Tuberculosis biomarker discovery and translation into point-of-care tests

Novel N Chegou, PhD
Division of Molecular Biology & Human Genetics
Novel@sun.ac.za
Outline

• Introduction
• Summary of my research journey
  • Reasoning behind the different projects
  • Where we are currently in some of the projects
• Important things that have contributed to our journey so far
Who am I?

- Born in Cameroon
- Bachelor in Medical Laboratory Science (4-year)
- 2005: BSc Honours at SU
- 2007: MSc upgraded to PhD
- 2009: PhD at SU
- 2010: Postdoctoral fellow
- 2014: Senior Researcher
- 2019: Associate Professor
Not as straight-forward as it seems

Born in Cameroon

- Lost both parents before completing secondary school

Bachelor in Medical Laboratory Science (4-year)

- 2 years helping in family small businesses
- Hospital laboratories (part-time)

2004: Arrival in SA

- Wait for one year before beginning studies
- Selling in a street market

2005: BSc Honours at SU

- Selling at the street market
- ELISAs in the weekends

2007: MSc upgraded to PhD

2009: PhD at SU
Research journey

Interferon Gamma Release Assays (IGRAs)

- More operational advantages over the skin test (TST)
  - No-inter-observer variability (esp QFTs)
  - Single visit
  - No reactivity to BCG
  - Results within 24hrs
  - No boosting on serial testing**

*Pai et al, Lancet Inf Dis 2004*
Research journey

• 2005-2007
  • The use of Interferon-gamma release assays (IGRAs; then new blood tests) in the diagnosis of MTB infection & disease
    • Can IGRAs assist in the diagnosis of pleural TB?
      • Standard IGRA (Quantiferon Gold)
    • What if we use pleural fluid or cells instead of blood in the tests?
  • Can IGRAs assist in the diagnosis of MTB infection in adults & children in our high burden settings?
    • How do these tests compare with each other (Quantiferon & T SPOT.TB) and with the skin test in HIV + and HIV - individuals

High level of discordant IGRA results in HIV-infected adults and children
A. M. Mandalakas, A. C. Hesseling, N. N. Chegou, H. L. Kirchner, X. Zhu, B. J. Marais, G. F. Black,
N. Beyers, G. Walzl
INT J TUBERC LUNG DIS 12(4):417-423

Highly discordant T cell responses in individuals with recent exposure to household tuberculosis
A. C. Hesseling, A. M. Mandalakas, H. L. Kirchner, N. N. Chegou, B. J. Marais, K. Stanley, X. Zhu, G. Black, N. Beyers, G. Walzl

Evaluation of Adapted Whole-Blood Interferon-γ Release Assays for the Diagnosis of Pleural Tuberculosis
Novel N. Chegou, Gerhard Walzl, Chris T. Bolliger, Andreas H. Diacon,
Michel M. van den Heuvel
Respiration 2008;76:131–138
What the field was working on at the same time

- Utility of IGRAs in the diagnosis of MTB infection
- Sensitivity of IGRAs, MTB infection, active TB, etc
  - Adults
  - Children
  - HIV infected individuals
  - Evaluation of different cut-offs for the assays
  - Agreement between the TST and IGRAs
  - Systematic Reviews & Meta-analyses on the use of IGRAs and TST

- Common statement in most of the then publications:

  “IGRAs are useful and have many advantages over the TST. However, they can not discriminate between latent TB infection and active TB disease…”
Our thought process: Can we develop alternative IGRA-like assays for active TB?

- For T-cell-based (IGRA-like) active-TB diagnostic tests to be developed, **new host markers** – *other than IFN-γ* and/or **New antigens -other than those used in IGRA*s (ESAT-6/CFP-10/TB7,7), need to be identified.

- What would we need in order to develop such an assay?
  - Looked at our environment: ongoing studies and equipment

  - **Luminex XMAP technology:**
    - Biomarker discovery platform, could evaluate 100 different biomarkers *other than IFN-γ* in little amounts of patient specimens
    - Antigens that were being investigated as vaccine candidates in a Gates-funded study
      - Access to collaborators who could provide **new antigens -other than those used in IGRA*s
    - Can we build on the existing, well validated IGRA platform?
Can Host Markers Other than IFN-γ or New Ags Other than ESAT6/CFP10 Differentiate Between Pulmonary TB and LTBI?

<table>
<thead>
<tr>
<th>Pulmonary TB patients (n=23)</th>
<th>HHC (n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard QFT test</td>
<td>TST pos: 82% (28) Not Read: 6% (2)</td>
</tr>
</tbody>
</table>

29-plex Luminex kit
- 10 QFT pos TB cases
- 9 QFT pos HHCs

Customized 8-plex kit
Remainder of participants

Assess diagnostic accuracy
- individual markers
- multi-marker models

Evaluation of 118MTB antigens
(23 TB, 20 HHC per Ag)

7-day WBA

EGF, MIP-1β, IL-1α, TGFα, VEGF, sCD40L, TNFα, IFNγ

IFN γ ELISA

Assess diagnostic accuracy
- individual Ags
- multi-Ag models

Chegou et al, BMC Pulmonary Medicine, 2009
Chegou et al, BMC Infectious Diseases, 2012
Validation studies in other African countries: The African-European Tuberculosis Consortium

Recruited/followed up 1384 “TB Suspects” (356 HIV+, 1028 HIV-), 7 African field sites: Collection of various sample types (serum, plasma, PBMCs, saliva, urine, paxgene tubes etc) for biomarker studies
The “test” system that we used then

1) Collect blood
2) Dilute with culture medium
3) & 4) Mix with antigens in plates
5) Prepare for incubation
6) Incubate for overnight or for 7 days, then
   Harvest culture supernatants
7) Test for biomarkers in culture supernatants

Whole blood assay pictures taken by NN Chegou in 2007
## The African European Tuberculosis Consortium (AE-TBC)- Some Highlights

### Africa-wide evaluation of host biomarkers in QuantiFERON supernatants for the diagnosis of pulmonary tuberculosis


### Evaluation of cytokine responses against novel Mtb antigens as diagnostic markers for TB disease


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**Fig. 4:** Assessment of IP-10 and CCL-2 CUG-LF assay at seven African institutes.
Pilot Study: -Serum biomarkers, No Antigen stimulation, no overnight or longer term culture

SUN & AHRI- Ethiopia

- 148 individuals with signs and symptoms suggestive of TB
- 19 markers evaluated
- Five marker biosignatures showed promise
- Top 5-marker model: IFN-γ, TNF-α, transthyretin, complement C3 and MMP-2
- Training set:
  - Sensitivity = 86%
  - Specificity = 91%
- Test set:
  - Sensitivity = 86%
  - Specificity = 90%

- ROC curves showing the accuracies of individual host serum markers
- Frequency of analytes in the top 20 diagnostic models
- Regardless of HIV infection status
AE-TBC Serum Biomarker Validation Study (Gambia, Uganda, Malawi, Namibia, SA)

Study design

Eligible patients (n=716)
- Completion of CRF
- Collection of samples

Clinical and laboratory assessment/Reference standard (n=716)

Excluded patients (n=9)
- Pregnant (n=1)
- Data capture issues (n=8)

Host markers evaluated (n=707)

Definite TB (n=185)

Probable TB (n=29)

No-PTB (n=487)

Questionable TB (n=6)

TB (n=214)

No-PTB (n=487)

Excluded from final analysis (n=6)

Data Analysis
- ROC curve analysis
- Random allocation into a training set (70%) and test set (30%)

Training set (n=491; 168 TB, 323 No-PTB)

Test set (n=210; 77 TB, 133 No-PTB)

- Identification of a 7-marker serum protein biosignature for active TB disease
- No antigen stimulation of cells required
- Serum samples

Identification of a 7-marker serum protein biosignature for active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB

Chegou et al, Thorax 2016;71:785-794
### Accuracy of the Seven Marker Serum Protein Biosignature (ApoA-I, CFH, CRP, IFN-γ, IP-10, SAA, Transthyretin) in the Diagnosis of TB Disease

#### Training set (n=491)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>%, (n/N)</td>
<td>86.7 (130/150)</td>
<td>85.3 (291/341)</td>
<td>72.2</td>
<td>93.6</td>
</tr>
<tr>
<td>95% CI</td>
<td>(79.9-91.5)</td>
<td>(81.0-88.8)</td>
<td>(65.0-78.5)</td>
<td>(90.1-95.9)</td>
</tr>
</tbody>
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#### Test set (n=210)

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<tr>
<td>%, (n/N)</td>
<td>81.3 (52/64)</td>
<td>79.5 (116/146)</td>
<td>63.4</td>
<td>90.6</td>
</tr>
<tr>
<td>95% CI</td>
<td>(69.2-89.5)</td>
<td>(71.8-85.5)</td>
<td>(52.0-73.6)</td>
<td>(83.9-94.8)</td>
</tr>
</tbody>
</table>

**Accuracy of the biosignature after selection of cut-off values optimized for sensitivity**

#### Training set (n=491)

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<th>NPV</th>
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</thead>
<tbody>
<tr>
<td>%, (n/N)</td>
<td>90.7 (136/150)</td>
<td>74.8 (255/341)</td>
<td>61.3</td>
<td>94.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>(84.5-94.6)</td>
<td>(69.8-79.2)</td>
<td>(54.5-67.6)</td>
<td>(91.2-97.0)</td>
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#### Test set (n=210)

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</thead>
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<tr>
<td>%, (n/N)</td>
<td>93.8 (60/64)</td>
<td>73.3 (107/146)</td>
<td>60.6</td>
<td>96.4</td>
</tr>
<tr>
<td>95% CI</td>
<td>(84.0-98.0)</td>
<td>(65.2-80.1)</td>
<td>(50.3-70.1)</td>
<td>(90.5-98.8)</td>
</tr>
</tbody>
</table>

Chegou et al, Thorax 2016;71:785-794
The ScreenTB Project

-Funder: EDCTP2;  -PI: Gerhard Walzl; -Duration: 04/2016 - 06/2019

-Trial Sites:

- SU –IRG (South Africa)
- MRC (The Gambia)
- UNAM (Namibia)
- Makerere (Uganda)
- AHRI (Ethiopia)
- LUMC – Netherlands
- LSHTM – UK
- LinQ Management-Germany
- Eurice - Germany

- Develop a point-of-care test that can measure seven protein biomarkers simultaneously in serum samples

- Adapt the test to make use of finger-prick blood
Multi-Biomarker Finger-prick test for TB

1. Fingerprick (quantitative)
2. Dilute sample in assay buffer
3. Lateral flow
4. Scan and analyze

ScreenTB
Some of the things that have helped in getting us this far

- Know your research topic, read original research papers, systematic reviews, meta analyses, identify the gaps in the field
- What are the known key/difficult problems in your research area that have been difficult to crack?
- Do not only work on projects that are based on findings in other settings “currently no data in our setting/country”
- Do not be ‘married’ to one idea! Your initial idea does not always have to work and you should be able to walk away from it and work on other things
- Objectivity; “We are scientists, not salesmen” (G. Walzl, 2005). Do not waste your time trying to get it done when overwhelming evidence suggests that it will not work
- An appropriate environment for your research:
  - The study team
  - Resources
  - Collaborative partners
  - Where your freedom is valued
- Good mentor(s)
- Have your own personal development plan
Acknowledgements

• Prof Gerhard Walzl (SU, Dept. of Biomedical Sciences)
  • PI: AE-TBC & ScreenTB

• Stellenbosch University Immunology Research Group
  • Clinical team
  • Research assistants
  • Students (ChegouLab)

• The AE-TBC Consortium

• The ScreenTB Consortium