Review

New tuberculosis diagnostics and rollout

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ARTICLE INFO

SUMMARY

Early detection and effective treatment are crucial for tuberculosis control, but global case detection rates remain low. The diagnosis of paediatric and extrapulmonary disease is problematic and there are, as yet, no rapid screening tests to assist active case finding in the community. Progress has been made in clinic-based detection tools with the introduction of Xpert MTB/RIF, a nucleic acid amplification test that combines sample processing and analysis in a single instrument to provide a diagnostic result and detection of resistance to rifampicin in under 2 h. Enthusiasm for Xpert MTB/RIF has been high and global rollout has been facilitated by donor agencies. However, concerns remain about access and sustainability due to the high cost and infrastructure requirements. Although more sensitive than smear microscopy, early studies suggest the impact of the new test on case detection rates and patient survival has been limited. Alternative technologies are being developed, including non-sputum-based tests to assist the detection of extrapulmonary disease. Evaluation studies are needed to provide evidence of the impact of the new technologies on patient outcomes. This will enable appropriate placement of new diagnostic products in the healthcare system to support the control and eventual eradication of tuberculosis disease.

1. Introduction

The old adage of 'prevention being better than cure', first enunciated by Hippocrates two and a half thousand years ago, endures to this day as tuberculosis (TB) control programs worldwide strive to prevent onward transmission of the disease. Fundamental to their success is early case detection and access to effective treatment.[1] World Health Organization (WHO) data suggest that global case detection rates are disappointing, with an estimated three million cases failing to be notified each year.[2,3] As shown in Figure 1, during 2013 the WHO Africa region experienced the lowest case detection rate, estimated at just 52% of new cases, while in Southeast Asia an estimated 1.3 million TB cases failed to be notified.

Until recently, knowledge of infection with *Mycobacterium tuberculosis* was sufficient to administer cure, but the emergence of strains resistant to anti-TB drugs means that for some patients

additional information is needed to access effective therapy.[4,5] TB case detection is beset by numerous problems. The slow onset and lack of specific symptoms makes the disease difficult to recognize in the early stages and patients may delay for weeks or months before seeking medical assistance, during which time they may transmit the disease to others.[6,7] When patients seek care at their local health centre, access to treatment may be delayed due to the lack of effective diagnostic tools, with detection of early-stage disease, extrapulmonary, HIV co-infected, and paediatric cases being particularly problematic. Screening tools based on clinical assessment and patient history have been developed, but may be of more value in monitoring treatment than for early diagnosis.[8–10]

There are two opportunities where intervention with improved diagnostic tools might aid case detection and reduce transmission: firstly in screening to detect new cases in the community in order to avoid delay in health-seeking behaviour, and secondly to improve the investigation of symptomatic patients presenting at the clinic. Technical specifications for the two scenarios differ considerably. A screening test should have high sensitivity, but specificity is less critical if confirmatory tests will be performed. Screening tests must be inexpensive, easy to use, and rapid, with

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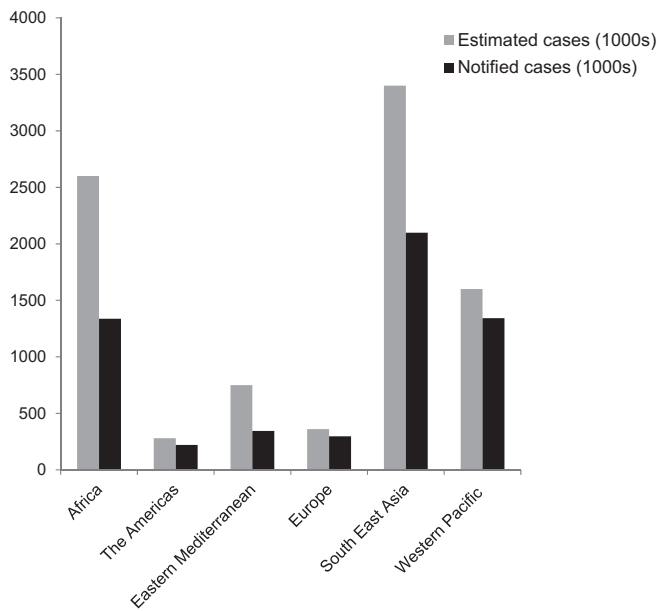E-mail address: Ruth.Mcnerney@gmail.com (R. McNerney).

Figure 1. Tuberculosis case detection in 2013. Estimated number of incident TB cases and number of notified cases by World Health Organization (WHO) region during 2013. Compiled with data from the WHO TB Control Report 2014.[3]

results available at the point of contact. In contrast, the diagnostic algorithm used at the point of care should be highly specific to avoid false-positive diagnoses and inappropriate treatment.

2. Testing at the point of care

Treatment for TB entails a program of multi-drug therapy for a period of at least 6 months, preferably with direct observation for the first 2 months. Patients need instruction, advice, and counselling, and the point at which TB treatment is initiated is usually a clinic, health centre, or hospital. Diagnosis in such settings is based on clinical examination, patient history, and a range of diagnostic tools, dependant on their availability. For patients attending clinics in TB endemic countries, the choice of diagnostic tests is often limited to smear microscopy, a low cost technology of limited diagnostic utility due to the paucity of bacteria in clinical specimens.[2,11]

The emergence of nucleic acid amplification tests (NAATs) as a diagnostic tool in the 1990s resulted in a new generation of diagnostic tests. However, TB proved a challenging disease, as extensive chemical and physical treatment was required to extract the bacteria, release the DNA, remove inhibitors, and concentrate the samples.[12] NAATs were found to be less sensitive than culture for diagnosing TB, but were highly specific and had the ability to detect new TB cases in hours.[13,14] NAATs are used widely in Europe and two tests received approval from the United States Food and Drug Administration (US FDA) to assist the diagnosis of TB: the AMPLICOR M. tuberculosis test (Roche Diagnostic Systems, USA), and the Amplified Mycobacterium Tuberculosis Direct test (MTD) (Gen-Probe, Inc., USA).[15] The commercial tests performed well during research projects in Africa,[16,17] but the high cost and level of technical support needed prevented widespread adoption in TB endemic countries.

2.1. Second-generation nucleic acid detection

Recognition that the failure to detect TB on a global scale is preventing effective control of the disease encouraged investment from public and philanthropic sources for the adaptation of

technology initially developed for homeland security and the detection of anthrax in the USA.[18] The GeneXpert analyser (Cepheid, USA) is a NAAT platform that integrates sample preparation, amplification, and detection of DNA, removing the need for laboratory facilities or specialist technical skills. The Xpert MTB/RIF assay detects M. tuberculosis DNA in under 2 h and detects mutations that cause resistance to the key drug rifampicin. Initial studies by the test developers suggested high sensitivity and specificity for detecting both disease and drug resistance,[19,20] but subsequent concerns regarding false-positive resistance results have led to recommendations in some jurisdictions that samples found resistant be confirmed by a second Xpert MTB/RIF test or, as in the case of South Africa, a line probe assay (LiPA) and phenotypic testing.[21–23]

As with previous NAAT technologies, the Xpert MTB/RIF test is less sensitive than culture but more sensitive than microscopy, and the ability to safely detect TB and resistance to rifampicin without referral to a specialist laboratory has been hailed as a game-changer in TB diagnostics.[18] The test has been approved by the US FDA for patients who have received less than 3 days of treatment, with the recommendation that culture also be performed.[24] The WHO endorsed the technology in 2010 and it has been promoted heavily in TB endemic countries for use at, or near the point at which care is provided.[25] Numerous studies have now been published demonstrating the test to be more sensitive than smear microscopy, and recommendations have been issued for its use to investigate paediatric and extrapulmonary cases. However, some frustrations have been expressed about the inability to monitor treatment due to the persistence of bacterial DNA in patient sputum,[26,27] a problem common to all NAAT tests.[28]

Studies on the impact of the new technology have been less conclusive and expectations that the implementation of Xpert MTB/RIF would lead to dramatic increases in case detection with improved cure rates have yet to be borne out. A multi-country study in Sub-Saharan Africa found that although the new test facilitated access to same-day initiation of treatment, the benefits did not translate into lower TB-related morbidity.[29] Similarly, a randomized controlled trial in Zimbabwe found screening with Xpert MTB/RIF did not reduce the rate of antiretroviral therapy-associated TB and mortality, as compared with fluorescence microscopy.[30] This is in part due to the practice of prescribing anti-TB therapy on clinical presentation and history, despite samples being negative in tests for the bacteria. In such cases the NAAT result has no bearing on treatment outcome.[31] Impact is also limited by the positioning of the technology within clinics as it does not address patient delay in seeking a diagnosis. Studies to assess the impact of rapid detection of drug resistance are ongoing, as in settings where second-line therapies are available, the rapid detection of resistance may prove beneficial for patient outcomes and lowered transmission. When used in a routine operational setting in Cape Town, South Africa, it decreased the time to commencement of second-line treatment by 25 days to a median time of 17 days.[32] However, should clinicians be reluctant to use the test when no, or only substandard, multidrug-resistant (MDR) TB treatment is available, then incorporation of a drug resistance test may constitute a barrier to implementation.

In addition to assessing clinical performance, rollout has exposed limitations of the technology and has provided increased understanding of how the test should be applied.[26] The test requires a trained and computer-literate operator, a stable supply of electricity, and in some settings air conditioning to moderate operating and storage temperatures. Throughput is moderate to low, depending on the model of instrument purchased. Concerns have been expressed about sustainability of the technology due to the high cost of manufacture. Agreement has been reached between the manufacturers of the test, Cepheid Inc., and a

consortium of donor agencies (UNITAID, the Bill & Melinda Gates Foundation, the United States Agency for International Development, and the United States President's Emergency Plan for AIDS Relief) to reduce the cost of a reaction cartridge to USD 9.98 for low-income countries, and UNITAID has invested USD 25.9 million to supply instruments and cartridges for 21 high-burden countries in Africa, Asia, and Eastern Europe. Rollout through donor funding resulted in considerable challenges for distribution and supply when large orders were placed in line with donor funding cycles, without due consideration of training requirements, installation, and start up times or the shelf-life of the reagents. Following stock-outs and a period during 2012 when the company could not satisfy demand, concerns have been expressed about reliance on a single supplier.[26]

2.2. Next-generation nucleic acid amplification

The next generation of TB NAAT products aim to acquire market share by virtue of their reduced cost, decreased time to result, and improved robustness and portability. A summary of NAAT technologies in development is presented in Table 1.

Miniaturization is being exploited as a means of minimizing reagent costs and increasing the speed of the amplification reaction. Isothermal amplification methods have been developed, where the thermocycling steps required for PCR are replaced by a single constant temperature step, which shortens the assay time and reduces the complexity of the device. Typically reaction temperatures are 62–65 °C. Efforts to reduce dependency on an electrical supply have resulted in accessory products that exploit exothermic chemical reactions to provide elevated temperatures.[33] An alternative development is recombinase polymerase amplification, an enzyme-dependant reaction that functions at temperatures between 25 and 42 °C. The detection of M. tuberculosis in processed sputum was achieved with high specificity in less than 20 min at 39 °C, with reported sensitivities of 91.4% (95% confidence interval (CI) 85–97.9%) and 87.5% (95% CI 81.7–93.2%), dependant on the DNA insertion element targeted.[34]

However, whilst advances have been made in nucleic acid amplification technology, sample handling and extraction of the DNA remain stumbling blocks. Attempts to replace the sophisticated and expensive technology used by the Xpert MTB/RIF with cheaper alternatives have yet to be proven in independent evaluation studies. To date three new rapid diagnostic tests have been placed on the market, as outlined below.

Truenat MTB (Molbio Diagnostics, India) is a miniaturized chip-based real-time PCR run on a handheld battery-operated device that reports results in less than an hour. Sputum is processed using a battery-operated sample preparation device that extracts nucleic acids using a nanoparticle-based protocol without the need for any additional equipment. Truenat MTB has been reported to have sensitivity and specificity similar to Xpert MTB/RIF.[35,36]

The EasyNAT Diagnostic Kit for Mycobacterium tuberculosis Complex (Ustar Biotechnologies, China), is an isothermal amplification kit with a 60-min amplification reaction step at 63 °C and 30-min visual detection using a lateral flow device.[37] When used to test thinned and concentrated sputum, the reported sensitivity compared with culture on Lowenstein–Jensen were 84.1% (95% CI 79.5–88.6%) and 97.8% (95% CI 97.1–98.5%), respectively, and the sensitivity in smear-negative cases was 59.8% (95% CI 49.8–69.8%).[38] A sample extraction kit is sold separately, but was not used in the study.

The VereMTB assay (Veredus Laboratories, Singapore) has been released for research use. Used with the VerePLEX Lab-on-Chip platform it combines PCR and microarray technology to detect M. tuberculosis, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium simiae/kansasii/scrofulaceum, Mycobacterium abscessus/chelonae, Mycobacterium xenopi, and Mycobacterium fortuitum.[39] It also detects resistance to rifampicin and isoniazid. The time to result is reported as less than 3 h, but sample extraction is not included.

Evidence from initial studies on these new tests is promising, but further data are needed before their potential to assist TB control can be judged. Independent studies are required in settings representative of the intended use of the device.

Table 1
Nucleic acid amplification technologies intended for use at the point of care for the diagnosis of tuberculosis

Technology	Amplification reaction	Operational features	Target	Time (min)	Stage of product development	Test developers
EasyNAT	Cross priming amplification	Isothermal 65 °C Instrument free visual output Instrument free DNA extraction	IS6110	<90	Released to market	Ustar Biotechnologies Ltd, China
Xpert TB/RIF	PCR	Automated sample extraction Resistance to rifampicin	rpoB	<90[a]	CE mark and US FDA approval, WHO endorsement	Cepheid Inc., USA
NEAT	Nicking enzyme amplification reaction	Isothermal 55 °C to 59 °C			In development	Ionian Technologies, Inc., USA/Alere, USA
RPA	Recombinase polymerase amplification	Isothermal 39 °C	IS6110 and IS1081	<20	Proof of concept study published	TwistDx, UK/Alere, USA
Truenat	PCR	Miniaturized chip-based Semi-automated DNA extraction	Ribonucleoside diphosphate reductase gene	<60	Released to market CE mark	Molbio Diagnostics Pvt. Ltd, India
VerePLEX Lab-On-Chip	PCR	Microarray technology Rifampicin and isoniazid resistance plus nine non-TB mycobacteria	IS6110 16S RNA	<180	Released for research use	Veredus Laboratories, Singapore
Genedrive	PCR	Paper-based DNA extraction technology Rifampicin resistance	REP13E12 rpoB	60	Field trials	Epistem Ltd, UK

PCR, polymerase chain reaction; CE mark, conformity mark for products sold within the European Economic Area; US FDA, US Food and Drug Administration; WHO, World Health Organization; TB, tuberculosis.
[a] Includes sample extraction.

2.3. Next-generation microscopy

Microscopy remains the workhorse of the diagnostic laboratory. It is a cheap and rapid test of reduced sensitivity compared to culture or NAAT, and can be used for diagnosis or to monitor treatment. It is a subjective test, reliant on the aptitude of the operator. Replacement of the Ziehl–Neelsen stain with fluorescent alternatives and the introduction of LED microscopes has reduced the burden on microscopists, but it remains a labour-intensive activity.

To increase throughput without compromising accuracy, an automated system has been developed. TBDx (Signature Mapping Medical Sciences, USA) incorporates robotic loading of stained slides with automated high-resolution digital image analysis to provide a result in minutes. The system has a 200-slide capacity, freeing technical resources and eliminating operator fatigue. Early studies suggested improved sensitivity over the human eye, but reduced specificity, and manual review of positive slides was necessary.[40] The application of a stepwise classification system for identifying objects has reduced the false-positive rate while maintaining high sensitivity, and suggests an accuracy of 90% can be attained.[41] Performance studies are underway in Nigeria and South Africa, with further studies planned for Asia.

A second innovation to assist the microscopist is CellScope, a portable digital fluorescence microscope that provides enlarged digitalized images for review.[42]

2.4. Next-generation blood tests

Commercial tests for antibody in sera have proved disappointing, with poor sensitivity and specificity.[43,44] The lack of a distinctive antibody response and inability to differentiate latent infection from active disease, compromises current immunoassay technology to such an extent that in 2010 the WHO issued a negative endorsement, urging practitioners not to use serological tests.[45] Given the multifaceted and unpredictable nature of the infection and complexities of the immune response, the absence of dominant host biomarkers is not surprising. Proteomic studies have identified a large number of potential markers in the serum of TB patients.[46–48] Similarly, attempts to map host RNA transcriptional signatures to detect and differentiate active disease have revealed a complex picture.[49,50]

Proteomic analysis and measurement of unstable RNA transcripts at the point of care is not feasible in TB endemic countries due to technical and cost constraints. However, robust technologies for detecting proteins are available. Proof of concept for diagnosis and accurate differentiation of disease from latent infection has been obtained using soluble cluster of differentiation ('sCD') biomarkers.[51] This proprietary technology can be implemented using traditional immunoassay platforms to test peripheral blood, which raises the possibility of developing a test for use at the point of care.

It has been suggested that measuring local markers of the immune response in samples taken from the site of disease may prove more effective at detecting active disease than testing peripheral blood. Promising results have been obtained testing bronchial lavage with improved sensitivity for detecting pulmonary disease, although some reduction in specificity was observed.[52,53] However, sample collection may prove problematic in some settings.

2.5. Extrapulmonary TB

Diagnostic tools for patients with extrapulmonary disease are limited, and frequently the only assays available are those developed for testing sputum. The sensitivities of these tests when applied to samples such as blood, urine, and tissue is often low due to the paucity of bacteria in the samples, and assays may be affected by interference or inhibition from the sample matrix. Some patients with severe immunosuppression secrete TB antigens in their urine. The Alere Determine TB LAM Ag tests urine for lipoarabinomannan (LAM), a cell wall component of mycobacteria. The assay is a 'rule in' test, as a negative result does not exclude the possibility of TB. The test was found cost-effective in Sub-Saharan Africa when used for patients with CD4 counts per mm^3 of less than 100.[54,55] The lateral flow devices are easy to perform, rapid (less than 30 min), and may be used at the point at which care is provided for TB or HIV. However the test is susceptible to false-positive results from contamination with dust or faeces, and care should be taken during sample collection.[56] The test lacks accuracy if used for patients with CD4 counts per mm^3 over 200 or children.[57,58]

Testing for immune markers may offer a means of diagnosis if undertaken at the site of disease[59] and promising results have been obtained for pleural TB.[60–62] Assessing interferon gamma (IFN-γ) in pleural, pericardial, ascitic, and cerebrospinal fluid has also shown promise, and in a study undertaken in South Africa the approach was found significantly more sensitive than Xpert MTB/RIF for pleural TB.[63] The Intergam Rapid Immuno Suspension Array, IRISA-TB (Antrum Biotech Ltd, South Africa) takes less than 2 h and is based on a multi-well plate ELISA format.

2.6. Drug resistance

Patients with MDR-TB (resistance to at least isoniazid and rifampicin) who are prescribed first-line drug therapy may fail treatment and remain infectious, and are at high risk of developing further resistance. The early detection of MDR-TB is therefore important for both individual and public health. Resistance to additional first-line drugs further compromises treatment success.[64–66] Resistance to fluoroquinolones and aminoglycosides used to treat MDR-TB results in extensively drug-resistant TB (XDR-TB).[5] Drugs used in the treatment of MDR-TB and XDR-TB are expensive, of higher toxicity, and outcomes are poor.[4,67,68] Adverse reactions are common and may be severe and irreversible.[68,69] Poor tolerance leads to reduced compliance, which can result in the amplification of resistance.[70] Knowledge of the full drug susceptibility profile would enable tailored treatment to improve efficacy and reduce exposure to ineffective toxic drugs.[66]

The genetic basis of resistance to rifampicin is well understood, and sensitive molecular tests have been developed. Rifampicin resistance has the additional advantage of being a good proxy for MDR-TB. The genetic basis of resistance to other anti-TB drugs is less well defined and further work is needed to determine the clinical impact of putative drug resistance mutations.[22] Whole genome sequencing provides the most efficient means of obtaining a complete resistance profile of the bacteria,[71,72] but the cost and infrastructure required preclude its use at the point of care in TB endemic countries.

Rapid laboratory-based tests for individual or selected drugs have been developed, with priority given to the drugs involved in MDR-TB and XDR-TB.[73] These include the line probe assays where, following PCR using labelled primers, hybridization to a panel of immobilized oligonucleotide probes indicates the presence or absence of mutations.[74] The first test to be used at the point of care was the Xpert MTB/RIF (Cepheid), a combined diagnostic and drug resistance test.[20] The company is reported to be developing an additional test for XDR-TB. A new product reported to be close to the market is the Epistem Genedrive system, which in addition to diagnosing TB detects resistance to rifampicin.[75] Sample extraction is undertaken using a novel paper-based digestion, followed by PCR and detection with labelled probes. The VereMTB test (Veredus Laboratories, Singapore) uses chip-based technology to

detect resistance to rifampicin and isoniazid, in addition to detecting *M. tuberculosis* and nine other mycobacteria.[39] The test has been released for research use and is undergoing evaluation. Both of these tests are new to the market; clinical performance data are not yet available, and their cost and infrastructure requirements are not known.

2.7. Barriers to the market

The case for new diagnostic tests for TB has been well made and is supported by the STOP TB Partnership and WHO.[76] However, investment remains low and the estimated global total of USD 42.4 million spent on diagnostics research during 2012 was less than a third that spent on basic research and one fifth that spent on developing new drugs, representing just 13% of the annual spend called for by the STOP TB Partnership.[77] Financial return on investment to create and manufacture tests for the control of TB is constrained by the drive to provide technology that is affordable in TB endemic countries. The lack of market incentives creates a social responsibility to encourage and facilitate the entry of beneficial new products into the market. Endorsement by the WHO has encouraged the adoption of new technologies, but market acceptance of individual products is dependent on regulatory approval, a costly and lengthy process that requires studies to determine clinical performance at the site of intended use.[78]

The arrival of new products on the market means that health providers will soon have a choice of technologies and products for diagnosing TB. A welcome effect of competition may be moderation of prices, as seen in malaria and HIV where multiple tests are already available. Technology assessment programs will be required during which the benefits and potential impact of diagnostic tools and strategies are compared. Programs for global rollout of a single TB diagnostic may be consigned to history, to be replaced by procurement decisions based on local need. It is imperative that continued research and funding efforts are targeted towards the development of diagnostic modalities that translate to improved patient outcomes and reduced transmission.

3. Conclusions

Improved tools for detecting TB are urgently needed. New tests have been developed for use in laboratories and clinics, but as yet little is available to assist early case finding in the community. The introduction of an easy to use molecular test that also detects resistance to rifampicin is a welcome step forward, but its high cost limits accessibility and increases dependency on donor support. The Xpert MTB/RIF test continues to be evaluated; studies to date suggest that although the test is more sensitive than smear microscopy for diagnosis, it has limited impact when used in settings where presumptive treatment is practised. Several alternative products have been developed and other technologies are being explored, including some innovative new approaches to assist the diagnosis of extrapulmonary disease. Sustained investment will be needed to ensure that these new tests reach the market. Studies to assess the effectiveness of new tests are needed to ensure appropriate placement within the health systems of TB endemic countries and to maximize the impact of the new technology on efforts to control and eradicate the disease.

Conflict of interest: RM, PH, and AZ declare no conflict of interest. JC is named on a patent application relating to soluble biomarkers for the detection of TB and holds share warrants related to this. She has received travel, childcare, and associated subsistence expenses from ProteinLogic.

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