Diagnosis of HIV-Associated Tuberculosis

Stephen D. Lawn

Desmond Tutu HIV Centre
Institute of Infectious Disease and Molecular Medicine
University of Cape Town

Dept. Of Clinical Research,
Faculty of Infectious & Tropical Diseases,
London School of Hygiene & Tropical Medicine, London, UK

NO CONFLICTS OF INTEREST TO DECLARE
Lawn & Zumla. Lancet 2011
OUTLINE

1. Defining the need
2. Revisiting the old
3. Ushering in the new
4. Looking to the future
Lab Confirmation of TB Diagnoses vs Age

- **Smear +**
- **Smear - Culture +**
- **No Lab Confirmation**

Age (years):
- 0 to 4 (n=2,853)
- 5 to 9 (n=722)
- 10 to 14 (n=393)
- 15 to 19 (n=1,436)
- 20 to 24 (n=3,184)
- 25 to 29 (n=4,294)
- 30 to 34 (n=4,284)
- 35 to 39 (n=3,762)
- 40 to 44 (n=2,840)
- 45 to 49 (n=2,245)
- 50 to 54 (n=1,479)
- ≥55 (n=1,986)
TB / LTBI Spectrum

Infection eliminated without priming antigen-specific T cells

Infection eliminated in association with T cell priming

Infection controlled with some bacteria persisting in non-replicating form

Bacterial replication maintained at a subclinical level by immune response

Clinical disease

Innate immune

Acquired immune

Quiescent infection

Active infection

Disease

Bacterial load?

Young et al. Trends Microbiol 2009
Lawn et al Clin Devel Immunol 2011
So What Do We Need?

Rapid detection of resistance to 1st and 2nd line drugs

Monitoring response to TB Rx

Point-of-care!

Low-cost

Simple and feasible
OUTLINE

1. Defining the need
2. Revisiting the old
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4. Looking to the future
HIV Status, CD4 Count and PTB vs EPTB

- PTB
- PTB + EPTB
- EPTB

Percentage of Notifications

CD4 count

- <50 (n=2,123)
- 50-99 (n=1,965)
- 100-149 (n=1,721)
- 150-199 (n=1,426)
- 200-349 (n=2,535)
- 350-499 (n=1,163)
- ≥500 (n=883)

(HIV+)

(HIV-)
HIV Status, CD4 Cell Count and Sputum Smear-Positive Grade

Percentage of Notifications

CD4 (cells / µl)

HIV+  HIV-
HIV Status, CD4 Count and Smear Status

Smear Positivity

CD4 Count

HIV+

HIV-

<50 (n=1,530) 50-99 (n=1,409) 100-149 (n=1,255) 150-199 (n=1,076) 200-349 (n=1,896) 350-499 (n=882) ≥500 (n=629) (n=9127)
Increasing diagnostic sensitivity of in the very lowest CD4 cell count strata:
- Sputum Xpert MTB/RIF
- Urine LAM
- Urine Xpert MTB/RIF

Lawn et al. JAIDS 2012
OUTLINE

1. Defining the need
2. Revisiting the old
3. Ushering in the new
   - Symptom screening
   - Diagnostic assays
4. Looking to the future
Yield of HIV-associated tuberculosis during intensified case finding in resource-limited settings: a systematic review and meta-analysis

Katharina Kranzer, Rein M G J Houben, Judith R Glynn, Linda-Gail Bekker, Robin Wood, Stephen D Lawn

Intensified case finding is the regular screening for evidence of tuberculosis in people infected with HIV, at high risk of HIV, or living in congregate settings. We systematically reviewed studies of intensified case finding published

Figure 2: Prevalence of tuberculosis among individuals screened in different settings in countries with generalised epidemics of HIV

VCT=voluntary counselling and testing. PMTCT=prevention of mother-to-child transmission.
Screen for presence of ≥1 of the following symptoms:
1. Current cough
2. Fever
3. Night sweats
4. Weight loss

Sensitivity: 78.9%
Specificity: 49.6%
NPV: high if TB prevalence <10%
Sensitivity of Symptom Screening for TB Pre-ART

Lawn et al IJTLD 2012; in press
Culture

Rapid molecular assays

Antigen detection

Electronic ‘noses’ /
‘breathalysers’

Biomarkers
Culture

Automated liquid culture and phenotypic DST: gold standard

Microcolony culture techniques:
MODS / Thin Layer Agar
- Interim low-cost solution
- MODS commercialization
- Minion et al. Lancet Infect Dis 2010
- Leung et al. IJTL 2012
- Feasibility / scale / impact?
Rapid Molecular Diagnostic Assays
For TB Diagnosis and DST

**Line-Probe Assays**

- WHO-approved in 2008
- Eg Hain Lifesciences MTBDRplus
  - Culture isolates: YES
  - Smear-positive sputum: YES
  - Smear-negative sputum: NO

Technical complexity

New ‘GenoQuick MTB’ (Moure et al J Clin Micro 2012)
Xpert MTB/RIF: real-time PCR using thermocycling and molecular beacon technology

Lawn & Nicol. Future Microbiology 2011
Sensitivity of Xpert MTB/RIF Assay (FIND Multi-Country Evaluation)

Boehme et al NEJM 2010
<table>
<thead>
<tr>
<th>Type of TB</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>Sputum smear-positive</td>
<td>98-100%</td>
</tr>
<tr>
<td>Sputum smear-negative</td>
<td>57-83%</td>
</tr>
<tr>
<td>Extrapulmonary (range of clinical samples)</td>
<td>25-95%</td>
</tr>
</tbody>
</table>

Lawn & Nicol, Future Microbiology 2011
Lawn & Zumla, Exp Rev Anti-Infect Ther 2012
# Xpert and EPTB

## Table 1. Summary of studies (n = 8) published before 7 March 2012 in which the diagnostic accuracy of Xpert® MTB/RIF for extrapulmonary TB was assessed.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Country</th>
<th>TB gold standard diagnoses (n)</th>
<th>TB not diagnosed (n)</th>
<th>Main sample types testing positive for TB (n)</th>
<th>Gold standard for TB diagnosis</th>
<th>Xpert sensitivity, % (95% CI)</th>
<th>Xpert specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index study</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Tortoli et al. (2012)</td>
<td>Italy</td>
<td>268</td>
<td>1206</td>
<td>Tissue biopsies/fine-needle aspirates (94); pleural fluid (18); gastric aspirates (61); pus (55); CSF (14); urine (16); peritoneal/synovial/pericardial fluid (10)</td>
<td>Culture (solid and liquid) or suggestive radiology/histology with documented positive response to TB treatment</td>
<td>81.3 (76.2–85.8)</td>
<td>99.8 (99.4–100)</td>
</tr>
<tr>
<td><strong>Other studies</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Armand et al. (2011)</td>
<td>France</td>
<td>32</td>
<td>NA</td>
<td>LNs (16); pleural (7); bone (5)</td>
<td>Culture (solid and liquid media)</td>
<td>53.1 (34.7–70.9)</td>
<td>NA</td>
</tr>
<tr>
<td>Causse et al. (2011)</td>
<td>Spain</td>
<td>41</td>
<td>299</td>
<td>Tissue biopsies (18); CSF (6); gastric aspirates (8); pleural fluid (4); purulent exudates (5)</td>
<td>Culture (solid and liquid media)</td>
<td>95.1 (83.5–99.4)</td>
<td>100 (98.8–100)</td>
</tr>
<tr>
<td>Friedrich et al. (2011)</td>
<td>South Africa</td>
<td>20</td>
<td>5</td>
<td>Pleural fluid (25)</td>
<td>Culture (liquid media)</td>
<td>25.0 (8.7–49.1)</td>
<td>100 (47.8–100)</td>
</tr>
<tr>
<td>Hillemann et al. (2011)</td>
<td>Germany</td>
<td>45</td>
<td>476</td>
<td>Tissue (30); gastric aspirate (8); urine (5)</td>
<td>Culture (solid and liquid media)</td>
<td>77.3 (60.5–87.1)</td>
<td>98.2 (96.0–98.9)</td>
</tr>
<tr>
<td>Ligthelm et al. (2011)</td>
<td>South Africa</td>
<td>30</td>
<td>18</td>
<td>Fine-needle aspiration LN biopsy</td>
<td>Composite standard: positive cytology + AFB and/or culture of MTB</td>
<td>96.6 (86.6–100)</td>
<td>88.9 (69.6–100) (note: only 18 samples)</td>
</tr>
<tr>
<td>Moure et al. (2011)</td>
<td>Spain</td>
<td>108</td>
<td>41</td>
<td>All smear-negative. Pleural fluid (26); LNs (34); abscess aspirates (17); tissues (12)</td>
<td>Culture (solid and liquid media)</td>
<td>58.3 (48.5–67.8)</td>
<td>100 (91.4–100)</td>
</tr>
<tr>
<td>Vadwai et al. (2011)</td>
<td>India</td>
<td>283</td>
<td>250</td>
<td>Tissue biopsies (105); pus (98); body fluids (24)</td>
<td>Composite of smear, culture, clinical, radiology and histology</td>
<td>80.6 (75.5–85.0)</td>
<td>99.6 (97.8–100)</td>
</tr>
</tbody>
</table>
High Diagnostic Yield of Tuberculosis From Screening Urine Samples From HIV-infected Patients with Advanced Immunodeficiency Using The Xpert MTB/RIF Assay

Stephen D. Lawn, MBBS, MRCP, MD, DTM&H,*† Andrew D. Kerkhoff, MSc,*‡ Monica Vogt, DipMedTech,* and Robin Wood, MMed, FCP*

JAIDS 2012
Screening for HIV-Associated Tuberculosis and Rifampicin Resistance before Antiretroviral Therapy Using the Xpert MTB/RIF Assay: A Prospective Study

Stephen D. Lawn¹,²*, Sophie V. Brooks¹, Katharina Kranzer¹,², Mark P. Nicol³,⁴, Andrew Whitelaw³,⁴, Monica Vogt¹, Linda-Gail Bekker¹, Robin Wood¹,⁵

45% increase in case detection

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![Bar chart showing sensitivity and specificity comparison between Microscopy and Xpert assays.](chart.png)

- **Sensitivity**: Microscopy > Xpert
- **Specificity**: Microscopy < Xpert
Detection of Rifampicin Resistance

- Problem of false +ves
- New G4 cartridges launched Dec 2011
Xpert MTB/RIF: A ‘game-changer’?

• **Pros include**
  – Exceptional performance for TB diagnosis
  – Rapid RIF Resistance screening
  – Near patient technology

• **Some cons**
  – RIF resistance specificity
  – Xpert-negative TB
  – Expense
    • 4 bay machine $17,000
    • 1 cartridge approx $15
  – Simple – but not simple enough
  – Will it be used at point-of-care?
Rapid Diagnosis of Tuberculosis with the Xpert MTB/RIF Assay in High Burden Countries: A Cost-Effectiveness Analysis

Anna Vassall¹,², Sanne van Kampen¹, Hojoon Sohn³, Joy S. Michael⁴, K. R. John⁵, Saskia den Boon⁶, J. Lucian Davis⁷, Andrew Whitelaw⁸,⁹, Mark P. Nicol⁸,⁹, Maria Tarcela Gler¹⁰, Anar Khaliqov¹¹, Carlos Zamudio¹², Mark D. Perkins¹³, Catharina C. Boehme¹³, Frank Cobelens¹*

The cost-effectiveness of routine tuberculosis screening with Xpert MTB/RIF prior to initiation of antiretroviral therapy in South Africa: a model-based analysis

Jason R. Andrewsᵃ,b,d,e, Stephen D. Lawnᶠ,g, Corina Rusuᶜ,d, Robin Woodᶠ, Farzad Noubaryᶜ,d,e, Melissa A. Benderʰ, C. Robert Horsburghⁱ, Elena Losinaᵇ,c,d,e,j,k, Kenneth A. Freedbergᵃ,b,c,d,e,i,l and Rochelle P. Walenskyᵃ,b,c,d,e

AIDS 2012
Characteristics and Early Outcomes of Patients With Xpert MTB/RIF-Negative Pulmonary Tuberculosis Diagnosed During Screening Before Antiretroviral Therapy

Stephen D. Lawn,1,4 Andrew D. Kerkhoff,1,5 Monica Vogt,1 Yonas Ghebrekristos,3 Andrew Whitelaw,2,3 and Robin Wood1

- Xpert-negative TB patients had:
  - very early TB disease
  - less advanced HIV
  - good prognosis

Lawn et al Clin Infect Dis 2012
Impact of Xpert in South Africa?
<table>
<thead>
<tr>
<th></th>
<th>Xpert-NEG (n=25)</th>
<th>Xpert-POS (n=64)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive and in-programme</td>
<td>21 (84.0)</td>
<td>54 (84.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Dead</td>
<td>0</td>
<td>6 (9.4)</td>
<td>&lt;0.179</td>
</tr>
<tr>
<td>LTFU</td>
<td>4 (16.0)</td>
<td>8 (12.5)</td>
<td>0.733</td>
</tr>
<tr>
<td>Transfer-out</td>
<td>0</td>
<td>1 (1.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Started TB Rx</td>
<td>17 (68)</td>
<td>49 (76.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Time to TB treatment</td>
<td>32 (26-48)</td>
<td>9 (6-18)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Lawn et al Clin Infect Dis 2012
Correspondence

Location of Xpert® MTB/RIF in centralised laboratories in South Africa undermines potential impact

Trébucq and colleagues have provided an important overview of the pros and cons of implementation of the Xpert® MTB/RIF rapid molecular diagnostic assay by national tuberculosis (TB) programmes in low-income countries.¹ A key attraction of this technology is that it can be used near the site of patient care.²
Living with HIV, dying of TB
We need a POC TB test!
Urine Antigen Detection

Lipoarabinomannan (LAM)
Sensitivity of LAM ELISA for TB Screening Pre-ART

- **Microscopy**
- **LAM ELISA**
- **LAM ELISA + Microscopy**

**Sensitivity**

- CD4 >100
- CD4 50-100
- CD4 <50

Specificity 100%

Determine TB-LAM Ag

Control band
Patient sample result
Sample pad
Determine TB-LAM Ag

Negative  Interm. positive  Strong positive
Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study

Stephen D Lawn, Andrew D Kerkhoff, Monica Vogt, Robin Wood
Agreement between two readers?

Overall agreement
514/516
99.6% (95% CI 98.6-100)

Kappa= 0.97
(95%CI, 0.88-0.99)
Agreement between TB-ELISA and Determine TB-LAM Strips?

Overall agreement
507/516
98.3% (95%CI, 96.7-99.2)

Kappa = 0.84
(95%CI, 0.72-0.92)
Sensitivity of LAM POC test

Specificity >98%
all strata

- CD4 >150
- CD4 = 50-150
- CD4 <50

Diagnostic sensitivity (%)

- Smear
- LAM
- Smear + LAM
Sensitivity of TB diagnostics among patients with a CD4 <100 cells/µL

- Liquid Culture 100%
- Xpert MTB/RIF 76%
- Sputum AFB 35%
- Determine TB-LAM 52%
- AFB + LAM = 66%
Clinical significance of lipoarabinomannan (LAM) detection in urine using a low-cost point-of-care diagnostic assay for HIV-associated tuberculosis

Stephen D. Lawn\textsuperscript{a,b}, Andrew D. Kerkhoff\textsuperscript{a,c}, Monica Vogt\textsuperscript{a} and Robin Wood\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>LAM-Negative (n=36)</th>
<th>LAM-Positive (n=23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4 count Median (IQR)</td>
<td>115 (69-160)</td>
<td>37 (182-379)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hb Median (IQR)</td>
<td>11.6 (10.0-12.5)</td>
<td>8.0 (7.5-9.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sputum smear-positive (%)</td>
<td>8 (27.2)</td>
<td>10 (43.5)</td>
<td>0.084</td>
</tr>
<tr>
<td>Days to culture positivity</td>
<td>17 (14-24)</td>
<td>12 (9-17)</td>
<td>0.005</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0</td>
<td>5 (21.7)</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Liquid culture

AFB Sputum Microscopy

Sputum Xpert MTB/RIF

Determine TB-LAM

Deaths
OUTLINE

1. Defining the need
2. Revisiting the old
3. Ushering in the new
4. Looking to the future
Culture

Rapid molecular assays

Antigen detection

Electronic ‘noses’ / ‘breathalysers’

Biomarkers
LAMP: Loop Mediated Isothermal Amplification

PCR is slow and requires thermocycling
LAMP at stable elevated temperature
Visual read-out
Multiplexing not possible
Second prototype: STAG 2012
‘Fast-Followers’ to Xpert: .......Cheaper and Simpler

Amplification at lower temps
Low energy requirements
Quick
Smart-phone sized hardware
Multiple drug resistance mutations

...WATCH THIS SPACE!
Electronic ‘Noses’ / Breathalyzers

- Electronic detection of volatile biomarkers using chemical sensors + pattern recognition system
- Rapid detection of Ag85B

Kolk et al J Clin Micro 2010
McNerney et al BMC Infect Dis 2010
The ‘Omics’ Era

- Transcriptomics
- Proteomics
- Metabolomics
An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis

Matthew P. R. Berry¹, Christine M. Graham¹*, Finlay W. McNab¹*, Zhaohui Xu⁶, Susannah A. A. Bloch³, Tolu Oni⁴,⁵, Katalin A. Wilkinson²,⁴, Romain Banchereau⁹, Jason Skinner⁶, Robert J. Wilkinson²,⁴,⁵, Charles Quinn⁶, Derek Blankenship⁷, Ranju Dhawan⁸, John J. Cush⁶, Asuncion Mejias¹⁰, Octavio Ramilo¹⁰, Onn M. Kon³, Virginia Pascual⁶, Jacques Banchereau⁶, Damien Chaussabel⁶ & Anne O’Garra¹

Whole blood 393-transcript signature

TB vs LTBI vs healthy controls

TB before and after Rx

Berry et al Nature 2010
CONCLUSIONS
CONCLUSIONS

• Huge progress over the past 5 years
• Major progress in rapid molecular techniques
• Xpert MTB/RIF
  – is a huge step forward
  – far from the prefect solution
  – has triggered a huge amount of development
• Determine TB-LAM
  – a POC niche for those with v. low CD4 counts
• Impact data needed
So What Have We Got?

- **TB Notifications**
  - Age strata: 0-4, 5-9, 10-14, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, 50-54, 55-59, 60-64, 65-69, 70-74, 75+
  - Y-axis: Number of TB notifications
  - Color codes:
    - HIV positive
    - HIV unknown
    - HIV negative

- **Prevalence rate**
  - X-axis: Age, years
  - Y-axis: Prevalence rate
  - Graph shows the prevalence rate over age groups.

- **Rapid detection of resistance to 1st and 2nd line drugs**
- **Monitoring response to TB Rx**
- **Point-of-care**!
- **Low-cost**
- **Simple and feasible**
Acknowledgments

- **Cape Town:** Robin Wood, Linda-Gail Bekker, Andrew Kerkhoff, Sophie Brooks, Ankur Gupta, Rishi Gupta, Monica Vogt, Katharina Kranzer, Landon Myer, Matthew McNally, Pearl Pahlana + staff at Hannan Crusaid clinic
- Mark Nicol, Andrew Whitelaw + NHLS staff
- **Harvard:** Jason Andrews, Rochelle Walensky, Ken Freedberg
- FIND – preferential pricing of cartridges
- Alere – supplied LAM tests
- IIDMM, UCT, LSHTM, WT Bloomsbury Centre