Diagnosis of HIV-Associated Tuberculosis: *Spit or Pee?*

Stephen D. Lawn

No conflicts of interest to declare.
### INDIA vs S. AFRICA 2012

<table>
<thead>
<tr>
<th></th>
<th>INDIA</th>
<th>SOUTH AFRICA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total TB cases (est)</td>
<td>2.5M</td>
<td>0.56M</td>
</tr>
<tr>
<td>% Global TB burden</td>
<td>29%</td>
<td>6.5%</td>
</tr>
<tr>
<td>TB incidence rate</td>
<td>176 / 100,000</td>
<td>1,003 / 100,000</td>
</tr>
<tr>
<td>TB/HIV cases</td>
<td>130,000</td>
<td>530,000</td>
</tr>
<tr>
<td>% Global burden of</td>
<td>12%</td>
<td>30%</td>
</tr>
<tr>
<td>TB/HIV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TB/HIV Deaths</td>
<td>42,000</td>
<td>88,000</td>
</tr>
</tbody>
</table>
TB in Cape Town 2009 (n=29,478)

Wood and Lawn PLoS ONE 2011
Proportion of TB/HIV Notifications With Laboratory Confirmation in Cape Town 2009 (Pre-Xpert)

- No Lab Confirmation, 47.9%
- Smear POS, 32.6%
- Smear NEG Culture POS, 19.6%
CD4 Counts of Cases of HIV-Associated TB (n=11,816) in Cape Town

Gupta / Lawn 2012; IJTLD 2013
1. Mortality due to failure of diagnosis of TB/HIV
2. Reasons for diagnostic failure
3. Rapid urine-based diagnostics for TB/HIV
1993

Kumasi, Ghana, W. Africa
The mortality and pathology of HIV infection in a West African city

Primary Causes of In-Patient HIV-Related Deaths (n=247), Abidjan, Cote D’Ivoire.

<table>
<thead>
<tr>
<th>Primary cause of death</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Tuberculosis</td>
<td>80 (32%)</td>
</tr>
<tr>
<td>Bacteraemia</td>
<td>26 (11%)</td>
</tr>
<tr>
<td>Cerebral toxoplasmosis</td>
<td>24 (10%)</td>
</tr>
<tr>
<td>Pyogenic pneumonia</td>
<td>19 (8%)</td>
</tr>
<tr>
<td>Purulent meningitis</td>
<td>12 (5%)</td>
</tr>
<tr>
<td>Non-specific enteritis</td>
<td>8 (3%)</td>
</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Cryptococcosis</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Kaposi’s sarcoma</td>
<td>4 (2%)</td>
</tr>
</tbody>
</table>
Histology

- TB was disseminated in 89%
- Histology: predominantly non-reactive and multibacillary
TB prevalence: 32% - 67%

The Prevalence and Drug Sensitivity of Tuberculosis among Patients Dying in Hospital in KwaZulu-Natal, South Africa: A Postmortem Study

Ted Cohen¹,², Megan Murray¹,²,³, Kristina Wallengren², Gonzalo G. Alvarez⁴, Elizabeth Y. Samuel⁵, Douglas Wilson⁶

Pathology and causes of death in a group of 128 predominant HIV-positive patients in Botswana, 1997–1998


Causes of Death on Antiretroviral Therapy: A Post-Mortem Study from South Africa

Emily B. Wong¹,²,³, Tanvier Omar⁴, Gosetsemang J. Sethlako¹,², Regina Osih¹, Charles Feldman⁶, David M. Murdoch⁶, Neil A. Martinson⁷,⁸, David R. Bangsberg⁹, W. D. F. Venter¹
The Spectrum of Clinical and Pathological Manifestations of AIDS in a Consecutive Series of 236 Autopsied Cases in Mumbai, India

Dhaneshwar Namdeorao Lanjewar

Sir J. J. Hospital and Grant Medical College, Byculla, Mumbai, Maharashtra 400008, India

Patients with AIDS 1988 – 2007

TB was primary cause of death in 63%

In those with TB, 94% had disseminated disease.
Reflects abject failure of the TB diagnostic process in HIV-infected medical in-patients
Problems With Existing Diagnostic Approaches

1. Investigation of ‘suspects’

2. Poor tools

3. Investigation for pulmonary TB with sputum as primary sample
Much HIV-Associated TB Lies
Ante-Mortem Studies:
HIV+ Patients in a South African Township
Prevalence of Undiagnosed Sputum Culture-Positive TB in Unselected HIV+ Patients Pre-ART

Lawn et al AIDS 2009
Problem #1. Investigation of ‘suspects’

Proportion of TB diagnoses (%)

- Induced sputum #2
- Induced sputum #1
- Spot sputum #1

All patients

<table>
<thead>
<tr>
<th>Proportion of TB diagnoses (%)</th>
<th>WHO screen</th>
<th>Any cough</th>
<th>Cough &gt;2 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of TB diagnoses</td>
<td>80</td>
<td>60</td>
<td>40</td>
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</tbody>
</table>

Lawn et al IJTLD 2012
An Ante-Mortem Study of TB Diagnosis in Unselected HIV+ Medical In-Patients

427 new HIV+ admissions
2,391 samples (median – 3 body compartments)
3,471 diagnostic tests for TB

Lawn et al CROI 2014
TB Prevalence

- TB diagnoses = 139 (Xpert or culture of any sample)
- TB Prevalence = 32.6% (28.1-37.2)

Median CD4 Count in TB cases = 80 cells/µL

Cough >2 weeks in just 13% of TB cases

WHO symptom screen positive in 92% of total patients studied: NOT predictive for TB
<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td><strong>ALL patients</strong></td>
<td>32.6%</td>
</tr>
<tr>
<td><strong>TB suspected on admission</strong></td>
<td>45.6%</td>
</tr>
<tr>
<td><strong>TB not suspected</strong></td>
<td>17.9%</td>
</tr>
<tr>
<td><strong>Cough &gt;2/52</strong></td>
<td>52.9%</td>
</tr>
<tr>
<td><strong>No cough &gt;2/52</strong></td>
<td>30.8%</td>
</tr>
<tr>
<td><strong>WHO symptom screen negative</strong></td>
<td>11.1%</td>
</tr>
<tr>
<td><strong>CD4 count &lt;100</strong></td>
<td>46.3%</td>
</tr>
<tr>
<td><strong>CD4 count &lt;101-200</strong></td>
<td>36.8%</td>
</tr>
<tr>
<td><strong>CD4 count &gt;200</strong></td>
<td>17.2%</td>
</tr>
<tr>
<td><strong>ART-naive</strong></td>
<td>39.8%</td>
</tr>
<tr>
<td><strong>Ever received ART</strong></td>
<td>27.3%</td>
</tr>
</tbody>
</table>
Conclusion

Where prevalence is so high and presentation so non-specific, ALL HIV+ patients should be investigated for TB.
Problem #2: Diagnostic Tools Function Poorly

Fluorescence microscopy
Sensitivity 15%-30%

Up to 30% cases normal CXR

Liquid culture
Median time to positivity >3 weeks for smear-neg samples

Lawn et al AIDS 2009
1. Sputum liquefaction and inactivation with 2:1 sample reagent

2. Transfer of 2 ml material into test cartridge

3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)

4. Sample automatically filtered and washed

5. Ultrasonic lysis of filter-captured organisms to release DNA

6. DNA molecules mixed with dry PCR reagents

7. Seminested real-time amplification and detection in integrated reaction tube

8. Printable test result

Time to result, 1 hour 45 minutes
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

PLoS Med 2011; 8: e1001067

45% increase in case detection

![Graph showing Sensitivity and Specificity](image)

- **Microscopy**
- **Xpert**
Problem #3: We are obsessed with testing sputum!
Pulmonary vs Extrapulmonary Disease Among In-Patients With TB/HIV (n=139)

PTB in 54.0% (n=51)

EPTB in 82.7% (n=64)

17.3% (n=24)

Median CD4 count = 80 cells/μL

Lawn et al CROI 2014
Urine Antigen Detection

Lipoarabinomannan (LAM)
Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis

C. Boehme, E. Molokova, F. Minja, S. Geis, T. Loscher, L. Maboko, V. Koulchin, M. Hoelscher


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

Sensitivity of LAM ELISA for TB Screening Pre-Art

Specificity 100%

Lawn et al. AIDS 2009.
Determine TB-LAM Ag

Control band
Patient sample result
Sample pad

Negative Positive Positive

Lawn et al Lancet Infect Dis 2012
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%
Urine LAM Diagnoses
TB in Sickest Patients
Lawn et al. AIDS 2012
Xpert MTB/RIF Testing of Urine - a New Rapid Means of Diagnosis of HIV-Associated TB
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*

*Sensitivity (%)

CD4 cell count strata

LAM

Xpert

Lawn et al. JAIDS 2012
WHY URINE?

Peter et al. Curr Opin Pulm Med 2010
Overlap Between Positive
Urine LAM / Urine Xpert / Blood cultures

- **Urine Xpert+**
  - = renal TB
- **Urine LAM**
- **Blood culture**
Of those with generalised TB, 65.2% had evidence of renal involvement.
Research Article

The Spectrum of Clinical and Pathological Manifestations of AIDS in a Consecutive Series of 236 Autopsied Cases in Mumbai, India

Dhaneshwar Namdeorao Lanjewar

Sir J. J. Hospital and Grant Medical College, Byculla, Mumbai, Maharashtra 400008, India
Rapid Urine-Based Diagnosis of TB in HIV-Infected Medical In-Patients
ASPIRATIONAL GOAL?

Microbiological Diagnosis of HIV-Associated TB Within 24 Hours of Hospital Admission
AIMS

To determine:

1. Prevalence undiagnosed TB ✓

2. Diagnostic yield
   - Urine-based screening
   - Sputum-based screening
   - Other non-respiratory samples
Day 1: Study TB Screen

Day 2 onwards: Routine Investigations

Study samples:
- Sputum
- Urine
- Blood cultures

Routine samples:
- Sputum
- Pleural fluid / biopsy
- CSF
- Lymph node aspirates / biopsies
- Urine culture
- Etc

Total TB Diagnoses by Culture or Xpert
1. Sputum Microscopy
2. Non-Respiratory Samples
3. Urine

- Urine Xpert (neat)
- Urine Xpert (concentrated)

2 mls

40 mls

Microscopy
Xpert
Culture
Determine TB-LAM
Proportion of Patients Able to Produce Urine / Sputum Samples

Proportion (%)

<table>
<thead>
<tr>
<th></th>
<th>Urine: Day 1</th>
<th>Sputum: Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion (%)</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>
Analysis #1
What Proportion of Cases Can Be Diagnosed Using Rapid Diagnostics on Urine and Sputum Samples Obtained Within the First 24 Hours?
Diagnostic Yield From Rapid Testing of Sputum and Urine Obtained in First 24 Hrs
Potential Cumulative Yield in First 24 Hours

- **Urine LAM**: Within 1 Hour
  - Potential Cumulative Yield: 1/3

- **Urine LAM / Xpert**: Within hours
  - Potential Cumulative Yield: 2/3

- **LAM / Xpert on urine + sputum**: Within 24 hours
  - Potential Cumulative Yield: 4/5
Analysis #2

Compare diagnostic yields of:

1. Urine obtained in 1\textsuperscript{st} 24 hours
2. All sputum samples
3. All other non-respiratory samples
Final Diagnostic Yields (n=139) From Three Types of Samples Obtained During Admission

- Sputum = 54.0%
- Urine Xpert and LAM = 69.1%
- Non-respiratory sample cultures = 49.6%

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Diagnostic Yield</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>54.0%</td>
<td>24</td>
</tr>
<tr>
<td>Urine Xpert</td>
<td>69.1%</td>
<td>26</td>
</tr>
<tr>
<td>Non-respiratory</td>
<td>49.6%</td>
<td>14</td>
</tr>
</tbody>
</table>
CD4 <100 cells/µL (n=74)

Urine (Xpert + LAM) 85.1%

Sputum (Xpert + culture) 51.4%

Non-respiratory (culture) 52.7%
Rapid Diagnosis of HIV-Associated TB: ‘Pee’ or ‘Spit’?
FAVOUR URINE
• Diagnostic yield
• Yield even greater at lower CD4 counts
• Ease of obtaining sample (even if comatose)
• No risk of nosocomial TB transmission
• Ease of laboratory handling
• Time to diagnosis
• Point of care (LAM)

FAVOUR SPUTUM
• Provides an index of infectious risk
Conclusions

Need a paradigm shift in diagnostic approach:

– Active routine investigation of ALL HIV+ medical in-patients regardless of symptoms

– Consider routine use of urine-based rapid tests (Determine TB-LAM and urine Xpert MTB/RIF)
Acknowledgements

• University of Cape Town: Robin Wood, Linda-Gail Bekker, Graeme Meintjes, Andrew Kerkhoff, Monica Vogt, Pearl Pahlana, Charlotte Schutz, Mark Nicol

• National Health Laboratory Service: Mark Nicol + NHLS staff

• GF Jooste District Hospital: Rosie Burton, Gavin van Wyk, hospital and laboratory staff

• Provincial Government of the Western Cape:
## WHO ENDORSEMENT AND RECOMMENDATIONS

### Xpert MTB/RIF

<table>
<thead>
<tr>
<th>Type of TB</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum smear-positive</td>
<td>98%</td>
</tr>
<tr>
<td>Sputum smear-negative</td>
<td>67%</td>
</tr>
<tr>
<td>Extrapulmonary median (range)</td>
<td>77% (range 19%-100%)</td>
</tr>
</tbody>
</table>

London School of Hygiene & Tropical Medicine

University of Cape Town