studies reported a pooled sensitivity of 56% (95% CI: 40-71%) and specificity of 95% (95% CI: 77-99%).74 A key observation is that diagnostic accuracy increases with more advanced degrees of immunosuppression.7275 This probably relates to higher mycobacterial load and more disseminated disease leading to greater probability of renal involvement.756768 Studies stratifying urine LAM by CD4 cell count have shown sensitivities of 56-85% among patients with CD4 <50 cells/µl.7985 Similarly, sensitivity has been found to be higher among hospital inpatients than among outpatients (58-67% versus 17-32%),79818486 reflecting the strong relationship between higher sensitivity and greater disease severity.57
Some variation in specificity of urine-LAM assays has been reported between studies. Most report excellent specificity (96-100%), but some report lower specificities (86-89%). The most likely reasons are: (i) inadequate reference standard using only sputum culture underestimates true prevalence of HIV-TB due to difficulty obtaining quality sputum samples and missing HIV-TB without pulmonary involvement; (ii) using the ‘Grade-1’ cutoff for the TB LAM assay - a very faint band on the test strip that is difficult to read accurately. Using ‘Grade-2’ rather than ‘Grade-1’ cutoffs for TB LAM improves specificity with only small decreases in sensitivity. Consensus among investigators is that only ‘Grade-2’ cutoffs should be used, and since January 2014 the manufacturer of TB LAM has removed the old ‘Grade-1’ cutoff from reference cards. Disease due to NTM or urine contamination with NTM could also theoretically cause false-positive results. The use of the urine LAM lateral-flow assay will be subject to a WHO expert review in 2015.

Urine Sampling May Increase Overall Diagnostic Yield

Urine-LAM testing identifies a different population of HIV-TB patients compared with sputum-based diagnostic assays, raising the possibility of combining them to screen for or diagnose HIV-TB. Studies using both urine and sputum-based diagnostics have demonstrated increased diagnostic yield compared to sputum alone, identifying up to 70-90% of diagnosable TB (Figure 3). Similarly, urine LAM identified a different population to those treated empirically for HIV-TB. In addition to increasing diagnostic yield, urine LAM appears to identify those patients who have higher mortality risk. It is these patients who are most likely to benefit from interventions such as early commencement of TB treatment. By virtue of its low cost (approximately US$3.50 per test), technically simple operation, and rapid results, TB LAM appears to be well suited for use in low-resource settings with high HIV-TB burden.

Figure 2: Photograph of a Determine TB LAM test strip showing the sample pad to which 60 μl of the test urine is applied, patient sample result, control band, and manufacturer’s reference card. TB: tuberculosis; LAM: lipoarabinomannan. Reproduced from Lawn with authors’ permission.
Given its modest sensitivity and potential overlap with NTM, its role in resource-rich settings is less clear, although it could be utilised as a ‘rule-in’ test to expedite TB treatment when NAATs have failed and cultures and speciation are pending.  

### OTHER FUTURE DIAGNOSTIC TECHNOLOGIES

Several evolving technologies offer potential benefits for HIV-TB. Serological assays have a long history and have transformed the diagnosis of HIV and other infectious diseases. Current TB serological assays have limited accuracy and WHO has issued strong guidance against their use. However, this remains an important area for further research and development since improved serodiagnosics offer huge potential to be developed as PoC tests, which could diagnose EPTB (useful for HIV-TB). HIV-TB presents particular challenges for serological assays, for example the heterogeneity of immunological responses to MTB.

A recent case-control study examined antibody responses to several MTB proteins in HIV-TB and demonstrated moderate sensitivity. Serological positivity was also common in HIV-infected controls, but the cross-sectional design was unable to differentiate poor specificity from early, subclinical disease that may have progressed to active TB. It appears that a pooled group of antigens gives a superior performance, and multiplex PoC diagnostics are being developed which can assess up to 57 MTB antigens with whole blood added directly to a cartridge. MTB’s production of specific volatile organic compounds has the potential to be used in PoC tests to discriminate patients with active TB infection. Other ‘breath tests’ have already shown that they can discriminate isoniazid-susceptible MTB, isoniazid-resistant MTB, and other common lung pathogens.

### DOES EMPIRICAL TUBERCULOSIS TREATMENT NEGATE THE NEED FOR NEW TUBERCULOSIS DIAGNOSTICS?

There was much enthusiasm following the promising results of early assessments of the Xpert MTB/RIF assay and WHO endorsement. Subsequent substantial investment by donors and governments has led to rapid global implementation. Several studies have modelled a positive impact of scaling-up of Xpert on economic, individual, and population-level outcomes. However, whilst data from high HIV-
burden settings have demonstrated that Xpert has superior diagnostic accuracy to sputum-smear microscopy and can improve time to diagnosis, any impact on morbidity or mortality is yet to be demonstrated.108-112

One major hypothesis for this failure to observe a beneficial impact is the use of ‘empirical’ treatment for TB clinically diagnosed by clinicians in the absence of positive TB diagnostics, especially in the context of HIV coinfection.113 Empirical TB treatment (defined as the decision to commence TB treatment in the absence of bacteriologically confirmed TB using smear microscopy, culture, or WHO-endorsed rapid diagnostics114) remains poorly studied but is likely to be done in a non-standardised fashion and be influenced by several factors, including poor sensitivity of traditional TB diagnostics, high TB prevalence (a high pre-test probability), high mortality in HIV-TB (therefore little to lose by treating TB), cadre of healthcare worker, and availability of adjunctive therapies. A meta-analysis of the WHO algorithm13 for smear-negative TB in high HIV-prevalent settings estimated pooled sensitivity of 61% (95% CI: 55-67%) and specificity was 69% (95% CI: 66-72%).115 It is difficult to standardise empirical treatment, and therefore difficult to estimate accuracy and account for it in models.

The TB-NEAT study110 noted that of the smear-negative patients detected by Xpert, 93% were treated empirically by clinicians. A legitimate concern is that Xpert may simply be confirming a TB diagnosis in those who would receive TB treatment anyway, negating the potential benefit of Xpert (or other new diagnostics) on individual and population-level outcomes.113 Models attempting to adjust for empirical therapy have also reflected this.116 However, empirical TB treatment also has potential harms through overtreatment, including cost to patients and healthcare systems, unnecessary drug toxicities which are more common in the context of concomitant antiretroviral therapy, and inappropriate treatment of unsuspected MDR-TB. Post-mortem studies also demonstrate that empirical treatment misses large numbers of HIV-TB cases.114-116 Further studies from a wide variety of settings are required to understand empirical TB treatment, in particular the impact of new diagnostics such as Xpert. However, there is a real danger that certain features of the design and conduct of TB diagnostics intervention trials may actually increase the likelihood of empirical TB treatment being prescribed.117 Thus, future trial designs for new diagnostics must account for empirical treatment, as should studies modelling implementation and scale-up.

CONCLUSION

In conclusion, HIV-TB remains a major public health burden. Improved diagnostics are considered crucial to addressing this public health challenge, especially in resource-limited settings. At present the most progressive technologies are the Xpert MTB/RIF assay and urine TB LAM assays, although clinical impact at an individual or population level has yet to be demonstrated. After decades of neglect the TB diagnostics pipeline looks promising, with several new diagnostic technologies that could be applicable to the challenges of HIV-TB. However, future diagnostics need evidence of impact beyond simple diagnostic accuracy before scale-up and widespread implementation. This includes clinical trials assessing clinical outcomes in a variety of settings and must take into account the use of empirical treatment.

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