Challenge of Diagnosing Smear-Negative TB

Department of Infectious Diseases
Outline of Talk

- Impact of HIV on TB
- Overview of diagnostic tests for TB
- Rational approach to SNTB in RLS
Impact of HIV on TB

- HIV mimics TB.
- Increased risk with rapid progression to disease.
- Alters clinical presentation.
- Alters radiological appearance.
- Affects diagnostic tests.
  - Smear, culture
  - Histology
- Affects treatment: drug toxicity, drug interactions,
- More paradoxical reactions.
- Higher relapse of TB (~4 increased)
- Increased mortality (~4 fold)
Making a diagnosis of an infection

- Clinical presentation
- See the organism
- Grow the organism
- Organism product
- Organism nucleic acid
- IR to the organism
- IR to product of the organism
- IR to damage caused by org
- Response to treatment

Organism

Immune Response
Clinical Presentation

- Symptoms
- Signs

Investigations:
- CXR
- Ancillary investigations
  - CRP
  - Hb
  - Albumin
Making a diagnosis of an infection

- Characteristic clinical presentation
- See the organism
- Grow the organism
- Demonstrate organism product
- Demonstrate organism nucleic acid
- Demonstrate IR to the organism
- Demonstrate IR to product of the organism
- Demonstrate IR to damage caused by organism
- Response to treatment
Definitive Diagnosis

- Smear
- Culture (MGIT, MODS)
- Nucleic acid amplification
- Ag detection in urine (LAM)
Seeing the Organism
Sputum Smear

- HIV negative - sensitivity of 2-3 smears » 50-70%
- HIV positive sensitivity of 2-3 smears » 30-40%
Why Smear negative in presence of infection

10 μL sputum (1ml = 1000μl therefore 10μl is 100^{th} of ml)

Area is 200mm^2

1hpf = 0.02mm^2

Total of 10000 hpf (200/.02)

1/100 hpf = 100 org on slide

100 in 10μL = 10000 in mL sputum

Should take 15-20 minutes to read slide ⇒ 30/40 slides/day using ZNS
Fluorescence microscopy:

- Use low-power (250x)
  - Field size ~ 0.34mm² (17x 0.02mm²)
  - Same area scanned in less time or scan larger area in the same time
- Microscopist can properly examine at least 100 smears/day (compare 30-40 ZNS).
- Increases the smear positive call by 10%
ZN Stain vs. Auramine
Culture

- 100 fold more Sn than smear
- Pick up 100 org/mL sputum
- Culture: median time to positivity is 3 weeks
Microscopic Observation Drug Susceptibility (MODS)

- Growth observed long before naked eye can visualize colonies
- Median time to positivity ~7 days
- Characteristic growth (tangles, cording) under the inverted light microscopy
- Incorporate anti-TB drugs into broth enables direct susceptibility testing
Inverted Microscopy
FIG. 1. Characteristic serpentine structure of young *M. tuberculosis* colonies grown in Middlebrook 7H9 broth for MODS, as seen under an inverted-light microscope (original magnification, ×20).
Molecular Testing

- Genetic basis for ID and resistance
- Amplification $\Rightarrow$ sequencing $\Rightarrow$ rapid ID
- critical mutations $\Rightarrow$ predict resistance
- 95% rif resistant d/t mutations in 81 bp region of $\beta$-subunit of RNA polymerase gene
- INH resistance associated with mutations at several points in several genes
- MTB vs. MOTT
- Live vs. dead
Gene Expert- MTB/RIF Test

- Fully automated
- Amplifies MTB specific DNA sequences
- Detects MTB and rifampicin resistance
- Specimens: sputum, CSF & other sterile fluids. **NOT BLOOD**
- Minimal bio-safety requirements and training
1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result:

   **Assay Name**: MTB-RIF
   **Test Result**: MTB DETECTED LOW; RIF Resistance NOT DETECTED

Time to result, 1 hour 45 minutes
GeneXpert

Smear-positive Sn: TB 98.2%
Smear-negative TB:
  1 sample Sn: 72.5%
  2 samples Sn: 85%
  3 samples Sn: 90.2%
Sp: 99.2%
Making a diagnosis of an infection

- Characteristic clinical presentation
- See the organism
- Grow the organism
- Demonstrate organism product
- Demonstrate organism nucleic acid
- Demonstrate IR to the organism
- Demonstrate IR to product of the organism
- Response to treatment
Detect immune response

- **TST:**
  - DTH response to PPD
  - Lacks specificity
  - LTBI vs. active disease
  - In HIV » poor sensitivity

- **IGRA: LTBI vs. active Dx**

- **INy & ADA levels serositis**

- **TB Ab's: poor Sn & Sp**

- **Histology: non-specific**
How do you make a diagnosis of an infection

- Characteristic clinical presentation
- See the organism
- Grow the organism
- Demonstrate organism product
- Demonstrate organism nucleic acid
- Demonstrate IR to the organism
- Demonstrate IR to product of the organism

- Response to treatment
In summary

Major factor hampering the diagnose TB in HIV is lack of sensitive, specific & rapid point-of-care diagnostic test.
Improving the diagnosis and treatment of smear-negative pulmonary and extrapulmonary tuberculosis among adults and adolescents

Recommendations for HIV-prevalent and resource-constrained settings

World Health Organization
WHO Recommendation

- Best approach to reduce time to Rx of SNTB is to use a **clinical approach**, based on **case definitions**.
- Imposes evaluation over time
- HCW is expected to be a clinician, think and use discretion.
Cardinal symptoms of TB

- Fever x $>2/52$
- Night sweats x $>2/52$
- Weight loss $>2.5\%$ over $1/12$
- Cough x $>2/52$. 

IJTLD 2004 8(6):792-795
Reliability of Symptoms

The presence of any 2 symptoms:

- **Sensitivity** - ~100% (all pts with TB have symptoms)
- **Specificity** - 88% (12% that don’t have TB had symptoms)
- **PPV** - 44% (of pts with symptoms 56% falsely diagnosed with TB)
- **NPV** - ~100% (absence of symptoms excludes TB)
- **CXR** not sensitive at excluding TB.
- **Absent symptoms** reliably excludes TB.

IJTLD 2004 8(6):792-795
Clinical assessment: This is a critical step in the diagnostic process, particularly in the absence of any bacteriological confirmation of tuberculosis. It must be based, as far as possible, on supportive investigations and sound clinical judgement in order to arrive at a correct diagnosis without undue delay and prevent excess mortality from undiagnosed tuberculosis. It is also useful for the diagnosis and management of nontubercular conditions during all evaluations of the patient. Sound clinical judgement will be essential for: classifying the patient as ambulatory or seriously ill on the basis of danger signs; classifying the patient of unknown HIV status as HIV-positive or negative; starting the patient on broad-spectrum antibiotics or antituberculosis drugs on the basis of his/her clinical condition and presentation; assessing, managing and/or referring the patient for treatment for other diseases. Because performing these activities is part
Classify Clinical State: Based on Danger Signs

Danger signs: The adult patient will be classified as seriously ill if one or more of the following danger signs are present:

- unable to walk unaided
- respiratory rate over 30 per minute
- fever of more than 39 °C
- pulse rate of over 120 per minute.
Antibiotics trial

Context: There is limited evidence for the use of empirical antibiotic treatment to rule out tuberculosis as a cause of cough in HIV-infected persons. Although non-response to antibiotics increases the likelihood of tuberculosis, the converse is not true; response to antibiotics does not exclude tuberculosis in tuberculosis suspects living in HIV-prevalent settings. Inappropriate use of broad-spectrum antibiotics may also lead to drug resistance, treatment delay and death of patients because of prolonged symptoms.
The primary role of antibiotics should not be as a diagnostic aid; they should be used to treat concomitant bacterial infection in people living with HIV/AIDS with cough or serious illness (Strength: A–IV).

When indicated, one course of broad-spectrum antibiotics, including coverage for typical and atypical causes of community-acquired pneumonia, should be used to reduce the time delay for tuberculosis diagnosis (Strength: A–IV). In such circumstances, fluoroquinolones should be avoided, as they may cause undue delay in the diagnosis of tuberculosis (Strength: A–II).
Role of CXR’s

- Chest X-rays play a significant role in shortening delays in diagnosis and should be performed early in the course of investigation of a tuberculosis suspect (Strength: A–II).

- Sound clinical judgement is needed to put a seriously ill patient with negative sputum smear results on antituberculosis treatment using only suggestive radiographical findings. In such circumstances, the clinical response of the patient has to be monitored and tuberculosis diagnosis should be confirmed at least by clinical response to antituberculosis treatment and preferably by culture (Strength:
Chest X-ray

- Pattern recognition
- Distribution
- Characteristic picture
  - Predominantly upper lobe involvement
  - Breakdown and cavitation
  - Pleural effusion
Bilateral hilar/mediastinal LAN
Ambulatory patient with cough 2–3 weeks and no danger signs

1st Visit

AFB HIV test

HIV+ or status unknown

2nd Visit

AFB-positive

Treat for TB
CPT
HIV assessment

TB likely

AFB-negative

CXR
Sputum AFB and culture
Clinical assessment

TB unlikely

3rd Visit

Treat for PCP
HIV assessment

Response

No or partial response

4th Visit

Reassess for TB

Response
Algorithm for the diagnosis of tuberculosis in seriously ill HIV-positive patient

Seriously ill patient with cough 2–3 weeks and danger signs

- Referral to higher level facility
- Immediate referral not possible

Parenteral antibiotic treatment for bacterial infection
- Sputum AFB and culture
- HIV test
- CXR

No tuberculosis

Treat tuberculosis

Parenteral antibiotics for bacterial infection
- Consider treatment for PCP
- Sputum AFB and culture
- HIV test

HIV+ or unknown

AFB-positive

AFB-negative

Improvement after 3–5 days

No improvement after 3–5 days

Start TB treatment
Complete antibiotics
Refer for HIV and tuberculosis care

Reassess for other HIV-related disease

TB unlikely

Reassess for tuberculosis

h
Response to ATT at 8/52 to Diagnosis TB

- Response to ATT is an effective way to diagnose HIV-associated SNTB

- Clinical criteria to monitor @ 8/52:
  - Wt gain of ≥ 5%
  - Hb increase ≥ 1g%
  - Reduction in CRP by >60%
  - Increase in KPS
  - Improvement in ≥ 50% of initial symptoms.

IJTLD 2006; 10(1):31
Response to ATT at 8/52 to Diagnosis TB

- ≥ 2 response criteria at 8/52 has 97.5% sensitivity for confirmed TB.
- Patients with suspected SNTB who do not meet this criteria are unlikely to have TB:
  - TB treatment should be discontinued
  - Referred to the next level of care for diagnostic evaluation.
Conclusion

Current diagnostic tests either lack sensitivity/specificity or robust, rapid applicability at point of care in RLS.

Use of clinical case definitions, diagnostic algorithms and response to treatment criteria are the most pragmatic tools available in RLS at the moment.

Implementation requires a reasonably efficient health system with appropriately trained staff.