High mortality risk among individuals assuming to be TB-negative in Guinea-Bissau can be predicted using a simple test.
suPAR

- **soluble urokinase Plasminogen Activator Receptor**
- soluble form of the protein uPAR
- Found in:
  - Blood, urine, ovarian cystic fluids, malignant cysts and ascytic fluids
Overview

- Introduction
  - Background
  - Objectives
  - Methodology
- Results
- General Recommendations
- Future Perspectives
Introduction: Might suPAR be a useful marker?

- suPAR is increased in several diseases in particular HIV

- suPAR correlated with several immunological functions such as recruitment of leukocytes, regulatory effects and immune activation

- Plasma suPAR was also elevated in patients with active TB in Guinea-Bissau
Introduction (cont.)

- Recently, suPARnostic ELISA for measuring plasma suPAR became available

- The study was conducted in collaboration with ViroGates.
TB programmes in low-income countries send many patients away as assumed TB negative (aTBneg)

Unexpectedly, we observed a high post-consultation mortality among these individuals

We therefore examined whether suPAR was a prognostic marker for post-consultation mortality
Background: TB in Guinea-Bissau

- Incidence of TB: 471 per 100,000 PYO
- 177 cases of smear-positive per 100,000 PYO
- 40% TB/HIV co-infected

(Gustafson P. et al., Int J Epidemiol 2004)
General objectives

- Can suPAR identify mortality risk among aTBneg individuals?
Specific objectives:

- Mortality compared to the control population
- Plasma suPAR as prognostic marker for mortality
- Plasma suPAR as a marker in HIV-negative and HIV-positive individuals
Methodology: Where?
Methodology: The Bandim Health Project

study area

TB surveillance started 1996
Methodology (Cont.): Inclusion

- Apr 2004 - Dec 2006
- Prospective cohort study
- Adults ≥ 15 years
- Living in the BHP study area
- Outpatients: 3 health centres & National TB hospital
- Some inpatients at the National TB hospital
- Problem: Not allowed by the administration to take blood at the TB hospital
Methodology (Cont.) : Inclusion

- Individuals presenting with a cough of more than 3 weeks and other symptoms were classified as pulmonary TB suspects.
- A study number was assigned and demographic information recorded.
- Blood, urine, and 3 sputum samples for acid-fast bacilli (AFB) microscopy were collected.
Flowchart of screened individuals

All patients suspected of having pulmonary tuberculosis
N= 1682

Diagnosed With TB
N=466

Excluded
N=65

Included
401

Inclusion Plasma
N=278

TB patients

Assumed TB Negative
N=1216

Lost to follow-up
N=205

Included
N=1007

Plasma
N=958

aTBneg

Plasma & Urine
N=863

aTBneg

Age unknown
N=4
Methodology (Cont.): Enrolment in a TB neg study

- Consent to participate in TB suspect study
- TB suspects not diagnosed with TB

- A study of suPAR as a prognostic marker for mortality was not initially planned:
  - Information from clinical examination was not recorded
  - Other prognostic markers were not measured
Methodology (Cont.): Follow up of aTBneg individuals

- Follow-up home visit 3 months after inclusion was started 1 year following reanalysis of previous data suggesting high mortality among aTBneg patients.

- 3-monthly visit often delayed due to difficulties in identifying subjects.

- Obtained information:
  - Date of visit
  - Survival status
  - Date of death
Methodology (Cont.): Mortality in Control Population

- 4983 Matched controls from the DSS of BHP

**Selection criteria:**

- Under observation when the aTBneg individual was included
- Control has follow up after the inclusion date of aTBneg
- Matched on age within 5 years of the aTBneg individual
- 5 controls were randomly chosen for each aTBneg individual
Methodology (Cont.): Laboratory methods

Smear:
- Smear microscopy was performed by experienced laboratory technicians

HIV:
- Screening by Determine HIV 1/2 at the National Public Health Laboratory (LNSP)
- Two more test for reactive samples
  - Capillus anti-HIV 1+2
  - Immunocomb II BiSpot HIV 1&2
    (Differentiation)
- Plasma suPAR levels were determined using the suPARnostic ELISA kit
Results: Mortality in aTBneg

- **aTBneg**: Mortality Rate was 21 per 100 PYO
- **Controls**: Mortality Rate was 3 per 100 PYO
- Mortality Rate Ratio (MRR): 6.92 (95% CI 4.48-40.0)

- MRR in age groups:
  - 15-34: 16.4 (95% CI 7.05-40.0)
  - 35-54: 9.00 (95% CI 4.00-20.1)
  - 55+: 2.25 (95% CI 1.04-4.87)
Results: (Cont.)

<table>
<thead>
<tr>
<th>Plasma suPAR</th>
<th>Below 4th Quartile</th>
<th>Above 4th Quartile</th>
<th>Mortality Risk Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Negative</td>
<td>7/547 (1%)</td>
<td>13/124 (10%)</td>
<td>8.3 (3.4-20)</td>
</tr>
<tr>
<td>HIV Positive</td>
<td>1/156 (1%)</td>
<td>24/111 (22%)</td>
<td>33.7 (4.6-246)</td>
</tr>
</tbody>
</table>
Kaplan-Maier curves
Results (Cont.) Mortality:

- Receiver Operator Characteristics (ROC) curves showing sensitivity and specificity according to HIV-status. The area under the curve (AUC) is further shown.
Results (Cont.)

Results regarding Plasma suPAR

1 ng/ml increase in suPAR: mortality rate increased 46%

HIV negative:
- Best cut-off=4.4
- Sensitivity=0.70
- Specificity=0.83
- High/Low MRR=9.9

HIV positive:
- Best cut-off=5.0
- Sensitivity=0.96
- Specificity=0.70
- High/Low MRR=42.8
Limitations

- The study was not planned at the beginning
  - Clinical inclusion information was not recorded
- Diseases other than TB and HIV was not assessed and verbal autopsy was poorly executed
- Other disease or inflammatory markers were not measured
  - Haemoglobin, C-reactive protein, CD4 counts,
Conclusions

- High mortality in aTBneg compared to controls
- Plasma suPAR carries prognostic information on mortality
- Plasma suPAR is prognostic for both HIV+ & HIV-
General Recommendations

Low suPAR: HIV-neg send home with no further follow up as done currently

High suPAR: HIV-neg should receive further attention (refer to a higher level facility, perform further diagnostic procedures e.g. TB culture, clinical examination, antibiotic treatment for one week and repeat clinical examination and suPAR measurement at 2 weeks; if no decrease in suPAR after 2 treatment regimes with 2 different antibiotics the physician may judge to start TB treatment
Future Perspectives

- Key issues to be addressed
  - Definition of high suPAR levels
  - Establish a cut-off value for selecting individuals that could receive further diagnostic testing
  - Determine what diagnostic testing should be carried out
  - Develop guidelines for the inclusion of suPAR levels in the management of aTBneg

- Cost-effective studies to determine how suPAR and other possible biomarkers can be included in a clinical decision tree for the management of aTBneg

- We intend to develop a proposal for a randomized study including suPAR as decision marker in one arm
Thanks to Many!