Potential Diagnostic Biomarkers

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Overview

- TB-specific biomarkers (LAM)
- TB non-specific biomarkers (T cell assays, unstimulated Th1 cytokines)
‘In the Mortality Bills, pneumonia is an easy second to tuberculosis; indeed in many cities the death-rate is now higher and it has become, to use the phrase of Bunyan 'the captain of the men of death.’

Sir William Osler

*Medicine in the Nineteenth Century* (1904). In *Aequanimitas with Other Addresses to Medical Students, Nurses and Practitioners of Medicine* (1904),
Urine + LAM = TB LFA

Advantages:

- Almost always obtainable
- Low infectious collection risk
- Simple and non-viscous fluid – easy processing

17.5 kD glycolipid found in the outer cell-wall of mycobacterial species

Bricken V, Mol Microbiol, 2004
Hamasur B, Microbiol Methods, 2001
A. Sensitivity

Shah M, Cochrane Library, 2016
The mortality impact of point-of-care urine lipoarabinomannan testing to guide tuberculosis treatment initiation in HIV-infected hospitalised patients: a multi-country randomised controlled trial

Jonny G. Peter (PhD)*1,10,11,12, Lynn S. Zijenah (PhD)*2, Duncan Chanda (MD)*1,3,13, Petra Clowes (MD)*4,5, Maia Lesosky (PhD)1, Phindile Gina (MD)1, Nirja Mehta (MSc)1, Greg Calligaro (MD)1, Carl J. Lombard (PhD)6, Gerard Kadzirange (MD)7, Tsitsi Bandason (PhD)8, Abidan Chansa (MD)3,13, Namakando Liusha (MD)3,13, Chacha Mangu (MD)4, Bariki Mtafya (MD)4,8, Henry Msila (MD)4, Andrea Rachow (PhD)4,5,9, Michael Hoelscher (PhD)4,5,9, Peter Mwaba (MD)3, Grant Theron (PhD)1,14, Keertan Dheda (PhD)1,10,11,12
Compartment-specific LAM

- Not useful in pleural space (LAM ELISA): 8% sensitivity
  Dheda K, PloS One, 2010

- Pericardial TB: 80% HIV+ve; 33% sensitivity; 50% in CD4<100
  Pandie, Dheda & Mayosi, BMC Med, 2015

Cerebrospinal Fluid Research

Utility of a novel lipoarabinomannan assay for the diagnosis of tuberculous meningitis in a resource-poor high-HIV prevalence setting
Vinod B Patel¹, Ahmed I Bhigjee¹, Hoosain F Paruk¹, Ravesh Singh², Richard Meldau³, Cathy Connolly⁶, Thombi Ndung'u² and Keertan Dheda*³,⁴,⁵

- LAM ELISA sensitivity 64% (n=14) and specificity 69% (n=13)
Comparison of a Clinical Prediction Rule and a LAM Antigen-Detection Assay for the Rapid Diagnosis of TBM in a High HIV Prevalence Setting

Vinod B. Patel¹, Ravesh Singh², Cathy Connolly³, Victoria Kasprowicz², Allimudin Zumla⁴, Thungi Ndungu², Keertan Dheda⁴,⁵,⁶*
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- N= 150; 84 HIV+ve (median CD4= 132); n=39 culture +ve
- LAM ELISA CSF sensitivity= 31%; specificity= 94%
- CD4< 100; sensitivity 50%; specificity 95%

- However, clinical prediction rule (glucose; lymphocytes> 200, CD4< 200; CLAT-ve) = sensitivity 47%; specificity 98%
LAM in TBM: other studies

- Autopsy study in 91 HIV-infected adults
  LAM LFA sensitivity = 75% in definite TB (Xpert= 100%)
  sensitivity = 50% in definite and probable TB
  22% to 78% where not on anti-TB treatment

  Cox JA, JCM, 2015
Post Jan 2014: grade 2 became grade 1
LAM utility in the CSF

Dr Omar Siddiqi

- 418 patients recruited (n= 93 culture +ve); 225 males (53.6%)
- Median age - 35 years
- HIV prevalence - 80%
- Median CD4 count - 101 cells/µl
Preliminary Results

- CSF LAM performed best in CD4 < 100
  - Sensitivity 35%
  - Specificity 91%

- CSF LAM picked up 4 cases missed by Xpert (3 of these died).
- There were 9 cases of TBM that were detected by urine LAM and missed by both Xpert and CSF LAM (CD4= 62).

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>GeneXpert</td>
<td>48%</td>
<td>98%</td>
</tr>
<tr>
<td>CSF LAM</td>
<td>24%</td>
<td>95%</td>
</tr>
<tr>
<td>Urine LAM</td>
<td>29%</td>
<td>72%</td>
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Concentration of LAM in the urine (n= 70 HIV+ve)

- Incremental significant increase in sensitivity (Figure 1)
- Sensitivity increase dependent on CD4 count (Figure 2)
- No effect on specificity (n = 31 non-TB)
Other TB-specific biomarkers in the CSF
**The identification of tuberculosis biomarkers in human urine samples**

Brandy L. Young, Zandile Mlamia, Putuma P. Gqamana, Salome Smit, Teri Roberts, Jonathan Peter, Grant Theron, Ureshnie Govender, Keeran Dhead, and Jonathan Blackburn

**Affiliations:** Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, Division of Medical Biochemistry, Dept of Clinical Laboratory Sciences, University of Cape Town, Cape Town, South Africa. *Medecine Sans Frontieres, Geneva, Switzerland. Both authors contributed equally.

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**UCT patent: panel of novel urine TB-specific biomarkers**

**Pyruvate dehydrogenase E1 component**

OS=Mycobacterium tuberculosis

MASYLDPIDPEETSEWLESFDTLLQRGCSPRARYMLMRLLERAGEQRAVIPALTSTDYNTIPTELEPWFPGDEDVERRYRAWIRWNAAIMVHRAORPGGVGGGHISTYASAAALYEVGFMHFRRFKSHPGGDQFVIQGHASPGIYA

RAFLERGLTAEQLDGFQEHSHVGGLPSYPHPRLMPDFWEFPTVSMGGLPLAIYQARFNHYLHGDGIKDTD

DQHWWCFGLGDGMEDEPRESLAVGALVGAELDNLTFVINCNLQRDGPVRNGKIQQELESFFRAGWNIVKVV

WGREWDAHALRDGALVNLMNTTPDGYQTYKANDGGYVRDHFFGRDPRTKVALVENSMQDQDWNKLKRGG

HDYRKYVAAYRADAHDGKQPTVLAKTIKGYALGKHFEGRNATHEMKLTLEDELKEFRTDQRVPVSDAQLLENNPYP

LPPYHPGLNAPERYMLDRRALGGFPERRTKASKALTLPGRDIYPLKHSghqevattmatvrtfeKELRDKIQIPRIVPIIPDDERFQMSWPSLKIYRNRLQLYTAVDADLMLAYKESEBGVQILHHEGIEASGVDFIAAGTSYATHENMPIYIFSMGFQRTGDSFWAAADQMARG

**Database Searching‡**

A combined human, Mtb H37Rv and decoy database was used in X!Tandem and Omssa

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**Flowchart:**

1. Collect Urine protein
2. Flow through 50KDa MWCO
3. Collect concentrate
4. Load/run SDS PAGE gel
5. 1st Excise protein bands
6. HPLC-MS/MS Analysis
7. 2nd Digest protein
8. Database Searching‡
Mycobacterial-specific molecules detected by LC-MS

- 2 mycobactin siderophores and tuberculosinyladenosine
- Detected in 71% of CSF samples (n= 14)
- Suggest that detection of TB-specific proteins coupled with appropriate detection platforms holds promise
Other TB-specific antigens

- **ESAT-6**
  - Feng GD, AJRCCM, 2014
  - Kashyap RS, Infection, 2009

- **Ag 85A and Ag85B**

- **65kd HSP**
  - Mudaliar AV, BMCNeurol, 2006

- **Tuberculostearic acid**
  - French GL, Lancet, 1987
Host (non-specific) biomarker readouts for TB (OMICS) 

matabolomics, proteomics, transcriptomics

- TB presents as part of a **differential diagnosis** (several infectious or inflammatory diseases)
- Host or **non pathogen-specific** biomarker approach that can discriminate diseases **seems counter intuitive**
- Blood-based host transcriptional signatures can distinguish TB from other inflammatory or infectious diseases in adults and children (**infection-associated sepsis vs. SIRS**)

Bloom CI, PLoS One, 2013  
Anderson ST, NEJM, 2014  
Berry MP, Nature, 2010  
Roe JK, PloS One, 2016  
Zak DE, Lancet, 2016

Reviews: Clif JM, Imm Rev, 2015  
Human proteome in the CSF (iTRAC LC-MS/MS)

- Ou Q, BioScience Trends, 2013
- Mu J, Biochem Biophys Res Commun, 2015
- Li Z, Clinica Chimica Acta, 2016 (metabolomics)

- Inappropriate controls (healthy or selected disease phenotypes)
- Small numbers
- No proven diagnostic value demonstrated
Comparison of IFN-g with Xpert MTB/RIF in pleural fluid

- N= 93; suspected pleural TB (20% HIV-infected)
- 43% (40/93) TB culture- or biopsy-positive

Meldau & Dheda, BMC Infect Dis, 2014

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity using uncentrifuged pleural fluid</th>
<th>Sensitivity using centrifuged pleural fluid (15 ml)</th>
</tr>
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<tbody>
<tr>
<td>Smear microscopy</td>
<td>0% (0/40)</td>
<td>-</td>
</tr>
<tr>
<td>Xpert in all patients</td>
<td>25% (9/40)</td>
<td>23% (9/40)</td>
</tr>
</tbody>
</table>

- Uniformly good specificity of 98%

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<tr>
<th></th>
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<th>Specificity</th>
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<tr>
<td>IFN-γ in all patients</td>
<td>93% (37/40)</td>
<td>96% (46/48)</td>
</tr>
</tbody>
</table>
Pleural TB
IFN- gamma

- 22 studies; 2101 patients
- Pooled sensitivity = 89%; specificity = 97% (ROC = 0.98)

Jiang J, Chest, 2007
Pericardial TB and peritoneal TB unstimulated IFN-g is the most accurate assay

- **Peritoneal TB:** SENS = 97% SPEC = 100% (n=119)
  
  Sharma SK, J Interf & Cytokine Research, 2006

- **Pericardial TB:** SENS = 95.7% and SPEC = 96.3% (n=151)
  
  Pandie & Dheda & Mayosi, BMC Med, 2014
Compartment-specific transport mechanisms and trapping of biomarkers

- Transport across serosal membranes incompletely understood
- Some biomarkers equilibrate rapidly with blood; others not
- Not size related

- Ion channels and exchanges; osmotic pressures; transport proteins, receptor mediated, transcellular lipid pathways, adsorptive transcytosis, water soluble agents through tight junctions, active transport mechanisms, exosomes, membrane pores

- TB modulates these mechanisms ‘trapping’ IFN-g

IGRA (T-SPOT-TB ELISPOT) in TBM (using cells from the CSF; n= 150)

- Sensitivity = 82%; specificity = 100% (n= 140)
- Works very well in TBM when used in conjunction with CLAT and Gram stain

Patel and Dheda, AJRCCM, 2010
TBM: unstimulated IFN-g when combined with low cost POC lab tests (Gram stain and CLAT)
Unstimulated and unprocessed IFN-Y when used in conjunction with CLAT and Gram stain; (n= 140)

Sensitivity= 92% (78-98); specificity= 100% (78-100); NPV= 83%

Patel and Dheda, JCM, 2011

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>IFN-γ levels ≥0.244 iu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
<td>Sensitivity (94%)</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td></td>
<td>P value</td>
</tr>
<tr>
<td>GeneXpert (All)</td>
<td>61%</td>
<td>95%</td>
<td>0.002</td>
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N= 184 suspected TBM; NPV= 91% in preparation
Other biomarkers

Multicentre clinical trial (IFN-g) now in progress........
New diagnostics must have incremental yield over clinical findings + basic CSF lab tests including Gram stain and CLAT (high NPV)

Not a ‘one size fits all’

Urine LAM should be performed in hospitalised advanced HIV with or without suspected TBM when sputum tests are unavailable or negative (low cost and simple test)

CSF LAM- need more data; promising option if Xpert unavailable

Unstimulated IFN-g in combination with simple adjunct lab tests is a promising approach that requires further evaluation

Further work on the TB-specific proteome in combination with amplification technologies is required
UKZN: Dr Vinod Patel, Thumbi Ndungu, others

SA MRC: Cathy Connelley
Funding Agencies:

- Discovery
- NIH Fogerty
- South African National Research Foundation
- EDCTP
- South African MRC