

# Host Markers in Quantiferon Supernatants Differentiate Active TB from Latent TB Infection

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**TITLE:**

**Host markers in Quantiferon supernatants differentiate active TB from latent TB infection: preliminary report**

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# Background

- IGRAs do not differentiate between active TB and LTBI
  - Limited value in high burden TB settings
  
- To develop T-cell based assays for active TB
  - Alternative antigens other than ESAT-6/CFP-10/TB7.7
  - Alternative markers other than IFN- $\gamma$
  
- Development of the Luminex xMAP technology has made it possible to measure multiple biomarkers in little amounts of sample
  - Up to 100 analytes in 25 – 50 $\mu$ l

# *Hypothesis*

Stimulation of whole blood with *M. tb* specific antigens will result in the production of multiple biomarkers, some of which will be unique to either LTBI or active TB disease.

# Methods



Recruitment of participants and QFT testing

**Pulmonary TB patients**

(n=23)  
(TST not done)

**HHC**

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

Random selection into pilot **LUMINEX STUDY** based on QFT results

29-plex Luminex assays on selected supernatants to identify promising markers

**10 QFT pos TB patients**

**9 QFT pos HHC**

EGF    TNF- $\alpha$   
VEGF   IL-1 $\alpha$   
TGF- $\alpha$    MIP-1 $\beta$   
sCD40L   IFN- $\gamma$

Validation of promising markers by customized 8-plex Luminex kits

**Remaining pulmonary TB patients** (n=13)

**Remaining HHC** (n=25)

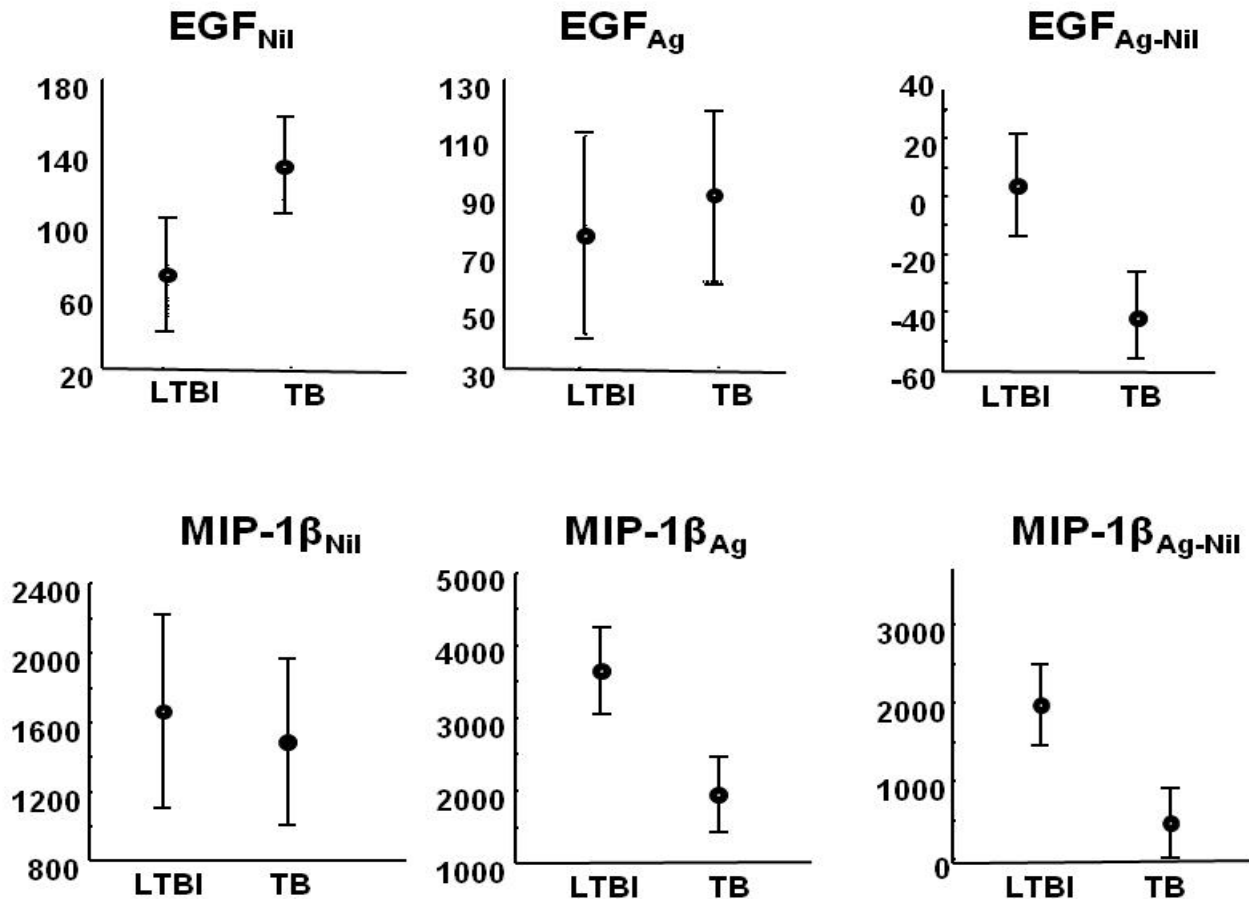
Data analysis

- QFT pos: active TB vs LTBI
- QFT pos and neg: TB vs Non TB

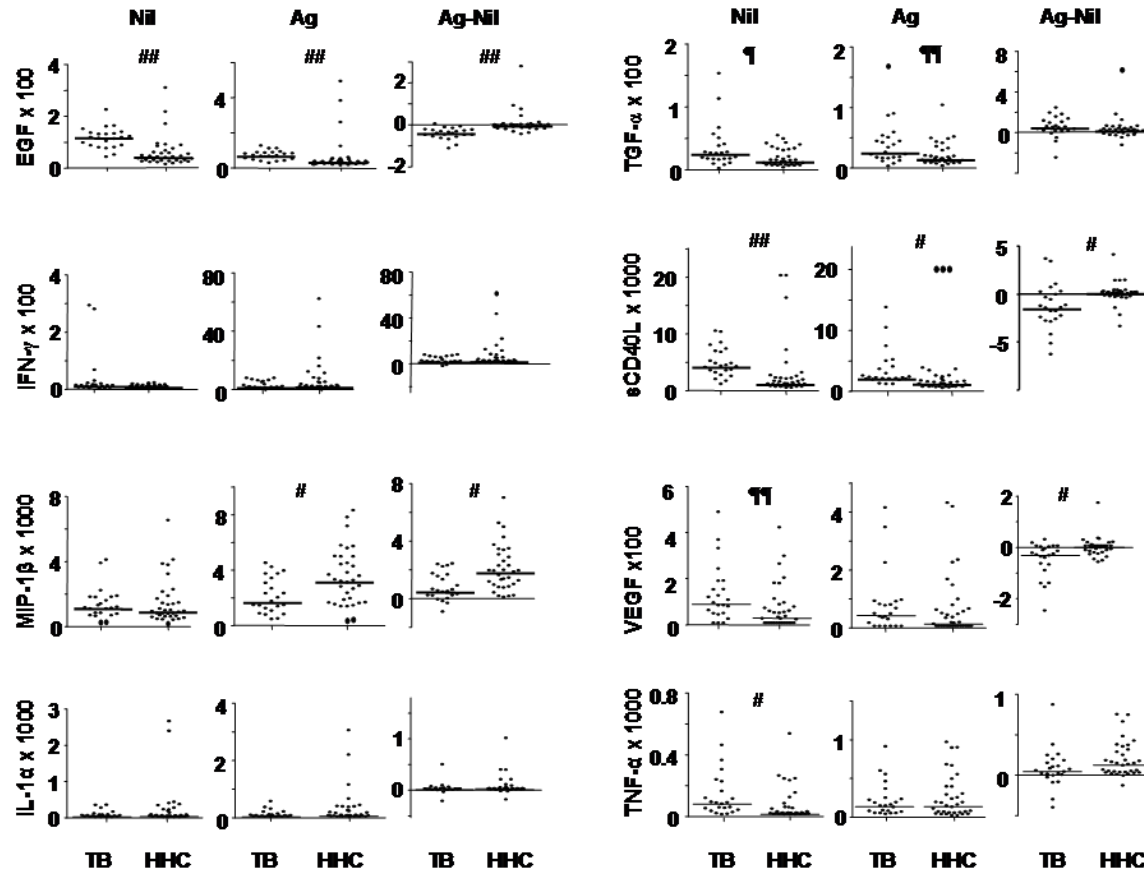
# Characteristics of study participants

	All	Pulmonary TB	Household contacts
<b>Number of Participants</b>	57	23	34
<b>Age, mean yr <math>\pm</math> SD</b>	31.2 $\pm$ 13.9	30.3 $\pm$ 13.6	31.8 $\pm$ 14.2
<b>Age range, yr</b>	10.1- 59.9	10.1 – 57.4	10.7 – 59.9
<b>Male/female ratio</b>	31/26	17/6	14/20
<b>TST mean, mm</b>	na	nd	22.8
<b>TST range, mm</b>	na	na	0.0-46.0
<b>TST pos, %</b>	na	na	87.5
<b>QFT pos, %</b>	82.5	95.6	73.5

# Levels of individual analytes in TB and LTBI



# Levels of individual analytes in all TB cases and contacts



##: p<0.0001, #: p<0.01, ¶¶: p=0.01, ¶: p=0.02

# Abilities of individual analytes to differentiate between TB disease & LTBI in QFT positive supernatants



Marker	Level in Pulmonary TB (n = 22)	Level in LTBI (n = 25)	Cut-off (pg/ml)	AUC	Sensitivity %	Specificity %
EGF <sub>Nil</sub>	115.5 (39.1-222.2)	34.5 (15.2-214.0)	76.16	<b>0.88</b>	90.9	84.0
EGF <sub>Ag</sub>	65.7 (23.4-121.8)	27.2 (12.2-487.9)	46.68	<b>0.87</b>	81.8	88.0
EGF <sub>Ag-Nil</sub>	-44.9 (-116.1-0.44)	-7.0 (-48.9-274.0)	-25.6	<b>0.90</b>	92.0	81.8
TGF- $\alpha$ <sub>Ag</sub>	22.2 (1.0-168.0)	13.4 (4.3-47.1)	13.78	<b>0.70</b>	81.8	60.0
sCD40L <sub>Nil</sub>	3995.0 (876.1-10245.0)	1002.0 (220.4->20000)	2307.54	<b>0.84</b>	86.4	80.0
sCD40L <sub>Ag</sub>	1974.0 (883.0-13493.0)	1040.0 (383.2->20000)	1563.66	<b>0.75</b>	86.4	72.0
sCD40L <sub>Ag-Nil</sub>	-1502.0 (-5339.0-3496.0)	10.6 (-3527.0-3927.0)	-471.06	<b>0.73</b>	88.0	68.2
MIP-1 $\beta$ <sub>Ag-Nil</sub>	458.5 (-1035-2321.0)	1833.0 (290.0-6890.0)	836.18	<b>0.79</b>	84.0	63.6
VEGF <sub>Nil</sub>	83.4 (0.0-482.3)	19.5 (0.0-415.8)	72.78	<b>0.73</b>	63.6	84.0

*Only analytes with sensitivity and/or specificity  $\geq 80\%$  are shown*

# Abilities of individual analytes to differentiate between TB cases & contacts regardless of QFT results



Marker	Level in Pulmonary TB (n = 23), pg/ml	Level in HHCs (n = 34), pg/ml	Cut-off pg/ml	AUC	Sensitivity %	Specificity %
EGF <sub>Nil</sub>	115.1 (39.1-222.2)	40.0 (11.7-306.8)	73.14	0.86	91.3	79.4
EGF <sub>Ag-Nil</sub>	-44.6 (-116.1-0.4)	-6.9 (-48.9-274.0)	-25.6	0.90	91.2	82.6
TGF- $\alpha$ <sub>Ag</sub>	23.8 (1.0-168.0)	13.0 (1.7-102.3)	13.78	0.70	82.6	58.8
sCD40L <sub>Nil</sub>	4040.0 (876.1-10245.0)	1047.0 (220.4->20000)	2307.54	0.83	86.9	79.4
sCD40L <sub>Ag</sub>	1950.0 (883.0-13493.0)	1087.0 (291.0->20000)	1563.66	0.75	87.0	70.6
sCD40L <sub>Ag-Nil</sub>	-1579.0 (-6452.0-3496.0)	-24.5 (-3527.0-3927.0)	-526.06	0.73	88.2	69.6
MIP-1 $\beta$ <sub>Ag-Nil</sub>	419.2 (-1035.0-2321.0)	1773.0 (-25.5-6890.0)	634.4	0.75	85.3	60.9

*Only analytes with sensitivity and/or specificity  $\geq 80\%$  are shown*

# ***Can the predictive ability of analytes be improved if used in combinations ?***

- Two different mathematical approaches, General Discriminant Analysis (GDA) and Support Vector Machines (SVM), were used to evaluate the abilities of combinations of analytes to differentiate between different *M. tb* infection states
  - Optimal prediction of TB infection states was achieved if analytes were used in combinations of three
  - the two approaches gave similar results

# ***Top three-analyte combinations differentiating between active TB and LTBI in QFT positives***

## **General Discriminant Analysis and Support Vector Machines:**

**$EGF_{Nil} / EGF_{Ag-Nil} + MIP-1\beta_{Ag-Nil} + IL-1\alpha_{Nil}$  or  $IL-1\alpha_{Ag}$**

## **Resubstitution classification matrix**

Up to 96% accurate prediction of active TB and 90% of LTBI

## **Leave-one-out cross validation**

-88% accuracy in either case

**Leave-one-out cross validation Misclassification in SVM: 10%**

*Chegou et al, 2009; BMC Pulmonary Medicine (Accepted)*

# ***Top three-analyte combinations differentiating between TB cases and non cases irrespective of QFT results***

## **General Discriminant Analysis and Support Vector Machines:**

Any one or 2 EGF conditions (**EGF<sub>Nil</sub>**, **EGF<sub>Ag</sub>**, **EGF<sub>Ag-Ni</sub>**)  
Plus 2 or one of **IL-1 $\alpha$ <sub>Nil</sub>**, **IL-1 $\alpha$ <sub>Ag</sub>**, and **MIP-1 $\beta$ <sub>Ag-Nil</sub>**

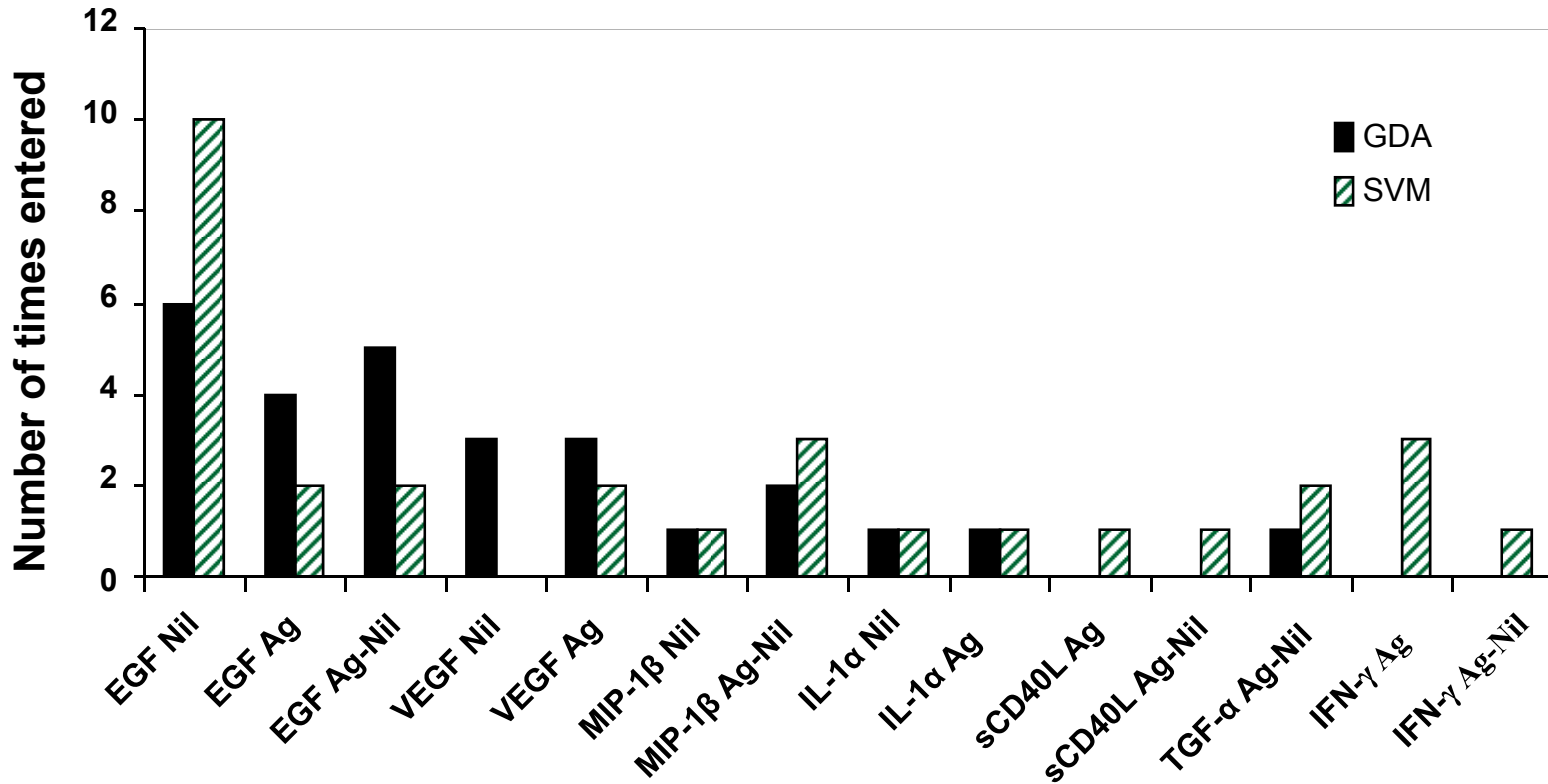
Accuracy for TB: **87.0% - 96.0%** After leave-one-cross validation:**82.6% - 87.0%**  
non TB cases:**85.3% - 94.1%** **:85.3% - 91.2%**

**Average leave-one-out cross validation  
misclassification rate in SVM**

**-: 10%**

*Chegou et al, 2009; BMC Pulmonary Medicine (Accepted)*

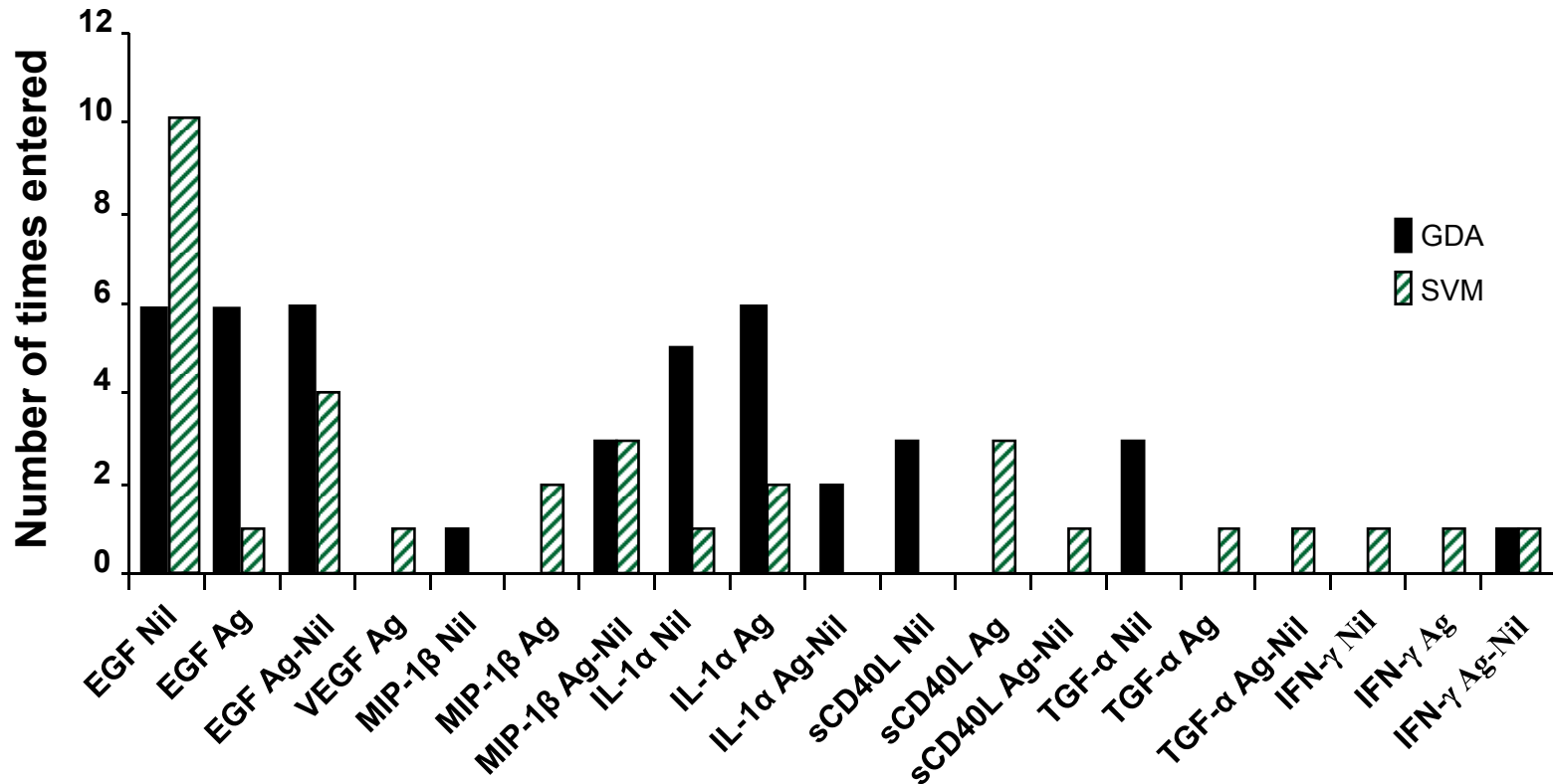
# Inclusions of individual analytes into the most accurate GDA & SVM 3-analyte models differentiating between TB disease & LTBI in QFT positive supernatants



GDA: General Discriminant Analysis, SVM: Support Vector Machines

*Chegou et al, 2009; BMC Pulmonary Medicine (Accepted)*

# Inclusions of individual analytes into the most accurate GDA & SVM 3-analyte models differentiating between TB patients & HHCs irrespective of QFT results



GDA: General Discriminant Analysis, SVM: Support Vector Machines

*Chegou et al, 2009; BMC Pulmonary Medicine (Accepted)*

# Main findings and conclusion

- **Multiple biomarkers measured in QFT supernatants show potential to discriminate accurately between active TB and the absence of active disease**
- Combinations of analytes are more promising than individual analytes
- The top individual analytes- **EGF<sub>Nil</sub>, EGF<sub>Ag</sub>, EGF<sub>Ag-Nil</sub>, sCD40L<sub>Nil</sub>, MIP-1 $\beta$ <sub>Ag-Nil</sub>**
- Most promising three-analyte model: **EGF<sub>Nil/Ag-Nil</sub> and MIP-1 $\beta$ <sub>Ag-Nil</sub>**
- Propose a 2-step test system
- IFN- $\gamma$  or IP-10 detection to diagnose *M.tb* infection
- Measure levels of EGF or MIP-1 $\beta$  etc to differentiate positive IFN- $\gamma$  results as active TB or LTBI

*Active TB may be accurately differentiated from LTBI utilizing adaptations of the commercial QFT test that includes measurement of EGF, sCD40L, MIP-1 $\beta$ , VEGF, TGF- $\alpha$  or IL-1 $\alpha$  in supernatants from QFT assays.*

*This approach holds promise for development as a rapid test for active TB.*

# *Future investigations*

- Assess the utility of analytes in smear negative TB, extrapulmonary TB, immune compromised subjects, children and people with other lung infections like acute bacterial pneumonia
- Evaluate additional biomarkers as new multiplex assays become available
- develop suitable point-of-care tests using simpler, easy-to-use, and less costly techniques

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