

The Tuberculosis Diagnostics Pipeline

By Erica Lessem

INTRODUCTION

Over the past decade, several new options for improving TB diagnosis have become available. The past year saw considerably more progress than the previous one. However, the reality of how most TB is diagnosed—or not—remains largely unchanged. The world failed to detect 3.6 million of the estimated 9.6 million new cases of TB in 2014.¹ Sputum smear microscopy—which misses over half of TB cases² and gives no indication of drug susceptibility to guide appropriate treatment—is still the diagnostic standard in most of the world, despite the availability of the far more sensitive GeneXpert MTB/RIF for six years.³ Late in 2015, the World Health Organization (WHO) approved Alere's TB lipoarabinomannan (LAM) test—a very affordable, simple, rapid, noninvasive, point-of-care (POC) rule-in test for people with HIV with very low CD4 counts—but no country has begun to implement it yet. New versions of line probe assays—Hain's MTBDR*plus* and MTBDR*sl* and a product from Nipro—received WHO recommendation, facilitating rapid drug susceptibility testing (DST), but the world is still a long way from universal DST, with an estimated 59 percent of cases of multidrug-resistant TB (MDR-TB) undetected.⁴

Research and development (R&D) for TB diagnostics features some promising developments since last year (see table 1). Improvements on nucleic acid amplification tests (NAATs) such as GeneXpert Omni and Ultra and Molbio's TrueNAT are being validated, positioning them for possible WHO recommendation. Further upstream, encouraging research into gene sets that can predict active TB disease and reliably distinguish it from latent TB and other infections may eventually underpin new blood tests (currently, there is no effective serological test for active TB). Incremental advances are being made to improve detection of pediatric TB (see "Extending quality," page 133).

Yet, overall, with a mere US\$65 million in 2014 funding out of an estimated annual need of \$340 million,⁵ the pipeline for evidence-based new diagnostics has largely remained stagnant (see table 2). Dismayingly, some companies continue to move forward with marketing for their products when the data are unavailable or scream that they should not, as is the case with Epistem, which is marketing GeneDrive in India despite the test's having flopped in studies.

With use of poor tests predominating, poor uptake of good extant options, poor evidence bases to support the introduction of new tests, and poor funding to support the development of better tests, it's no wonder we've made little headway in diagnosing TB.

Table 1. 2016 Tuberculosis Diagnostics Pipeline: Products in Later-Stage Development or on Track for Evaluation by the WHO with New Published Data or Policy Updates Since the 2015 Pipeline Report

Test	Type	Sponsor	Status	Comments
MOLECULAR/NAAT				
BD MAX MTB assay	qPCR for MTB in automated BD MAX	BD	In 16 <i>M. tuberculosis</i> samples, 100% sensitivity, 97.1% specificity ⁶	
Genedrive MTB/RIF	Portable RT-PCR for MTB + RIF resistance	Epistem	Worse sensitivity than smear [1] in 2016 study ⁷	Marketed in India
GenoType MTBDR _{plus}	Line probe assay for RIF + INH resistance	Hain Lifescience	WHO now recommends based on FIND evaluation ⁸	WHO guidance pending
GenoType MTBDR _s	Line probe assay for FQ + SLID resistance	Hain Lifescience	WHO now recommends ⁹	FIND's multicountry evaluation of MTBDR _s /version 2.0 from 2015 still unpublished
MeltPro	Closed-tube RT-PCR	Zeesan Biotech	New study from China of 2,057 smear-positive TB patients shows sensitivity of detecting resistance to rifampin 94.2%, isoniazid 84.9%, ofloxacin 83.3%, amikacin 75.0%, kanamycin 63.5% ¹⁰	
NTM+MDR-TB Detection Kit 2	Line probe assay for RIF + INH resistance	Nipro	WHO now recommends based on FIND evaluation ¹¹	WHO guidance pending
RealTime MTB/TB MDx m2000	Automated RT-PCR for MTB; can be added to HIV RNA platform	Abbott	Sensitivity 100%, 95% CI: 98.6–99.9 in smear-positive samples, similar to GeneXpert MTB/RIF ¹²	
Truenat MTB	Chip-based NAAT with RT-PCR on handheld device for MTB	Molbio Diagnostics, Bigtec Labs	FIND and ICMR studies underway	
Xpert MTB/RIF Ultra	Next-generation cartridge-based detection of MTB + RIF resistance	Cepheid	FIND study results anticipated end 2016	
Xpert Omni	Single-cartridge mobile platform that can use single MTB/RIF or Ultra cartridge	Cepheid	FIND study pending but delayed	
Xpert XDR	NAAT	Cepheid	FIND study anticipated 2018	
ANTIBODY/ANTIGEN DETECTION				
Determine TB LAM Ag	Urine dipstick for TB LAM protein	Alere	WHO recommended use in people with HIV with CD4 count ≤ 100 ¹³	

CI: confidence interval
 FLD: first-line drugs (isoniazid, rifampin, ethambutol, pyrazinamide)
 FQ: fluoroquinolone
 ICMR: Indian Council of Medical Research
 INH: isoniazid
 LAM: lipoarabinomannan
 MDR-TB: multidrug-resistant tuberculosis

MTB: *Mycobacterium tuberculosis*
 NAAT: nucleic-acid amplification test
 qPCR: quantitative polymerase chain reaction
 RIF: rifampin
 RT-PCR: real-time polymerase chain reaction
 SLID: second-line injectable drug (e.g., amikacin, capreomycin, or kanamycin)
 WHO: World Health Organization

Table 2. Later-Stage or Marketed TB Diagnostic Test Candidates with No New Published Evaluation Data

Test	Type	Sponsor	Status
MOLECULAR/NAAT			
EasyNAT	Isothermal DNA amplification/lateral flow to detect MTB	Ustar	No new data since poor in Tanzanian field study ⁴
FluoroType MTB	Semiautomated direct MTB detection; PCR in a closed system; results in 3 hours	Hain Lifescience	Marketed
FluoroType MTB RNA	MTB RNA for monitoring of anti-TB therapy	Hain Lifescience	No published data
GeneChip	RT-PCR for RIF + INH DR	CapitalBio	Marketed
LATE-PCR with Lights-On/Lights-Off Probes + PrimeSafe	Single-tube PCR to detect MTB, resistance to INH, RIF, EMB, SLID	Hain Lifescience/Brandeis University, Stellenbosch University	No published data
LiPA pyrazinamide	Line probe assay for PZA resistance	Nipro	Marketed
PureLamp	Manual NAAT by loop-mediated isothermal amplification for MTB detection	Eiken	WHO June 2015 review impeded by data quality issues; guidelines development group reexamined evidence January 2016; recommendations expected June 2016
REBA MTB-MDR	Line probe assay for RIF + INH resistance	YD Diagnostics	No new data; marketed
REBA MTB-XDR	Line probe assay for FQ + SLID DR	YD Diagnostics	No new data; marketed
TREK Sensititre MYCOTB MIC plate	Dry microdilution plate to detect MICs for FLD + SLD (except PZA)	TREK Diagnostic Systems, Thermo Fisher Scientific	No new evaluation data but used in study in Cameroon ¹⁵
TRC Rapid MTB	Automated rapid rRNA to detect MTB	Tosoh	No new data
VOLATILE ORGANIC COMPOUNDS			
Giant African pouched rats (<i>Cricetomys gambianus</i>)	Trained sniffer rats to detect MTB in sputum	Apopo Foundation	No new data
AUTOMATED IMAGING			
CAD 4TB	Digital CXR for TB screening	Delft Imaging Systems	Marketed; in 2016 WHO to review available evidence on computer-aided radiographic TB detection and organize a scoping meeting to determine research needs and if guidelines should be developed ¹⁶
ANTIBODY/ANTIGEN DETECTION			
MBio Array System	POC cartridge to measure ~57 simultaneous MTB antigen-antibody reactions	MBio Diagnostics, FIND	No new data

CXR: chest x-ray
 DR: drug resistance
 EMB: ethambutol
 FLD: first-line drugs (isoniazid, rifampin, ethambutol, pyrazinamide)
 FQ: fluoroquinolone
 INH: isoniazid
 MDR-TB: multidrug-resistant TB
 MIC: minimum inhibitory concentration
 MTB: *Mycobacterium tuberculosis*
 NAAT: nucleic-acid amplification test

PCR: polymerase chain reaction
 POC: point of care
 PZA: pyrazinamide
 RIF: rifampin
 RT-PCR: real-time polymerase chain reaction
 SLD: second-line drug
 SLID: second-line injectable drug (e.g., amikacin, capreomycin, or kanamycin)
 TB: tuberculosis
 WHO: World Health Organization

TROT, TROT TO MARKET

Perhaps the most exciting advance in TB diagnostics came in late 2015, when the WHO recommended Alere's Determine LAM Ag simple urine dipstick test for ruling in TB in people with HIV with CD4 counts below 100/mm³ or who are seriously ill.¹⁷ The test's imperfect sensitivity (56% pooled sensitivity based on five studies of people with CD4 counts below 100/mm³) means that a negative test must still be followed up with other testing to rule out TB.¹⁸ However, given the extremely high mortality of people with TB and HIV (TB is thought to be the cause of death in nearly 40% of HIV-positive patients, half of which is undiagnosed)¹⁹ and challenges in diagnosing TB in people with low CD4 counts, having an inexpensive (\$2.26 per test), simple, and noninvasive test to use in this very high-risk population is a major advance. In fact, the LAM test is the first TB test to ever demonstrate a mortality benefit in a randomized controlled clinical trial: among 578 people with HIV in hospitals in South Africa, Tanzania, Zambia, and Zimbabwe, using LAM was associated with an absolute reduction of all-cause mortality at eight weeks of 4% (95% confidence interval [CI]: 1%–7%) from 25% to 21%, and a relative risk reduction of 17% (95% CI: 4%–28%).²⁰ This difference appeared to be attributable to the test's allowing earlier initiation (by one day on average) of anti-TB therapy.²¹ Countries with large burdens of TB/HIV, including many countries in sub-Saharan Africa, should roll out LAM testing immediately, along with proper accompanying training to ensure the test is used only in the recommended population and that follow-up tests are done as necessary.²² Whoever ends up with the rights to the test—Abbott is trying to pull out of a putative acquisition of Alere—should ensure its continued manufacture as well as marketing.²³ Further upstream, funding from the Global Health Innovative Technology Fund (GHIT Fund, which is itself funded by the Japanese government, pharmaceutical companies, the Bill & Melinda Gates Foundation, and the Wellcome Trust) to FIND and Fujifilm will support the development of what is hoped to be a more sensitive LAM test.^{24,25,26}

Other policy advances can help improve timely detection of drug-resistant TB. In late 2015, the WHO extended its 2008 guidance, which recommended the use of the Hain version 1 line probe assay (LPA), to recommend the use of two alternative LPAs with the capability to detect TB and rifampin resistance: the Hain version 2 LPA (also called the GenoType MTBDR_{plus} assay) and the Nipro Assay.²⁷ The WHO still does not recommend using LPAs on smear-negative samples. In 2014 and 2015, FIND conducted a cross-sectional noninferiority study to compare the accuracy of these two tests to that of Hain Version 1 assay, evaluating their performance both on clinical isolates and on sputum specimens from people with pulmonary TB; both tests showed comparable performance in detecting *Mycobacterium tuberculosis* (the bacterium that causes TB infection and disease) and rifampin resistance in smear-positive samples: on clinical isolates, sensitivity and specificity compared with the phenotypic reference standard for Hain V1, HainV2, and Nipro were 90.3%/98.5%, 90.3%/98.5% and 92.0%/98.5%, respectively, for detection of rifampin resistance and 89.1%/99.4%, 89.1%/99.4%, and 89.6%/100.0%, respectively, for detecting isoniazid resistance. In sputum testing, sensitivity and specificity were 97.1%/97.1%, 98.2%/97.8%, and 96.5%/97.5% for rifampin resistance and 94.4%/96.4%, 95.4%/98.8%, and 94.9%/97.6% for isoniazid resistance.²⁸ The WHO will update its guidance on LPAs later in 2016. While this certainly reflects progress, Hain and Nipro launched these assays in 2011; it's taken five years to optimize and fully evaluate them.

In May 2016, the WHO also recommended and issued guidance on Hain's MTBDR_{sl}, an LPA capable of detecting resistance to fluoroquinolones and second-line injectables.²⁹ LPAs can produce results in 24–48 hours, much quicker than the two weeks that liquid culture or two to three months that solid culture take. As such, the MTBDR_{sl} LPA can guide appropriate treatment selection. The announcement of the WHO's MTBDR_{sl} recommendation accompanied its recommendation of the shortened or "modified Bangladesh" regimen, whose introduction the test can help facilitate, as the shortened (9- to 12-month) regimen is not suitable for fluoroquinolone- or injectable-resistant TB (pre-XDR-TB; see "Tuberculosis Treatment," page 163).³⁰ Countries and donors must scale up the introduction of this test and work with Hain to further reduce the price. FIND negotiated a public sector price of €7.50 (approximately \$10) per test strip in 138 countries; however, the total cost of running a test (which requires other laboratory supplies) can result in costs of \$20–\$30. The test equipment itself can cost \$8,000–\$40,000, depending on its size and whether it automatically reads results or not.³¹

Newer iterations of GeneXpert are moving closer to market. Recent investments may make it more suitable for use in a variety of settings and more sensitive. GeneXpert Omni, a smaller and more rugged single-cartridge version of the test that is dustproof and runs on batteries could be a point-of-care test for TB. The test device's anticipated cost is \$2,895.³² Cepheid claims that another new product, the GeneXpert Ultra cartridge, is more sensitive than the MTB/RIF, approximating the sensitivity of culture, and has a shorter processing time.³³ FIND is currently validating both the Ultra cartridge and Omni platform: Ultra results are expected at the end of 2016; Omni results have been further delayed. If studies show them to indeed be as promising as the company claims, the WHO will issue recommendations and formulate guidance accordingly. Data on the use of Ultra in smear-negative specimens are expected in 2017, which could inform recommendations on whether Ultra can be used to replace culture. Cartridge prices are expected to remain consistently high at \$9.99, as even though MTB/RIF sales volumes have increased,³⁴ those profits are said (by Cepheid) to have been reinvested in R&D. In 2017, Cepheid plans to release the XDR assay, designed to genotype resistance to isoniazid, fluoroquinolones, and second-line injectables when MTB/RIF (or Ultra) indicates rifampin-resistant TB, though no peer-reviewed data yet exist on this product.³⁵ A FIND evaluation is expected in 2018.

Molbio's TrueNAT, an Indian GeneXpert competitor that has been on the market since 2013, is finally being validated by outside parties (FIND and the Indian Council on Medical Research).³⁶ A recent study compared TrueNAT to Xpert MTB/RIF on 274 patient specimens, using culture as the reference standard. The assays had similar sensitivity on sputum-smear-positive samples: TrueNAT had 99% sensitivity (95% CI: 94.2%–99.95%) versus MTB/RIF's 100% (95% CI: 96.5–100.0%, respectively). With sputum-smear-negative, culture-positive samples, the sensitivity of the TrueNAT was 86.2% (95% CI: 74.1%–93.4%) as compared with 90.1% (95% CI: 88.7%–94.35%) for Xpert MTB/RIF.³⁷ The cartridge-based sample preparation extraction tool, TruePrep—which is rugged and portable—costs \$7,000, and each assay costs \$14; the public sector will receive a further discount.³⁸

Other products without the data to back them up continue to be marketed by unscrupulous manufacturers. Epistem's Genedrive performed dismally in a recent clinical study of 336 participants: sensitivity was 45.4% (95% CI: 35.2%–55.8%) versus 91.8% (95% CI: 84.4%–96.4%) for Xpert MTB/RIF and 77.3% (95% CI: 67.7%–85.2%) for smear microscopy. In smear-negative cases, sensitivity of GeneDrive was 0% (95% CI: 0, 15.4) versus 68.2% (95% CI: 45.1%–86.1%) for Xpert.³⁹ Yet just after those data were published, Epistem announced full commercial launch of Genedrive TB tests in India, claiming it “enables early detection of TB and antibiotic resistance without need for central laboratory facilities.”⁴⁰ The regulatory authority in India should ban the marketing of this test, and private providers should be extremely wary and not waste patients' time, money, and effort by subjecting them to it (see box).

Extending Quality and Affordability to the Private Sector

Many countries, including 12 of the 22 countries with the highest TB burdens (India, Pakistan, the Philippines, Bangladesh, Afghanistan, Kenya, Uganda, Vietnam, Indonesia, Myanmar, Nigeria, and Cambodia) have large private-sector markets for TB diagnosis and care.⁴¹ Ensuring access to affordable, quality diagnosis is both critical and challenging. The use of unvalidated tests, or using tests off-label, can endanger patients and their communities and, at best, wastes their money and time. Important tests, such as GeneXpert, are not commercially available in the private sector in Burma, Cambodia, Indonesia, Nigeria, Uganda, or Vietnam.⁴² But even when good tests are available in the private sector, patients pay dearly, as concessional prices are normally available only to the public sector, and some private practitioners are aiming to maximize profit. For example, in Afghanistan, Bangladesh, India, Kenya, Pakistan, and the Philippines, GeneXpert is available, but the average price charged by private laboratories is \$68.73 (range \$30.26–\$155.44).⁴³ Private diagnosis without case notification to the public program also impedes getting a true picture of local, national, and global TB epidemiology.

In India, where about half of patients seek TB diagnosis and care in the private sector, the Indian government and other actors have taken steps to mitigate the inappropriate use of TB diagnostics, such as banning the use of serological tests and discouraging the use of Quantiferon TB Gold (a test for latent TB that was being inappropriately marketed and used in India to screen for active TB).^{44,45} Efforts are underway to ensure best TB diagnostic practices among private-sector providers in India at an affordable price. The Initiative for Promoting Affordable and Quality TB Tests (IPAQT) offers WHO-recommended TB diagnostics to laboratories who agree to pass on these price reductions to patients by agreeing to a maximum ceiling price, participating in quality assurance programs, and notifying cases to the public program.⁴⁶

To date, IPAQT has involved 116 laboratories across India, with over 250,000 presumptive TB cases tested, and volumes climbing. IPAQT has notified 23,000 cases in five cities under a pilot program and plans to further involve more decentralized laboratories and to streamline notification, as well as to look into expanding into cross-disease diagnostic support, such as including the HIV1 viral load GeneXpert cartridge in the IPAQT framework. Other countries with robust private-sector activity in TB should follow suit. The Clinton Health Access Initiative's Nigeria team recently conducted an analysis to determine feasibility there.

IN DEVELOPMENT

Back in the lab, a promising development came from a team at Stanford University that identified a three-gene set indicative of active TB (*GBP5*, *DUSP3*, and *KLF2*) in whole blood across eight data sets containing over 1,000 samples from both adult and pediatric patients in ten countries. This gene signature could accurately separate people with active TB from healthy controls, from people with latent TB, and from people with other diseases. HIV status, bacillus Calmette-Guérin (BCG) vaccination, and drug resistance did not confound expression of the three-gene set. The gene set may be of use in monitoring treatment, as its expression increases with disease severity and decreases with time of treatment, though this must be validated prospectively. Further validation and development are required.⁴⁷

Researchers from the University of Washington and the University of Cape Town have received funding to further study a simple oral swab to test for TB DNA, and it detected TB well in 18 of 20 patients in a proof-of-concept study (90.0% sensitivity compared with GeneXpert MTB/RIF; 95% CI: 66.9%–98.2%).^{48,49}

Several researchers are exploring the value of immune activation markers such as C-reactive protein (CRP) to indicate active TB disease or to identify good responses to TB therapy. One small study pre- and post-treatment in Gambia showed that CRP levels showed the most significant decrease by two months of treatment ($P < .0001$), whereas two other markers, beta2 microglobulin and neopterin, showed little change by two months but a significant decrease by six months of treatment ($P = .0002$ and $P < .0001$, respectively).⁵⁰ A larger prospective study identified a seven-marker biosignature including CRP as well as transthyretin, interferon- γ , complement factor H, apolipoprotein-A1, inducible protein 10, and serum amyloid A that identified TB disease in the test set ($N = 210$) with a sensitivity of 93.8% (95% CI: 84.0%–98.0%) and a specificity of 73.3% (95% CI: 65.2%–80.1%), regardless of HIV infection status.⁵¹ CRP may be of particular interest for pediatric development (see “Tuberculosis Diagnostics Research for Children,” page 135).

DST research saw some developments. Development of a rapid colorimetric method for detection of resistance to pyrazinamide—one of the most important drugs to treat TB, for which DST development remains challenging due to the enormous number of resistance-associated mutations in the *M. tuberculosis pncA* gene—using a dye called 5-cyano-2,3-ditoyl tetrazolium chloride (CTC) progressed when initial testing in a small test of 50 isolates showed DST results could be available in four to six days with 97.1% sensitivity

and 81.3% specificity, in comparison with liquid culture.⁵² Unfortunately, research that could underpin DST development for bedaquiline has not yet been as successful—examining the 12 cases who had developed over fourfold increases in bedaquiline minimum inhibitory concentrations (MICs) in study C209 revealed that all had *M. tuberculosis* with mutations in the *Rv0678* gene, but there was no correlation between MIC change and treatment response, making it unclear what might be a clinically meaningful breakpoint.⁵³ Developing DST for bedaquiline will be important, as resistance to it has already started to develop.⁵⁴

RECOMMENDATIONS

With new products moving forward and interesting new leads to pursue, we are pleased to report progress with the TB diagnostics pipeline. But the world is still far from ensuring all those with TB get appropriate diagnostics. Both R&D and access need dramatic infusions of funding and political will. In particular:

- **National governments and donors must substantially increase funding for TB programs to allow for best diagnostic practices.** This includes the widespread scale-up of NAAT to supplant microscopy, universal DST using liquid culture or LPAs, digital X-ray, and the rapid adoption of LAM testing in areas with high HIV burdens.
- **National governments, donors, and the private sector must invest far more in TB R&D to advance better tests, including those for children.** This should include a commitment to rapidly and rigorously evaluating new technologies and to publishing peer-reviewed results. Greater resources are necessary to achieve the requirements set out in Target Product Profiles (TPPs).⁵⁵
- **National governments and donors should work closely with the nonprofit and private sectors to ensure only quality and affordable tests are used.** In countries with large proportions of care-seeking in the private sector, access to appropriate diagnostics is extremely limited and can be catastrophically expensive. Good programs such as IPAQT to address this exist and should be expanded and replicated.
- **Developers must commit to timely and rigorous validations of their tests prior to marketing, and health and regulatory authorities and private practitioners should hold them accountable for doing so.** Epistem and other companies who market ineffective or as-yet-unproven tests must cease doing so immediately. National governments should ban the import and use of inappropriate tests and enforce those bans. Those working in TB globally should call to task companies such as Epistem that inappropriately market them.

Tuberculosis Diagnostics Research for Children

By Lindsay McKenna

The ability to confirm the presence of tuberculosis (TB) bacteria in the body (microbiological diagnosis) underpins much of the existing technology and paradigm for diagnosing TB in adults, but this approach is problematic for children. An estimated 60 percent of children with TB go undiagnosed: in 2014, national TB programs reported 358,521 cases of TB among children to the World Health Organization (WHO),⁵⁶ yet credible models estimate that one million cases of incident TB occur among children each year.⁵⁷ TB is also likely a major unrecognized co-morbidity or cause of illness and death among children affected by pneumonia, meningitis, HIV, and malnutrition.⁵⁸ Children with the disease have fewer TB bacteria in their bodies (paucibacillary disease), difficulty producing sputum, and high rates of extrapulmonary TB. As a result, diagnosis is often empirical (presumed, rather than confirmed) and based on a combination of clinical and epidemiologic information.

The gold standard for diagnosing TB, microbiological confirmation using culture, is only obtained in 15–20% of children with clinically diagnosed TB disease.⁵⁹ Compared with culture, Xpert MTB/RIF has 62% pooled sensitivity when performed on induced or expectorated sputum (36% more sensitive than smear microscopy) and 66% pooled sensitivity when performed on gastric aspirate or lavage (44% more sensitive than smear microscopy).⁶⁰ Xpert MTB/RIF sensitivity in culture-negative children clinically diagnosed with TB is just 2% for induced or expectorated sputum.⁶¹ The WHO recommends Xpert MTB/RIF as the initial diagnostic test in children suspected of having multidrug-resistant TB (MDR-TB) or HIV-associated TB or, where resources allow, as the initial diagnostic test in all children suspected of having TB.⁶² These recommendations apply to both pulmonary and extrapulmonary specimens, with the exception of stool, urine, and blood, given the lack of data for the utility of Xpert MTB/RIF for these specimen types.⁶³ It is important to note that a majority of studies evaluating Xpert MTB/RIF's performance have been conducted at higher-level health facilities, where it is likely that sicker children with higher rates of smear-positive TB are present for evaluation. How Xpert MTB/RIF performs among children in an outpatient setting, and with different levels of TB disease severity, has not been well studied. That said, while Xpert MTB/RIF is superior to smear microscopy and helps provide rapid confirmation of disease, it should not be used as a rule-out test for TB in children: clinical evaluation remains important in diagnosing TB in children.

Research is ongoing to determine the most feasible and sensitive combinations of tests and specimen types (including urine and stool) for diagnosing TB in children⁶⁴; to compare the performance of smear, culture, and Xpert at baseline and during treatment; and to optimize specimen sample collection and processing to improve diagnostic yields in children. In addition to efforts to optimize existing tools, new technologies in the pipeline might also improve our ability to detect TB in children in the future. The WHO recently recommended molecular line probe assays (LPAs) for the rapid detection of resistance to second-line TB drugs, including fluoroquinolones and injectable agents in children with confirmed rifampin-resistant TB or MDR-TB, based on extrapolation from data in adults.⁶⁵ Xpert MTB/RIF Ultra, currently under evaluation in adults, is expected to have increased sensitivity and ability to detect paucibacillary TB disease, which is common in young children. Xpert XDR is expected to improve our ability to quickly diagnose resistance to first- and second-line TB drugs. These advances, though extremely important, are incremental. To radically improve diagnosis of all forms of pediatric TB, a rapid biomarker-based test that does not rely on sputum and can be used at the point of care is necessary.⁶⁶

The discovery and validation of biomarkers for TB diagnosis and treatment monitoring in children is an urgent research priority. A blueprint for pediatric TB biomarker identification and development, resulting from a 2014 U.S. National Institutes of Health (NIH)-convened workshop, is a call to action. The blueprint identifies critical research needs, including enhancing the detection of pathogen biomarkers and identifying host biomarkers, and calls for collaboration to advance the field.⁶⁷ Efforts by an NIH-organized working group are underway to harmonize pediatric biorepositories (specimen collection methods and clinical data collection) to optimize their use for the future discovery and development of TB biomarkers in children.

Compared with adults, children have increased risk of progression from infection to active disease.⁶⁸ While it is possible to diagnose TB infection in children using biomarker-based tuberculin skin testing (TST) and interferon-gamma release assays (IGRAs), these tests have shortcomings, including their inability to differentiate between TB infection and disease, cross-reactivity with other mycobacteria, particularly for TST, and increased false-negative tests among immune-compromised children. The ability to differentiate between infection and disease and to identify children at increased risk of progression to active disease would improve feasibility and make more efficient the targeted provision of preventive therapy to child contacts of TB patients in high-burden settings.

Efforts to identify and validate biomarkers of TB disease and risk of progression in adults and children are ongoing and, if proven, will greatly improve the reliability and ease of TB diagnosis. However, drug-susceptibility tests rely on microbiological samples. Where a microbiological sample cannot be obtained, biomarkers that enable treatment monitoring in children could provide an interesting opportunity to improve access to appropriate treatment. Select pediatric biomarker research highlights are presented below.

PEDIATRIC BIOMARKER RESEARCH HIGHLIGHTS

LAM

Because both children and people with HIV tend to have higher rates of extrapulmonary TB, it was expected that the lateral flow urine LAM assay (currently recommended in HIV-positive adults who have low CD4 counts or are seriously ill) would work well in children, too. However, the LAM test demonstrated poor sensitivity (48.3%) and specificity (60.8%) compared with culture in HIV-positive and HIV-negative children with TB.⁶⁹ The WHO recommendation for LAM in people with CD4 counts <100 (or with advanced HIV disease) does not extend to children, based on the generalization of data from adults, while acknowledging very limited data in children.

C-reactive protein

C-reactive protein (CRP), a nonspecific marker of inflammation detectable in blood and measurable with existing assays at the point of care, has shown potential for screening for TB disease and indicating response to TB treatment in adults (see “In Development” in this chapter, page 134). In one study, CRP demonstrated 98% sensitivity and 59% specificity for TB among South African adults with smear-negative, culture-positive TB with or without HIV.⁷⁰ A study to identify the expression patterns of biomarkers in the plasma of HIV-negative children in India with pulmonary and extrapulmonary TB compared with healthy controls found that children with active TB showed significantly elevated levels of CRP. These findings are not surprising, as a detectable difference in inflammation can be expected when comparing healthy and sick children. As such, CRP should be evaluated as a marker of active TB and for use in TB diagnostic algorithms in larger pediatric cohorts, inclusive of children with latent TB and other pulmonary infections and HIV.⁷¹

TAM-TB

Encouragingly, a novel T-cell activation marker-tuberculosis assay (TAM-TB) demonstrated 83.3% sensitivity and 96.8% specificity among children with TB symptoms compared with culture. The pediatric cohort (N = 113) in this prospective proof-of-concept study included HIV-positive and HIV-negative children 6 months to 16 years old. The combined use of the TAM-TB assay and Xpert MTB/RIF demonstrated 94% sensitivity compared with culture. TAM-TB is a rapid blood-based test with the potential to improve the detection of active TB in children; further refinement and testing, especially in HIV-positive children with low CD4 cell counts, are necessary.⁷²

RNA expression signatures

A genome-wide analysis of RNA expression in blood among children undergoing evaluation for TB, including those with HIV, identified a 51-transcript signature capable of distinguishing TB from other diseases and from latent TB infection. The 51-transcript signature demonstrated 82.9% sensitivity and 83.6% specificity for culture-confirmed TB (Xpert MTB/RIF sensitivity was 54.3%). For culture-negative TB where children are deemed to have highly probable, probable, or possible TB, the 51-transcript signature had an estimated sensitivity of 62.5–82.3%, 42.1–80.8%, and 35.3–79.6%, respectively (estimated

sensitivity for Xpert MTB/RIF was 25%–35.7%, 5.3%–13.3%, and 0%, respectively). The 51-transcript signature distinguished TB from latent infection with a sensitivity of 94% and a specificity of 100%. The 51-transcript signature identified higher proportions of culture-confirmed and culture-negative cases of TB than Xpert MTB/RIF; however, innovation is needed to translate transcriptional signatures into diagnostic tools for resource-poor settings—current methods used to detect RNA transcripts are complex and costly.⁷³

Gene expression signatures

A multicohort analysis of data sets available in two public gene expression microarray repositories identified a three-gene signature (*GBP5*, *DUSP3*, and *KLF2*) capable of diagnosing active TB in adults and children, irrespective of bacillus Calmette-Guérin (BCG) vaccination or HIV status. The TB score derived from the three-gene signature demonstrated 85 percent sensitivity and 93% specificity in a cohort made up of both healthy people and those with active TB, 80% sensitivity and 86% specificity in a cohort made up of people with latent or active TB, and 81% sensitivity and 74% specificity in a cohort made up of people with other diseases or active TB. The TB score showed a significant decreasing trend with progression of treatment, suggesting its potential as a biomarker of clinical response to treatment. However, TB scores in children with culture-negative TB were significantly lower than those in children with culture-positive TB. The TB score demonstrated 86% sensitivity and specificity for latent TB versus culture-positive active TB in children.⁷⁴ A blood-based test offers much advantage over what currently exists, but the ability to detect TB in culture-negative children is extremely important, limiting the potential utility of this three-gene signature for children with culture-negative TB, who account for 80% of children with TB.

RECOMMENDATIONS FOR PEDIATRIC TB DIAGNOSTICS

Much work remains to develop novel diagnostic technologies to accurately detect TB infection and disease; to predict disease progression in healthy, infected children; and to monitor treatment in children. The few tests that have been validated and recommended for use in children, including Xpert MTB/RIF, are sub-optimal and underutilized, partly due to difficulties with specimen collection. At the same time, efforts to identify children at risk for TB—especially within maternal and child health programs, where sick children often first present for care—and referral systems and decentralized capacity to diagnose childhood TB, clinically or with available tools, are urgently needed. We also need:

- To validate tests in adults and children in parallel to expedite access to improved diagnostic technologies for children. These evaluations should include a variety of sample types in children with and without HIV and should assess age-related performance;
- Increased investments in research to discover and validate biomarkers and innovation to translate these biomarkers into simple and affordable tests that can rapidly and accurately diagnose TB, monitor treatment, and predict disease progression in children. In 2014, less than \$2.3 million and \$2.8 million was spent globally on research and development for pediatric TB diagnostics and basic science, respectively;⁷⁵
- To establish and support harmonized and collaborative pediatric biorepositories important for biomarker discovery and development;
- To support and create networks of sites that support field evaluation of new diagnostics and can pool data to more rapidly demonstrate the impact of new tools;
- To scale up and decentralize the use of existing technologies and strategies to diagnose pediatric TB infection and disease, especially within maternal and child health programs; and
- To train health care workers to improve their ability and confidence to clinically diagnose children with TB when tests are unavailable or come back negative.

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