Diagnosis of TB in HIV-infected persons and children: Challenges and Solutions
Soumya Swaminathan, MD
Coordinator, WHO/TDR, Geneva

Global TB Burden - 2009

- 9.4 million new TB cases, 1.7 million deaths
- Estimated 10%–15% of cases
- 900,000-1.4 million cases
- But
  - Less specific symptoms, less likely to expectorate
  - Considered less infectious - receive lower priority
  - Specimens other than sputum – fewer bacilli and may include test inhibitors
  - Diagnostic tests developed for adult TB perform poorly in children

Global TB Report 2010
Tuberculosis in India - 2009

- Total TB cases notified 1.53 million
- Mortality rate 23/100,000
- 17% of TB patients knew their HIV status
- 12% of tested TB patients were HIV+
- 13,500 children with TB notified – no data on HIV co-infection
- In south Africa, active screening detected childhood TB at 400/100,000
- Burden higher than reported

www.tbcindia.org, Marais et al Infect Dis Clin NA 2010

Risk of Progression from Infection to Disease

- Natural history (1920-1950)
  - Exposure ↓ Infection ↓ Disease

Risk Factors
- Age (<2-3 years)
- Nutritional status
- Timing of infection
- Immune status (HIV)
- ? Others (exposure to indoor air pollution, cigarette smoke, infectious dose)

IJTLD 2004; 8: 392-402
Wallgren's timetable: 90% of disease occurs in 1\textsuperscript{st} year after infection

Bacilli excreted, irrespective of progression

<table>
<thead>
<tr>
<th>Infection</th>
<th>Months</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>1</td>
</tr>
</tbody>
</table>

I   Hypersensitivity
II  Miliary TB and TBM
III Lymph node disease / Pleural effusion
IV  Adult-type disease

Age and risk – modified by HIV
Pathogens in children failing antibiotics

McNally L. Lancet 2007;369: 1 440

<table>
<thead>
<tr>
<th>Younger than 1 year</th>
<th>1 year or older</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=99)</td>
</tr>
<tr>
<td>Pneumocystis jiroveci</td>
<td>32 (32%)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>15 (15%)</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>4 (4%)</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>2 (2%)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Other gram positive</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Stenotrophomonas spp</td>
<td>3 (3%)</td>
</tr>
<tr>
<td>Legionella spp</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Other gram negative</td>
<td>10 (10%)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>6 (6%)</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>Other viruses</td>
<td>11 (11%)</td>
</tr>
<tr>
<td>Aspergillus spp</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Streptococcus spp</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Escherichia spp</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

All data are number (%). Only children who had all study investigations and failure therapy are included (admissions and non-responder blood cultures, admission nasopharyngeal aspirate and NB-NR, or lung aspirate for viral immunofluorescence; and culture, induced sputum, and NB-NR in long aspirate for P. aeruginosa pneumonia and tuberculosis). Data regarding the tuberculous cases were not regarded as significant and therefore not included.

Table 5: Organisms isolated from children who were investigated for failure to respond to HIV status and age.

Lung diseases identified at necropsy

TB third most common

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total*</th>
<th>Adjusted % (SE)</th>
<th>HIV-positive (n=180)</th>
<th>HIV-negative (n=84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute pyogenic pneumonia</td>
<td>116 (44%)</td>
<td>39-1% (3-2)</td>
<td>74 (41%)</td>
<td>42 (50%)</td>
</tr>
<tr>
<td>POP</td>
<td>58 (22%)</td>
<td>27.9% (3.1)</td>
<td>52 (25%)</td>
<td>6 (7%)</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>54 (20%)</td>
<td>18.8% (2.5)</td>
<td>32 (15%)</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>CMV</td>
<td>43 (16%)</td>
<td>20.2% (2.8)</td>
<td>40 (22%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Interstitial pneumonitis</td>
<td>30 (11%)</td>
<td>11.8% (2.1)</td>
<td>15 (8%)</td>
<td>15 (18%)</td>
</tr>
<tr>
<td>Shock lung</td>
<td>27 (10%)</td>
<td>11.5% (2.2)</td>
<td>24 (12%)</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Pulmonary oedema</td>
<td>19 (7%)</td>
<td>6.4% (1.6)</td>
<td>10 (6%)</td>
<td>9 (11%)</td>
</tr>
<tr>
<td>Lymphocytic interstitial pneumonitis</td>
<td>10 (4%)</td>
<td>3.8% (1.2)</td>
<td>9 (5%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Ghon focus

Pleural effusion
Disseminated (miliary) disease

Clinical symptoms
Nutritional status
Contact history
Chest radiograph
Tuberculin skin test
A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis

A. C. Hesselink,* H. S. Schaaf,* R. P. Gle,* J. R. Starks,† N. Beyers*

- High sensitivity and very low specificity
- High specificity and very low sensitivity

- Screen most obvious cases
- Performs worse in most difficult cases
- Setting and stage at presentation
- Depend on available investigations
Table 4  Review of previous studies of scoring systems

<table>
<thead>
<tr>
<th>Scoring system</th>
<th>Type of study</th>
<th>Country</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>HIV?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edwards et al</td>
<td>Prospective</td>
<td>Zambia</td>
<td>88</td>
<td>25</td>
<td>Yes</td>
</tr>
<tr>
<td>Edwards et al</td>
<td>Retrospective</td>
<td>Papua New Guinea</td>
<td>62</td>
<td>95</td>
<td>No</td>
</tr>
<tr>
<td>Edwards et al</td>
<td>Prospective</td>
<td>India</td>
<td>91</td>
<td>88</td>
<td>No (excluded)</td>
</tr>
<tr>
<td>Edwards et al</td>
<td>Prospective</td>
<td>Brazil</td>
<td>84</td>
<td>97</td>
<td>No</td>
</tr>
<tr>
<td>Jones et al</td>
<td>Prospective</td>
<td>Brazil</td>
<td>56</td>
<td>94</td>
<td>No (excluded)</td>
</tr>
<tr>
<td>Brazil MOH</td>
<td>Retrospective</td>
<td>Brazil</td>
<td>89</td>
<td>87</td>
<td>No</td>
</tr>
<tr>
<td>Fortin/Union</td>
<td>Retrospective</td>
<td>Multiple</td>
<td>41 (0-4 years)</td>
<td>44 (0-4 years)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>62 (5-14 years)</td>
<td>50 (5-14 years)</td>
<td></td>
</tr>
</tbody>
</table>

**Poor agreement between scores**

Hesseling et al IJTLD 2007:11:245

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Algorithm for diagnosis of tuberculosis in children

1. Fever and/or cough for more than 2 weeks
2. Loss of weight or failure to thrive
3. Recent contact with an infectious case

[Diagram of algorithm with steps and decision points]

- Never treat pulmonary TB without an attempt to demonstrate AFB
- No role of GC/G test, ELISA or PCR
- L/x must be rule out of suspected case or microbiology test

IAP 2010
**Differential diagnosis of pulmonary TB in HIV infected children**

<table>
<thead>
<tr>
<th>Age ranges</th>
<th>Clinical features</th>
<th>Radiological features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TB</strong></td>
<td>All ages</td>
<td>Subacute onset(^a) persistent cough, weight loss or failure to thrive, persistent fever</td>
</tr>
<tr>
<td>Bacterial pneumonia</td>
<td>All ages</td>
<td>Rapid onset, high fever, elevated leukocyte count, tachypnoea</td>
</tr>
<tr>
<td>Viral pneumonia</td>
<td>More common in infants</td>
<td>Air trapping with wheezing, tachypnoea</td>
</tr>
</tbody>
</table>

\(^a\) Onset can occasionally be acute, especially in immunocompromised infants.  

(WHO/HTM/TB/2006.362)
Specimen Collection in Children

- Sputum Induction: outpatient setting, can be performed in young children and infants, no SAE, yield from one IS = 3 GL
- Nasopharyngeal aspiration: minimal facilities and training, yield similar to GA
- Stool: stringent decontamination procedures required, less sensitive
- String test: suitable for older children, time of string in stomach can be ~ 1 hour
- Lymph node aspiration: safe outpatient procedure, yield higher than respiratory specimens, should be done if palpable peripheral LNs
- Combined yield of multiple specimens (sputum, NPA, SI, GA) collected in 1 day similar to yield of specimens collected over consecutive days


Yield of *Mycobacterium tuberculosis* in culture using various specimen collection methods

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>Yield of <em>M.tb</em> in culture</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric lavage</td>
<td>40% - 92%</td>
<td>Difficult, invasive procedure, increased yield in infants and extensive disease, 3 consecutive specimens required after overnight fasting. Can be done by trained nurses</td>
</tr>
<tr>
<td>Broncho-alveolar lavage</td>
<td>4% - 43%</td>
<td>Extremely invasive, requires tertiary care facilities. Useful if performed with diagnostic bronchoscopy</td>
</tr>
<tr>
<td>Naso-pharyngeal aspiration</td>
<td>24% - 30%</td>
<td>Less invasive. Appropriate for low income countries with limited facilities</td>
</tr>
<tr>
<td>Laryngeal swab</td>
<td>27% - 63%</td>
<td>Useful in older children who are unable to expectorate</td>
</tr>
<tr>
<td>Induced sputum</td>
<td>20% - 30%</td>
<td>Yield comparable with gastric lavage and naso-pharyngeal aspiration. Requires training, can be done by nurses. Useful in hospital setting. Infection control procedures needed.</td>
</tr>
<tr>
<td>String test</td>
<td>Yet to be determined</td>
<td>Patients as young as 4 years tolerated the procedure well. Peak discomfort at the time of swallowing and mild during string retrieval. Further studies required.</td>
</tr>
</tbody>
</table>
### Differential Performance of Diagnostic Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Adults (%)</th>
<th>Children (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sputum</td>
<td>Gastric aspirate</td>
</tr>
</tbody>
</table>

#### Sensitivity

- **Smear positive**
  - Adults: 60-75%
  - Children: 10-20%
- **Culture positive**
  - Adults: 90%
  - Children: 10-60%
- **TST positive**
  - Adults: 80% (<50% HIV)
  - Children: 50-80%
- **NAAT positive**
  - Adults: 65-90%
  - Children: 25-85%

#### Specificity

- **Smear positive**
  - Adults: >95
  - Children: Low, variable
- **Culture**
  - Adults: 98-99
  - Children: >90
- **TST positive**
  - Adults: 10-20
  - Children: ~50
- **NAAT positive**
  - Adults: 98
  - Children: 85-98

### Factors affecting yield of culture

- Patient population (primary care vs referral hospital)
- Severity of illness
- Type of specimen
- Collection procedure and expertise of staff
- Transportation and decontamination procedures
- Lab quality
Detection of Mtb nucleic acid

- Commercial NAAT: Performance similar to smear neg TB (~60% sensitivity), high specificity, not well evaluated in children
- In house NAATs: heterogeneity in performance
- Nucleic acid in non-respiratory specimens:
  - Stool (poor sensitivity ~35%)
  - Transrenal DNA: needs evaluation
  - Blood: 26% of microbiologically confirmed children positive compared tp 26% children with LTBI and 7% without TB
Fully automated NAAT

- Xpert
- Recommended by WHO 2010
- Funding through Global Fund
- Results in 2 hours
- Multiple pathogens (individual cartridges)
- 98% sensitive in SM+
- 75% sensitive in SM-
- Equal results in HIV+ patients

Other Tests

- Volatile organic compounds in breath: will be simple and non invasive. Recent study in adults – 85% sens and 65% specificity. Needs improvement
Newer Diagnostic tests

- Liquid culture (MGIT): more sensitive than solid media
- MODS (microscopic observation drug susceptibility assay): needs validation
- Xpert TB (multiplex PCR, fully automated): 75% sensitive for smear neg TB in adults, studies required using different specimens from children
- Serological tests: no role
- IGRA (interferon gamma release assay): not recommended for diagnosis of active or latent infection

Need for Reference Standard

- Different from clinical case definition (used for management of patients)
- In research settings, accepted reference standard is culture (preferably with species identification)
- For initial proof of principle studies, important to use "gold standard"
- More pragmatic approaches for diagnostic test evaluation in operational settings
When there is no gold standard....

• Different approaches to creating a reference standard
  – Imputation of missing values
  – Discrepant analysis
  – Use multiple tests to create a standard
    • Rule based approach (use multiple tests to define subjects according to the certainty of diagnosis)
    • Panel based approach
    • Statistical methods eg latent class analysis
  – Overall goal is to decide whether test is providing true information (analytical validity), which is meaningful (clinical validity) and useful (clinical utility)

Latent Class Analysis

• Allows unbiased estimate of validity of diagnostic test in absence of reference std
  – Assumes variable of interest not observable (latent)
  – Subjects belong to mutually exclusive classes of latent variable
  – Observable variables measure the latent var
  – Latent variable determines association between observable variables
  – Also assumes conditional independence of latent classes
  – Has been used to estimate validity of serologic test for Chagas, DAT for visceral leishmaniasis etc
  – Needs to be applied to data set in children and tested
Other Needs for TB Diagnostics
Research in Children

• Standardized specimen collection, handling and processing (SOPs)
• Standard application of test techniques, study-related investigations, interpretation (CXR) and reporting
• Methods of sampling and participant selection
• Standard ascertainment of TB disease
• Systematic characterization of TB disease and comparison groups
• Cross-sectional studies preferable to case-control and minimum set of variables should be collected

Way Forward...

• TDR Diagnostic Expert Evaluation Panel (DEEP) guide on pediatric TB: Nat Rev Microbiol series
• Consensus standard SOPs to be developed for public domain website, explore LCA
• Engage test developers (FIND, academia, NGOs)
• Engage donors
• Increase visibility of childhood TB
• Integrate childhood TB in MDGs initiatives
• Conduct multi-centric evaluations
TB in HIV-infected

- Depends on stage of immunodeficiency
  - At high CD4 counts, typical clinical and radiographic presentation, positive smears
  - At low CD4 counts (<200 cells/mm), more smear negative and extra-pulmonary disease
- Paradox: high bacillary burden in tissues, very little in sputum
- Patients prone to many other infections: wide differential diagnosis
- Patients often very ill, diagnosis is urgent
- Urgent need: POC test

Rule in or Rule out TB

- Quality care of HIV-infected persons includes treatment AND prevention of TB
- Newly diagnosed and those on follow-up need periodic screening for TB
  - If TB diagnosed, start treatment
  - If no TB and no contra-indications, start IPT
Approaches to Diagnosis

- Clinical algorithm to rule out TB (cough or fever any duration or night sweats >3 weeks: 93% sensitivity, 36% specificity)
- Chest xRay: atypical, normal in ~15-20%
- Tuberculin skin test: <50% positive
- IGRA: ~70% sensitive
- Products of M.tb: transrenal DNA, urinary LAM – maybe more sensitive than in HIV-
- Screening with culture yields 5-25% in unselected patients with newly diagnosed HIV
- Culture: not sensitive in extrapulmonary disease, takes time


HIV+ Man with Fever, Painful Red Eye: Chest X-ray normal, 3 smears neg, CD4 86
Panopthalmitis due to TB in a patient with advanced AIDS and pulmonary TB

Diagnostic Issues

- More extra-pulmonary, atypical forms
- 4% asymptomatic HIV+ patients with normal chest x-ray and negative sputum smears have pos. sputum cultures for *M. tuberculosis* (Swaminathan et al IJTLD 2004)
- Active case-finding among antenatal women detected TB cases (AIDS 2003;17(9):1398-400)
- Normal x-ray does not rule out TB
- “Smear neg. TB” could be other OI’s
Immune Reconstitution Syndrome

- Occurs in 20-30% of patients starting ART
- TB commonest form of IRS: enlarging LNs, worsening chest x-ray, fever
- Crypto meningitis, herpes zoster and simplex also seen
- Due to exuberant cytokine response
- Risk factors: ARV within 6 wks, disseminated dis, low baseline CD4, rise in CD4%, fall in VL, ? High bacillary burden

Predictive value of combinations of symptoms

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cough+Fever</td>
<td>54</td>
<td>84</td>
<td>61</td>
<td>79</td>
</tr>
<tr>
<td>Cough+wt.loss</td>
<td>60</td>
<td>77</td>
<td>55</td>
<td>81</td>
</tr>
<tr>
<td>Cough+ abn X-ray</td>
<td>31</td>
<td>69</td>
<td>65</td>
<td>80</td>
</tr>
<tr>
<td>Cough+Fever +wt.loss</td>
<td>46</td>
<td>78</td>
<td>64</td>
<td>88</td>
</tr>
<tr>
<td>Cough+Fever + abn X-ray</td>
<td>43</td>
<td>98</td>
<td>88</td>
<td>79</td>
</tr>
<tr>
<td>Cough+wt.loss + abn X-ray</td>
<td>49</td>
<td>96</td>
<td>84</td>
<td>81</td>
</tr>
<tr>
<td>Cough+fever+wt. loss+ abn x-ray</td>
<td>37</td>
<td>98</td>
<td>89</td>
<td>78</td>
</tr>
</tbody>
</table>
## Cough, fever, weight loss, x-ray

<table>
<thead>
<tr>
<th>Combination</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any 1</td>
<td>94</td>
<td>33</td>
</tr>
<tr>
<td>Any 2</td>
<td>83</td>
<td>64</td>
</tr>
<tr>
<td>Any 3</td>
<td>66</td>
<td>84</td>
</tr>
<tr>
<td>All 4</td>
<td>37</td>
<td>98</td>
</tr>
</tbody>
</table>

NPV of all 3 symptoms being absent plus normal CXR 97%

TRC unpublished observations

## Radiographic Findings in Tuberculosis with and without HIV Infection

<table>
<thead>
<tr>
<th></th>
<th>HIVTB (n = 86)</th>
<th>TB (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>13.5</td>
<td>0</td>
</tr>
<tr>
<td>Parenchymal Opacities</td>
<td>56</td>
<td>90</td>
</tr>
<tr>
<td>Cavity</td>
<td>14</td>
<td>39</td>
</tr>
<tr>
<td>Pleural Effusion</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Miliary TB</td>
<td>13.5</td>
<td>1</td>
</tr>
</tbody>
</table>

Tuberculin skin test results in patients with active tuberculosis

<table>
<thead>
<tr>
<th>CD4 cell count cells/µl</th>
<th>Number of Patients per Strata</th>
<th>Positive TST &gt; 5 mm</th>
<th>Induration size Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>205</td>
<td>41%</td>
<td>0.0 (0.0-15.0)</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>107</td>
<td>70%</td>
<td>15.0 (0.0-20.0)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Overall, 51% of HIVTB patients had pos. TST
Swaminathan et al IJTLD 2008

Value of tuberculin skin test

- Positive correlation between Mantoux test and CD4 count
- Not much increase in sensitivity by using 5mm cut-off
- Mantoux poor diagnostic test for TB
- Misses 30-40% latent TB infection also
Implementation issues

For TB and HIV/AIDS national programmes:
- Where is the appropriate place of Xpert in health system?
- How will we make Xpert work in tiered health/laboratory services?
- What are the capacity strengthening needs for Xpert?
- Where are the gaps and needs for scale-up?

Research needs

- Do we need validation protocol that should be used in all sites?
- What is the programmatic impact of Xpert TB for rapid diagnosis of TB and DR TB among PLHIV?
- What are best operational laboratory models that include Xpert for HIV services?
TB REACH

- Based in Stop TB Partnership
- 120 million dollar fund
- Aim: Fund innovative ways to improve TB case finding
- Details available at website – deadline Feb 28 2011
- Good opportunity to team up and test new strategies especially in hard to reach populations, children, HIV-infected etc
Feeling Overwhelmed?

TIME AND OPTIONS FOR COLLABORATIVE INNOVATIVE ACTIONS

THANKYOU