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

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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

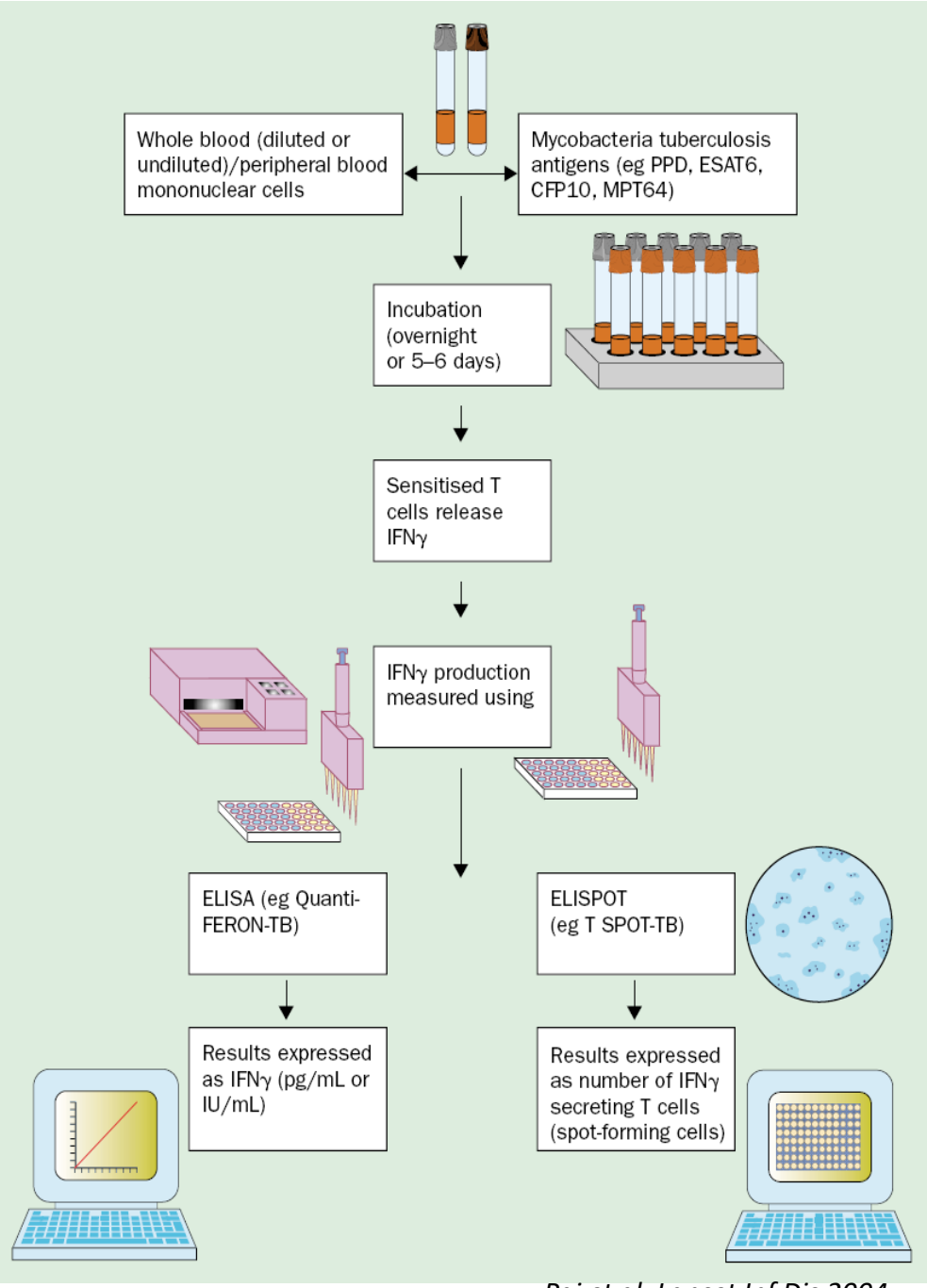
2007: MSc upgraded to PhD

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,[1] A M Mandalakas,[2] H L Kirchner,[3] N N Chegou,[4] B J Marais,[1] K Stanley,[4] X Zhu,[2] G Black,[4] N Beyers,[1] G Walzl[4]

Thorax 2009;**64**:840–846. doi:10.1136/thx.2007.085340

Evaluation of Adapted Whole-Blood Interferon-γ Release Assays for the Diagnosis of Pleural Tuberculosis

Novel N. Chegou[a] Gerhard Walzl[a] Chris T. Bolliger[b] Andreas H. Diacon[b] Michel M. van den Heuvel[b]

Respiration 2008;76:131–138

What the field was working on at the same time

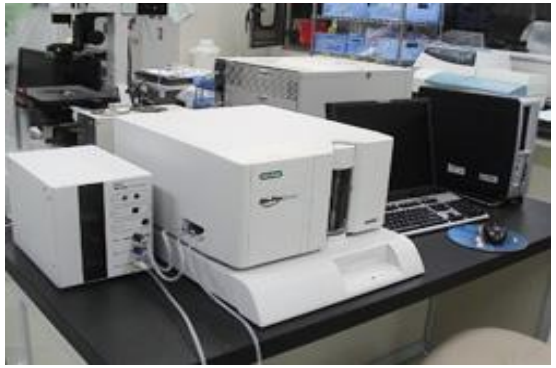
- Utility of IGRAs in the diagnosis of MTB infection
- Sensitivity of IGRAs, MTB infection, active TB, etc
  - Adults
  - Children
  - HIV infected individuals
  - Evaluation of different cut-offs for the assays
  - Agreement between the TST and IGRAs
  - Systematic Reviews & Meta-analyses on the use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

Pulmonary TB patients (n=23)
Standard QFT test

HHC (n=34)
TST pos: 82% (28)
Not Read: 6% (2)

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

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

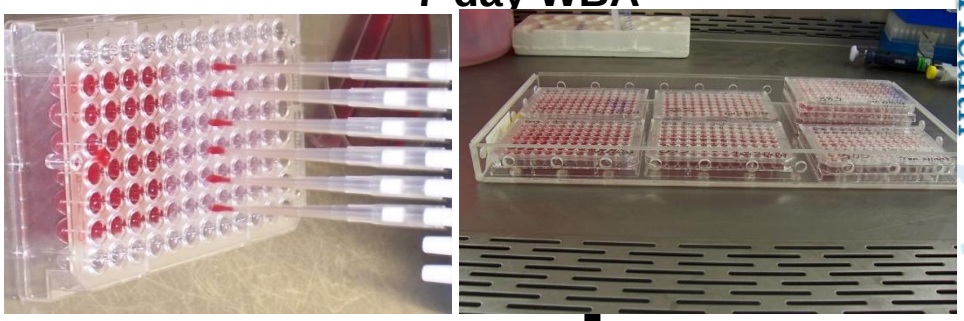
Customized 8-plex kit
Remainder of participants

Assess diagnostic accuracy
-individual markers
-multi-marker models

Chegou et al, BMC Pulmonary Medicine, 2009

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

7-day WBA



IFN γ ELISA

Assess diagnostic accuracy
-individual Ags
-multi-Ag models

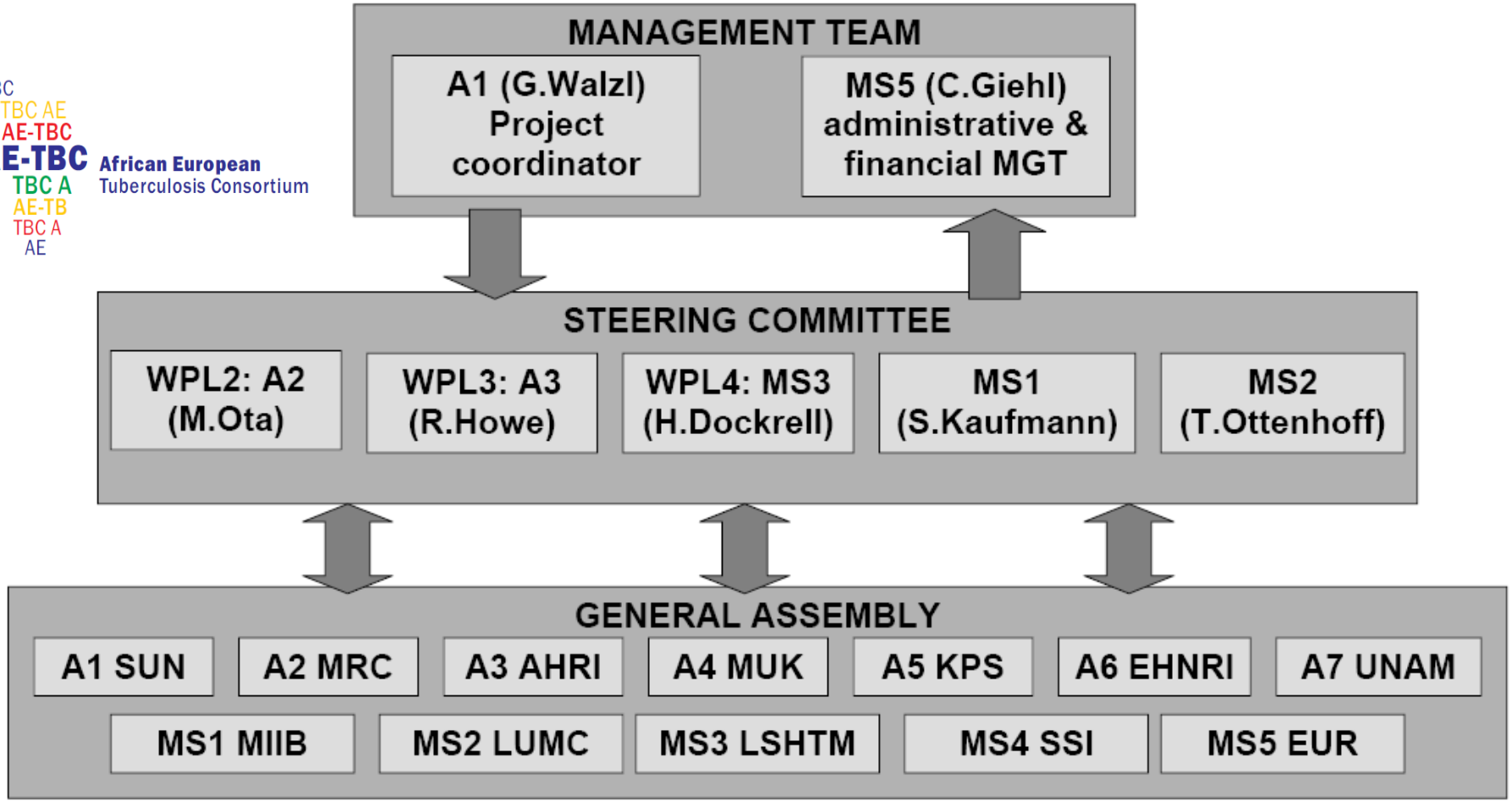
Chegou et al, BMC Infectious Diseases, 2012

Grand Challenges in Global Health #6-74

BIOMARKERS for TB

Validation studies in other African countries: The African-European Tuberculosis Consortium

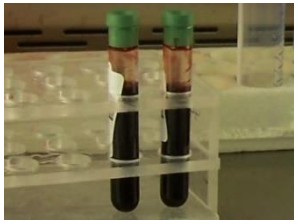
TBC
AE-TBC AE
TBC AE-TBC
AE-TBC African European
TBC A Tuberculosis Consortium
AE-TB
TBC A
AE



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

E D C T P

The "test" system that we used then

1) 

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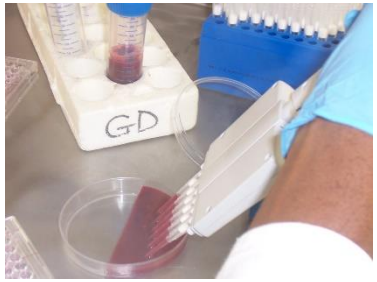
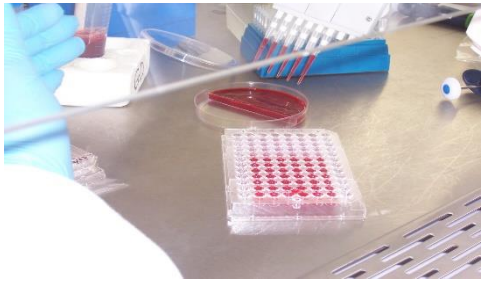
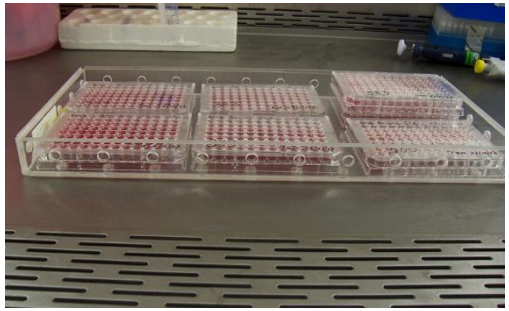
3) 

4) 

5) 

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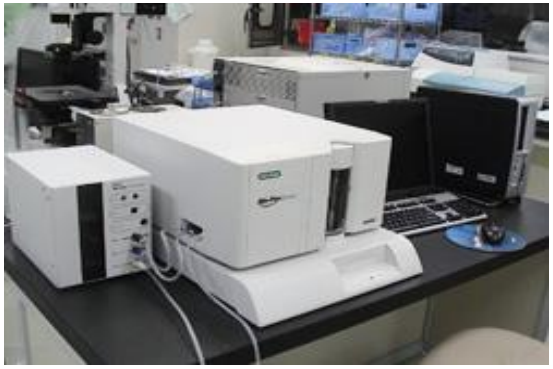
7) 

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

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

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

Received: 9 May 2017
Accepted: 25 January 2018
Published online: 08 February 2018

Novel N. Chegou[1], Jayne S. Sutherland[2], Anna-Ritah Namuganga[3], Paul LAM
Annemieke Geluk[5], Gebremedhin Gebremichael[6], Joseph Mendy[2], Stephanus Mall
Kim Stanley[1], Gian D. van der Spuy[1], Magdalena Kriel[1], Andre G. Loxton[1], Belinda
Felanji Simukonda[7,14], Yonas Bekele[8], Jacob A. Sheehama[9], Josefina Nelongo[9], Ma
van der Vyver[9], Atsbeha Gebrexabher[6], Habteyes Hailu[6], Maria M. Esterhuyse[10], Ic
Rosenkrands[11], Claus Aagard[11], Martin Kidd[12], Desta Kassa[5], Adane Mihret[8], Ra
& AE-

Journal of Infection (2016) 73, 219–230

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British Infection Association

www.elsevierhealth.c

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

Dolapo O. Awoniyi [a], Andrea Teuchert [a], Jayne S. Sutherland [b], Harriet Mayanja-Kizza [c], Rawleigh Howe [d], Adane Mihret [d], Andre G. Loxton [a], Jacob Sheehama [e], Desta Kassa [f], Amelia C. Crampin [g,h], Hazel M. Dockrell [h], Martin Kidd [i], Ida Rosenkrands [j], Annemieke Geluk [k], Tom H.M. Ottenhoff [k], P.L.A.M. Corstjens [l], Novel N. Chegou [a], Gerhard Walzl [a,*], the AE-TBC Consortium

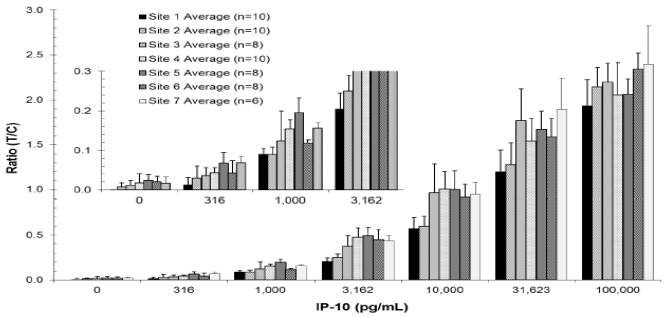


Fig. 4: Assessment of IP-10 and CCL4 UCP-LF assay at seven African institutes

Corstjens PL et al, Clinical Biochemistry 49 (2016) 22–31

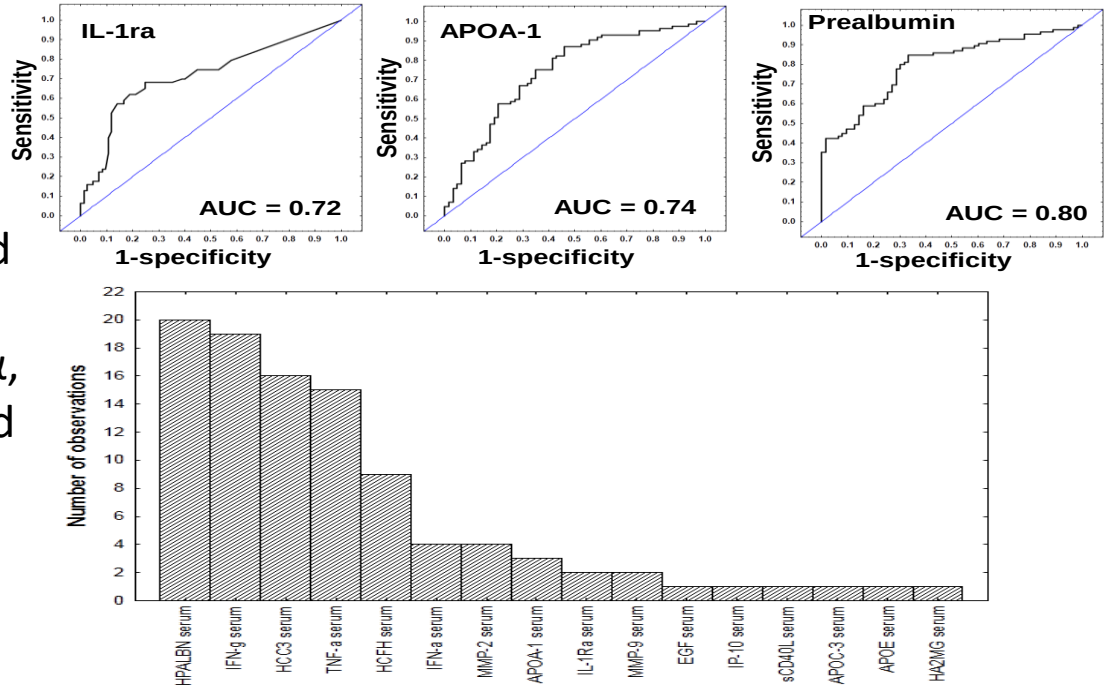
EDCTP

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

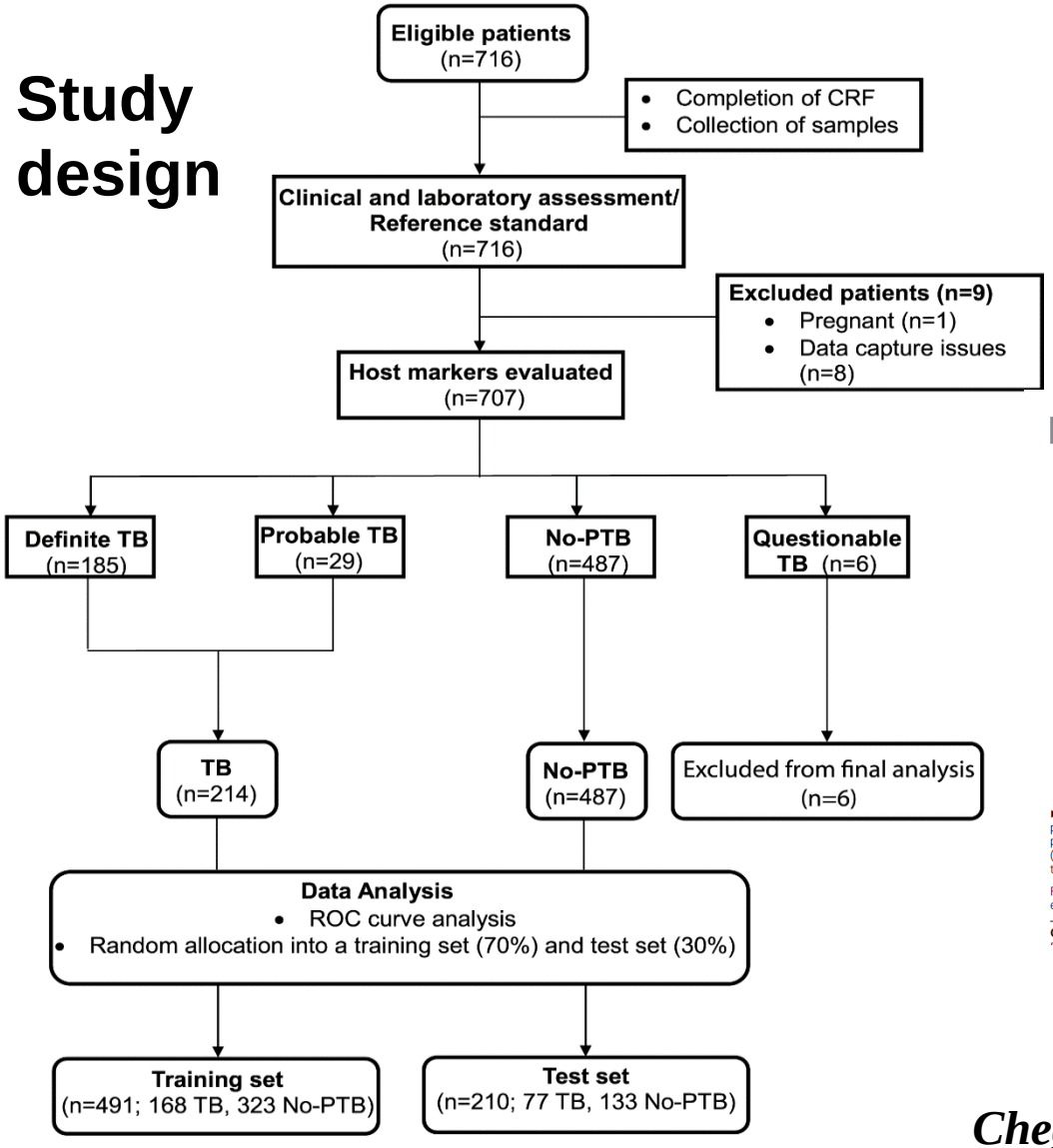
- 19 markers evaluated
- Five marker biosignatures showed promise
- Top 5-marker model: IFN-γ, TNF-α, transthyretin, complement C3 and MMP-2
- Training set:
  - Sensitivity = 86%
  - Specificity = 91%
- Test set:
  - Sensitivity = 86%
  - Specificity = 90%



-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



-Identification of a 7-marker serum protein biosignature for active TB disease

-No antigen stimulation of cells required

-Serum samples

Thorax Online First, published on May 4, 2016 as 10.1136/thoraxjnl-2015-207999

ORIGINAL ARTICLE

Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB

Novel N Chegou,[1] Jayne S Sutherland,[2] Stephanus Malherbe,[1] Amelia C Crampin,[3] Paul L A M Corstjens,[4] Annemieke Geluk,[5] Harriet Mayanja-Kizza,[6] Andre G Loxton,[1] Gian van der Spuy,[1] Kim Stanley,[1] Leigh A Kotzé,[1] Marieta van der Vyver,[7] Ida Rosenkrands,[8] Martin Kidd,[9] Paul D van Helden,[1] Hazel M Dockrell,[10] Tom H M Ottenhoff,[5] Stefan H E Kaufmann,[11] Gerhard Walzl,[1] on behalf of the AE-TBC consortium

▸ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2015-207999).

For numbered affiliations see end of article.

Correspondence to

ABSTRACT

Background User-friendly, rapid, inexpensive yet accurate TB diagnostic tools are urgently needed at points of care in resource-limited settings. We investigated host biomarkers detected in serum samples obtained from adults with signs and symptoms suggestive of TB at primary healthcare clinics in five African countries (Malawi, Namibia, South Africa, The

Key messages

What is the key question?

▸ Are there serum host marker signatures, which are suitable for point-of-care tests that differentiate between active pulmonary TB and

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

Accuracy of the Seven-Marker Serum Protein Biosignature (ApoA-1, CFH, CRP, IFN-γ, IP-10, SAA, Transthyretin) in the Diagnosis of TB Disease

Training set (n=491)				
	Sensitivity	Specificity	PPV	NPV
%, (n/N)	86.7 (130/150)	85.3 (291/341)	72.2	93.6
95% CI	(79.9-91.5)	(81.0-88.8)	(65.0-78.5)	(90.1-95.9)
Test set (n=210)				
%, (n/N)	81.3(52/64)	79.5(116/146)	63.4	90.6
95% CI	(69.2-89.5)	(71.8-85.5)	(52.0-73.6)	(83.9-94.8)

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

Training set (n=491)				
	Sensitivity	Specificity	PPV	NPV
%, (n/N)	90.7 (136/150)	74.8 (255/341)	61.3	94.8
95% CI	(84.5-94.6)	(69.8-79.2)	(54.5-67.6)	(91.2-97.0)
Test set (n=210)				
%, (n/N)	93.8 (60/64)	73.3 (107/146)	60.6	96.4
95% CI	(84.0-98.0)	(65.2-80.1)	(50.3-70.1)	(90.5-98.8)

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