T-Cell Marker Based Assays for the Diagnosis of Tuberculosis in Children

Annual meeting of Child and Adolescent TB Working Group

16.10.2020 | Dr. med. Laura Olbrich

Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich
Background

Immunooassays

**Tuberculin Skin Test (TST)**
- Induration after 48-72 h

**Interferon Gamma Release Assay (IGRA)**
- IFNγ production after 16-24 h

**T-Cell Activation Marker TB (TAM TB)**
- Cell marker for LTBI
  - CD27: Maturation (+)
  - CD38: Activation (-)

Graph adapted from N. Ritz
**Background**

**TAM TB Results from Endemic Settings**

<table>
<thead>
<tr>
<th>Paediatric TB suspects, Tanzania, CD27</th>
<th>Culture-confirmed tuberculosis (n=18)</th>
<th>Highly probable tuberculosis (n=8)</th>
<th>Probable tuberculosis (n=12)</th>
<th>Not tuberculosis (n=63)</th>
<th>Indeterminate (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay-positive cases</td>
<td>15 (83%)</td>
<td>3 (38%)</td>
<td>2 (17%)</td>
<td>2 (3%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Assay-negative cases</td>
<td>3 (17%)</td>
<td>5 (63%)</td>
<td>10 (83%)</td>
<td>61 (97%)</td>
<td>11 (92%)</td>
</tr>
</tbody>
</table>

*Table 2: T-cell activation marker-tuberculosis assay results by classification groups*

**IGRA+ Adults from Western Cape, RSA, CD38**

[Graph showing CD38+IFN-γ* and Mb-CW, ESAT6-CFP10](#)

*Courtesy of Dr. Christof Geldmacher Portevin et al 2014 Lancet ID, Adekambi et al 2015 JCI.*
Background

Detection of Culture-Negative TB Cases in Paediatric TB Suspects

Courtesy of Dr. Christof Geldmacher
Portevin et al 2014 Lancet ID
RefuScreen-AIDA-TB
TAM TB Evaluation in Adults and Children

- Study Design: Prospective TB diagnostic multi-site cohort study in Munich, Germany
- Analysis dataset: Patients suspected to have TB n=338 incl. <18yrs n=47
- TAM TB assay: Whole blood assay, CD38 Antigens: ESAT6/CFP10 and PPD

<table>
<thead>
<tr>
<th></th>
<th>Ref test +</th>
<th>Ref test -</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM TB +</td>
<td>63 (80.8%)</td>
<td>1 (1.8%)</td>
</tr>
<tr>
<td>TAM TB -</td>
<td>15 (19.2%)</td>
<td>55 (98.2%)</td>
</tr>
<tr>
<td></td>
<td>78 (100%)</td>
<td>56 (100%)</td>
</tr>
</tbody>
</table>

Sensitivity 80.8% (95% CI 70.3 – 88.8%)
Specificity 98.2% (95% CI 90.4 – 100.0%)
ROC AUC: 0.89 (95% CI: 0.85 – 0.94)

Preliminary Results - Data cleaning still ongoing
Development of TAM TB Commercialized Kit

Blood collected in Heparin anticoagulant

≈ 500µL

Blood activated using the Activation tube

Controls are run for a batch of patient samples not with each sample

Vortex the tube briefly & Add 2.5 mL of Optilyse C

Incubate 16 hrs to 20 hrs @ 37°C ± 2 °C

Spin@600g, 5 mins, Discard Supernatant

Act Blood

Incubate 10 -12 mins

Add 375µL of the permeabilization buffer, mix briefly and transfer to the staining tube

After incubation wash with 1xfix buffer and resuspend the cells finally in 300µL 1xfix buffer.

DXFLEX

Vortex for 10 seconds & Incubate 40-45 mins

Vortex before acquisition

Activation : CD3+ T-Cells

(42.43%) CD4+ T-Cells(57.57%)

CD4 PE-A

Count

10^4

10^5

0

Dr. Laura Olbrich | Division of Infectious Diseases and Tropical Medicine, University Hospital | 16.10.2020
Pilot Study

- Study Design: Prospective evaluation of TAM TB compared to microbiological reference standard (culture, Xpert®)
- Analysis dataset: 90 children recruited at Christian Medical College, Vellore, India
  - Age: 0.5 – 15 yrs
- Diagnostic classifications:
  - Microbiologically confirmed TB: 30 (33%)
  - Unconfirmed/Probable TB: 23 (26%)
  - Unlikely TB: 37 (41%)
- TAM TB assay:
  - Whole blood assay, CD38
  - Antigens: ESAT6/CFP10

<table>
<thead>
<tr>
<th>Ref test +</th>
<th>Ref test -</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAM TB +</td>
<td>24 (80%)</td>
<td>6 (16%)</td>
<td>30</td>
</tr>
<tr>
<td>TAM TB -</td>
<td>6 (20%)</td>
<td>31 (84%)</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>30 (100%)</td>
<td>37 (100%)</td>
<td>67</td>
</tr>
</tbody>
</table>

- Higher Specificity with more stringent unlikely TB classification

Courtesy of Prof. Joy M Michael & Prof. Valsan Verghese
**RaPaed-AIDA-TB**

**Consortium**

**Study Design:**
- Diagnostic validation study
- 8 new diagnostic tests incl. TAM TB
- 1,000 symptomatic children
- 20-25% target confirmation rate

**Consortium & Partners:**
- NIMR – MMRC, Mbeya, Tanzania
- INS, Maputo, Mozambique
- CoM, Blantyre, Malawi
- UCTLI, Cape Town, South Africa
- CMC, Vellore, India
- LMU, Munich, Germany
- FIND, Switzerland
- University of Melbourne, Australia
- Stellenbosch University, South Africa
- Karolinska Institute, Sweden
- Research Center Borstel, Germany
- NTP Tanzania, NTP Mozambique
- MoH, Malawi
- OVG, Oxford University, UK
RaPaed-AIDA-TB

TAM TB Performance

- Analysis dataset:
  - 571 children
  - 4.6 years median age, IQR 1.7 - 8.2
  - 91 HIV pos, 20.0%
  - TAM TB result available for analysis, n=171

- Diagnostic classifications:
  - Microbiologically confirmed TB, 129 (26.0%)
  - Unconfirmed TB, 202 (40.7%)
  - Unlikely TB, 165 (33.3%)

  Defined by investigators and NO TB Rx – Endpoint review pending

- TAM TB assay:
  - Whole blood assay, CD38
  - Antigens: ESAT6/CFP10

Preliminary Results - Data entry & Data cleaning still ongoing
# Results

## TAM TB Performance

<table>
<thead>
<tr>
<th>Stage</th>
<th>Sensitivity – all pos</th>
<th>Sensitivity – without single Xpert trace cases</th>
<th>Specificity</th>
<th>ROC area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>56.5% (41.1% - 71.1%)</td>
<td>75.8% (57.7% - 88.9%)</td>
<td>91.7% (77.5% - 98.2%)</td>
<td>0.74 (0.66 - 0.83)</td>
</tr>
<tr>
<td>0 - 1 yr</td>
<td>66.7% (34.9% - 90.1%)</td>
<td>88.9% (51.8% - 99.7%)</td>
<td>100.0% (29.2% - 100.0%)</td>
<td>0.82 (0.67 - 0.97)</td>
</tr>
<tr>
<td>1 - 5 yr</td>
<td>50.0% (24.7% - 75.3%)</td>
<td>77.8% (40.0% - 97.2%)</td>
<td>88.9% (65.3% - 98.6%)</td>
<td>0.71 (0.57 - 0.85)</td>
</tr>
<tr>
<td>5 - 10 yr</td>
<td>60.0% (26.2% - 87.8%)</td>
<td>66.7% (29.9% - 92.5%)</td>
<td>90.9% (58.7% - 99.8%)</td>
<td>0.75 (0.57 - 0.94)</td>
</tr>
<tr>
<td>10 - 14 yr</td>
<td>50.0% (15.7% - 84.3%)</td>
<td>66.7% (22.3% - 95.7%)</td>
<td>100.0% (39.8% - 100.0%)</td>
<td>0.75 (0.56 - 0.94)</td>
</tr>
</tbody>
</table>

*Preliminary Results - Data entry & Data cleaning still ongoing*
TAM TB for the Diagnosis of Paediatric TB

Conclusion

- TAM TB shows promising performance in a variety of settings, in both children and adults
- Simplified and standardized assay kit developed
- RaPaed-AIDA-TB promising test performance, particularly for infants
- Requires laboratory infrastructure, incl. incubation and flow cytometry
- Ongoing evaluation
  - RaPaed-AIDA-TB, Endpoint Review is being conducted
  - ERASE TB (incipient TB, household contacts, initiation of recruitment Q1 2021)

More RaPaed-AIDA-TB at the Union:

- „Performance of new screening and diagnostic tests in potential pediatric tuberculosis diagnostic algorithms: interim results from the RaPaed study“
- Friday, 23rd Oct, 13:05-13:15h (CET)
  Symposium „Towards a TB-free childhood: best practices to find, cure and prevent TB in children in Africa“
Acknowledgements

LMU: C. Geldmacher, M. Ibraheem, K. Held, C. Dalgarno, E. Saathoff, S. Mutuku, F. Rieß, M. Hoelscher, N. Heinrich


Funders: German Center for Infection Research (DZIF); European and Developing Countries Clinical Trials Partnership (EDCTP; RIA2016MC -1623)

Beckman Coulter Inc.: M. Adelmann, N. Girish, S. Chawla, S. Pattabhiraman, A. Jarare