DIAGNOSIS & INVESTIGATION OF TUBERCULOSIS

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Outline

- Natural History of Tuberculosis
  - Active vs Latent TB
- Diagnosis of Tuberculosis
  - History and Examination
  - Laboratory
  - Radiological
- Summary
Introduction

- In 2010, a total of 8.8 million people worldwide became sick with TB disease (new cases)
- TB is a leading killer of people living with HIV (PLHIV)
- TB is one of the world’s deadliest diseases
  - 1.1 million deaths among HIV-negative cases of TB
  - 0.35 million deaths among people who were HIV-positive in 2010
Transmission

Close contacts at highest risk of becoming infected
Need to identify the source to break the transmission
Natural history of TB infection

4-6 weeks

- Exposure
- Infection
- (innate response)
- (adaptive response)
- Elimination of bacteria
- Initial immune control of bacteria
- Latent TB
- Inability to control bacteria
- Onward transmission

Years-decades

- Elimination of bacteria
- Lifelong containment
- Reactivation
Active and latent tuberculosis

Exposure to a person with active TB

Person becomes infected

Sub-clinical infection controlled by T cells

Infection present until death

Insufficient exposure for MTB to become established. All MTB then cleared.

- No symptoms, but MTB still present but cannot be detected
- Chest X-ray/CT scan can be normal
- Diagnosis difficult
- Only detection method is TST

LATENT TB

Symptomatic disease. Subjects are infectious.

ACTIVE TB

- Pathogen may be detected using smear, culture or PCR
- Chest X-ray or CT may be abnormal
- Complicated in non-pulmonary TB (especially children)
Diagnosis of tuberculosis

- Radiological findings
- History and Examination
- Laboratory diagnosis
Diagnosis of tuberculosis

- History and Examination
- Radiological findings
- Laboratory diagnosis
History

Symptoms suggest pulmonary TB:

1. Cough > 2 weeks
2. Productive cough occasionally blood stained
3. LOW, LOA
4. Fever
5. SOB, Night sweats, chest pain

Symptoms suggest extra-pulmonary TB

1. Wide range of symptoms and sometime not specific
2. Prolonged fever
Examination

- Wide range of lung findings
- Hepatosplenomegaly, lymphadenopathy
- Cachexic
- ...etc
Diagnosis of tuberculosis

- Radiological findings
- History and Examination
- Laboratory diagnosis
Laboratory diagnosis

1. **Microscopy** - AFB smear
2. **Isolation / Culture**
   - solid media
   - liquid media
3. **Molecular - Nucleic acid testing**
4. **Newer test - Antibody testing**
   - Interferon gamma released assays
5. **Mantoux test**
Collection of Specimen

For optimal results, specimens should be collected in clean, sterile containers

Specimens:
- Sputum and bronchial washings
- Gastric aspirate
- Urine and stool
- Sterile body fluids: cerebrospinal fluid, synovial, pleural, pericardial, and peritoneal liquid.
- Abscesses
- Bone marrow
- Biopsies or tissues

Diagnostic Standards and Classification of Tuberculosis in Adults and Children,
Am J Respir Crit Care Med, 2000
Laboratory diagnosis

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Acid fast staining

Two procedures are commonly used for acid–fast staining:

1. Carbolfuchsin methods
   - Ziehl–Neelsen and Kinyoun methods

2. Fluorochrome methods
   - auramine-O, rhodamine-O or auramine–rhodamine dyes
Ziehl-neelsen/kinyoun stain

Organisms appear red on a blue / green background
Stains that are utilized are fluorescent or oramine-O or the oramine-rhodamine stain.

Bright yellow rods against a dark background.
### WHO/IUATLD Quantification scale

**Ziehl Neelsen**

<table>
<thead>
<tr>
<th>Number of AFB</th>
<th>Number of fields* examined</th>
<th>What to report</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AFB in 300 fields</td>
<td>300 fields</td>
<td>No Acid Fast Bacilli seen</td>
</tr>
<tr>
<td>1–9 AFB in 100 fields</td>
<td>100 fields</td>
<td>Record exact figure (1 to 9 AFB per 100 fields)</td>
</tr>
<tr>
<td>10–99 AFB in 100 fields</td>
<td>100 fields</td>
<td>1 +</td>
</tr>
<tr>
<td>1–10 AFB in each field</td>
<td>50 fields</td>
<td>2 +</td>
</tr>
<tr>
<td>More than 10 AFB in each field</td>
<td>20 fields</td>
<td>3 +</td>
</tr>
</tbody>
</table>
ZIEHL-NEELSEN/KINYOUN STAINING

Advantages:

- A simple convenient test - the easiest, quickest and inexpensive procedure
- Requires minimal infrastructure and equipment
- Provides the physician with a preliminary diagnosis
- Prioritizes infectious cases
- Smears may be prepared directly from clinical specimens
- Accessible to the majority of patients
Disadvantages:

- High bacterial load: requires 5,000 to 10,000 AFB per mL of specimen to allow the detection of bacteria in stained smears (In contrast, 10 to 100 organisms are needed for a positive culture)
- Mycobacteria other than M. tuberculosis are also AFB positive
- Can not distinguish between dead or live bacteria
- Limited sensitivity (46-78%) but specificity is virtually 100%
- Can not perform susceptibility testing
Fluorescence microscopy

- Increase test sensitivity - average, about 10 to 15%. The specificity similar to that of Ziehl-Neelsen.

- Shorter time needed -
  - The mean time per smear examination was only 1.41 minutes for LED FM compared to 2.48 minutes for Ziehl-Neelson microscopic examination.

- Limitation
  - The fluorescent bulbs are expensive and have short half-lives.

- Light emitting diode-based fluorescence microscopy (LED FM)
  - less expensive, having lower maintenance requirement and not requiring a dark room.
  - recommended by WHO.
NOTE

- A negative smear does not exclude the diagnosis of TB

- A poor specimen may also produce negative results
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
   Interferon gamma released assays
5. Mantoux test
Culture and sensitivity

- Cell culture techniques (where live bacteria are grown on a plate in the laboratory) are still seen as the gold standard for active TB.

- Also provide data on likely effectiveness of certain antibiotics.
Three different types of traditional culture media are available

- Egg based (Löwenstein–Jensen, Ogawa)
- Agar based (Middlebrook 7H10 or 7H11)
- Liquid medium (Middlebrook 7H9)
SOLID MEDIAS FOR CULTURE

Egg-based:
- Lowenstein-Jensen.
- Ogawa

Agar-based:
- Middlebrook (7H10 and 7H11).

*M. tuberculosis* bacilli are slow growing mycobacteria

They produce characteristic non-pigmented colonies, with a general rough and dry appearance simulating breadcrumbs.
SOLID MEDIAS FOR CULTURE

Advantages:
- Gold standard
- More sensitive than microscopy (Sensitivity 80-85%; Specificity 98%)
- Detect cases with low mycobacterial loads (as few as 10 bacteria/ml)
- Species identification – based on rate of growth, colony characteristic, biochemical properties
- Drug susceptibility testing especially for drug-resistant TB
- Used to monitor the effectiveness of treatment

Disadvantage:
- Long incubation period (6-8 wks)
LIQUID MEDIAS FOR CULTURE

- New automated systems
  - MB/BacT, BACTEC 9000, VersaTREK, and the Mycobacterial Growth Indicator Tube (MGIT)
- Faster result as early as 10 days
- More sensitive than solid media
- More prone to contamination by other microorganisms
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
   Interferon gamma released assays
5. Mantoux test
Molecular Methods

Nucleic Acid Amplification Tests (NAAT)

- provide rapid results within 24 - 48 hours and has greater PPV (>95%) with AFB smear positive specimens.
- Confirm rapidly the presence of Mycobacterium in 50 - 80% AFB smear negative, culture positive specimens
- Does not cross-react with NTM
Molecular Methods

Nucleic Acid Amplification Tests (NAAT)

- NAAT results may remain positive for months despite appropriate antitubercular therapy and a good clinical response.

- Thus, they assist in diagnosis but should not be used as a criterion for assessing infectivity or response to therapy
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
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5. Mantoux test
Rapid Methods (Serology Assays)

- All commercial tests for active PTB had highly variable sensitivity (0% to 100%) and specificity (31% to 100%)
- Cross reactivity to Bacille Calmette Guérin (BCG) or nontuberculous mycobacteria (NTM)
- Commercial serological assay should not be used to diagnose pulmonary and extrapulmonary TB
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
   Interferon gamma released assays
5. Mantoux test
Mantoux test & IGRAs

- Used to detect **Latent** TB Infection
- Not to diagnose **ACTIVE** tuberculosis infection
- Not needed if sputum AFB is already positive and TB treatment can be started without further delay
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
   - Interferon gamma released assays
5. Mantoux test
Interferon gamma released assays (IGRAs)

- Based on the principle that the **T-cells** of individuals who have acquired TB infection respond to re-stimulation with *Mycobacterium tuberculosis*-specific antigens by secreting **interferon gamma (IFN-γ)**.
  - T-SPOT.TB (Oxford, Immunotec)
  - QFT-GIT Test (Cellestis)
- Do not cross-react with the BCG and most NTM
Interferon gamma released assays (IGRAs)

i. As an alternative to TST for
   • Patients who are not expected to/could not come back for a reading of skin induration after 48 - 72 hours
   • Patients who had recent BCG vaccination or past NTM infection

ii. Where a 2-step test is considered (TST followed by IGRA)
   • Close-contacts whose TST is in the range of 5 - 9 mm
   • Patients who are offered LTBI treatment but are not convinced that they have LTBI
Laboratory diagnosis

1. Microscopy - AFB smear
2. Isolation / Culture
   - solid media
   - liquid media
3. Molecular - Nucleic acid testing
4. Newer test - Antibody testing
   Interferon gamma released assays
5. Mantoux test
Mantoux Testing Procedure

1. Wipe the arm with a sterile cotton swab.
2. Use 2 TU (0.1ml) of Purified Protein Derivative (PPD) RT 23 SSI which is given by intradermal injection to left forearm, using a specific TB needle/syringe. If done correctly there should be a bleb, raised about 7 mm in diameter, which disappears within an hour.
3. Read at 48 – 72 hours
Interpretation of Mantoux test

Table 2: Positive TST for LTBI

<table>
<thead>
<tr>
<th>Positive TST Reaction (Measurement)</th>
<th>Type of Individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥5 mm</td>
<td>• HIV-infected persons</td>
</tr>
<tr>
<td></td>
<td>• Organ transplant recipients</td>
</tr>
<tr>
<td></td>
<td>• Persons who are immunosuppressed for other reasons (such as those taking the equivalent of ≥15 mg/day prednisolone for ≥1 month or taking TNF-α antagonists)</td>
</tr>
<tr>
<td>≥15 mm</td>
<td>• Individuals from countries with low incidence of TB</td>
</tr>
<tr>
<td>≥10 mm</td>
<td>• All other high risk individuals</td>
</tr>
</tbody>
</table>
# Mantoux test

<table>
<thead>
<tr>
<th>False positive</th>
<th>False negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Infection with nontuberculosis mycobacteria</td>
<td>1. Viral infections- URTI, measles, influenza, HIV</td>
</tr>
<tr>
<td>2. <em>Previous BCG vaccination</em></td>
<td>2. Immunosuppressant due to disease or treatment including HIV infection.</td>
</tr>
<tr>
<td>3. Incorrect method</td>
<td>3. Severe tuberculosis disease</td>
</tr>
<tr>
<td>4. Incorrect interpretation of reaction</td>
<td>4. Poor nutrition</td>
</tr>
<tr>
<td></td>
<td>5. Corticosteroid therapy</td>
</tr>
</tbody>
</table>
Additional Procedures/Diagnostic Tests

**Pulmonary Tuberculosis**
- Sputum induction with nebulised hypertonic saline,
- Gastric lavage
- Fiberoptic bronchoscopy with bronchoalveolar lavage

**Extrapulmonary TB**
- Obtaining samples for *Mycobacterium tuberculosis* culture from the affected sites
- Presence of caseating granulomas, or granulomas with Langerhan’s giant cells on histology or cytology of the specimen is highly suggestive of tuberculosis but they are not specific.
Pleural TB
- Ziehl-neelsen staining of pleural fluid and biopsy specimens is **not helpful** as the sensitivity is only 0.0% and 3.8% respectively.
- The sensitivity of culture and PCR is higher for **pleural biopsy specimens** than for pleural fluid specimens
- Adenosine Deaminase (ADA) level in pleural fluid is useful

Tuberculous Meningitis
- High index of clinical suspicion
- Blood tests, CT scan or MRI brain, Lumbar puncture
- Lumbar puncture: High protein, low glucose and presence of inflammatory cells, predominantly lymphocyte
- Measurement of CSF Adenosine Deaminase level
- CSF PCR level could be helpful
Diagnosis of tuberculosis

- Radiological findings
- History and Examination
- Laboratory diagnosis
Imaging in PTB

- CXR remains the primary imaging modality for PTB in children and adults.

- A normal CXR may be seen in up to 15% of patients with proven primary PTB.

- Hallmark: consolidation with cavitation
# Tuberculosis classifications

## A) Pulmonary Tuberculosis

<table>
<thead>
<tr>
<th>Smear positive:</th>
<th>Smear negative:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TB in a pt with $\geq 2$ sputum AFB positive</td>
<td>1. At least 3 sputum AFB negative, with radiographic findings consistent with PTB, determine by the treating doctor</td>
</tr>
<tr>
<td>2. TB in pt with 1 sputum AFB positive + CXR abnormalities consistent with active TB</td>
<td>2. TB in pt with initial sputum for AFB were negative, who had sputum culture send and result is positive for M. tuberculosis.</td>
</tr>
<tr>
<td>3. TB in pt with $\geq 1$ sputum AFB positive + sputum culture for M. tuberculosis positive.</td>
<td></td>
</tr>
</tbody>
</table>
### Classifications....

<table>
<thead>
<tr>
<th>B) Extrapulmonary Tuberculosis</th>
<th>C) Pulmonary with extrapulmonary TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB of organs other than lung parenchyma. Diagnosis based on at least 1 culture positive or histological or strong clinical evidence consistent with active extrapulmonary TB</td>
<td>TB involving the lung parenchyma as well as any other part of body</td>
</tr>
</tbody>
</table>
Summary

- Diagnosis should include history, examination, laboratory and radiological findings
  - Active versus Latent TB
- Always try to get clinical specimen for AFB smear and C+S
- May consider trial of anti TB therapy ultimately
Thank You
SPUTUM
COLLECTION
Sputum collection

- Early-morning sputum specimens (not saliva) are usually recommended, obtained after a deep, productive cough (Satisfactory quality implies the presence of mucoid or mucopurulent material)
- Rinse his/her mouth with water before producing the specimen
- Never collect sputum in the laboratory or toilet
- Done in a separate open-air area (Rapidly dilutes aerosols & UV light rapidly inactivates the bacilli)
- Collect away from other people
- No one should be standing in front of the patient during expectoration
Sputum Collection

- Recommended to collect three times:
  - Spot specimen (1)
  - Early morning specimen (2)
  - Another spot specimen (3)

Diagnostic Standards and Classification of Tuberculosis in Adults and Children, Am J Respir Crit Care Med, 2000
WHO/IUATLD Recommendation
## Spot & collection specimens: smear & culture results

<table>
<thead>
<tr>
<th>Type of specimen(s)</th>
<th>Positive by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smear %</td>
</tr>
<tr>
<td>1 spot</td>
<td>66</td>
</tr>
<tr>
<td>1 collection</td>
<td>77</td>
</tr>
<tr>
<td>2 spot</td>
<td>76</td>
</tr>
<tr>
<td>1 spot + 1 collection</td>
<td>81</td>
</tr>
<tr>
<td>2 collection</td>
<td>83</td>
</tr>
<tr>
<td>2 spot + 1 collection</td>
<td>84</td>
</tr>
<tr>
<td>1 spot + 2 collection</td>
<td>84</td>
</tr>
<tr>
<td>2 spot + 2 collection</td>
<td>85</td>
</tr>
</tbody>
</table>
Sputum Collection

- Spot specimens to be collected immediately on first visit (AFB x 1)

- Then an empty pre-labeled sterile container is given to patient to be taken home.

- The early morning specimen is to be collected and brought to the lab (AFB x 2)

- Then on arrival to the lab, a third spot specimen is collected from the same patient (AFB x 3)
Additional Procedures/Diagnostic Tests

Pulmonary Tuberculosis

- Sputum induction with nebulised hypertonic saline,
- Gastric lavage
- Fiberoptic bronchoscopy with bronchoalveolar lavage
Sputum Induction Guidelines (Adults and Children)

**Indication**
1. Patients who are unable to spontaneously expectorate adequate sputum specimens
2. May be useful in the diagnosis of miliary TB and tuberculous pleural effusion

**Contraindications/Precautions**
1. Patients in whom severe coughing may be harmful including patients with:
   - haemoptysis of unknown origin
   - acute respiratory distress
   - unstable cardiovascular status (arrhythmias, angina)
   - thoracic, abdominal or cerebral aneurysms
   - hypoxia (SaO2 <90% on room air)
   - lung function impairment (FEV1 < 1.0 litre)
   - pneumothorax
   - pulmonary emboli
   - fractured ribs or other chest trauma
   - recent eye surgery
   - bleeding disorders

   The risks and benefits of the procedure should be discussed with patient before proceeding with it.
2. Patients who are unable to follow instructions or having reduced level of consciousness.
3. Inadequate fasting (<3 hours)

As hypertonic saline (3%) causes bronchoconstriction, the procedure should only be performed after pre-medication with salbutamol and under medical supervision in patients with asthma, suspected asthma or severely impaired lung function (FEV1 <1 litre)
**Preparation For The Procedure**

**Assess the patient for potential risk and explain the procedures to patient.**

1. Patient should rinse their mouth and gargle with water (to prevent specimen contamination).
2. Fill the nebuliser (preferably ultrasonic nebuliser) with 3% saline (such as 5 ml for children and 20 – 30 ml for adult).
3. Patient should sit upright, place the mouthpiece in the patient’s mouth, (apply nose clip) and turn nebuliser on.
4. Inhale and exhale through the mouthpiece only.
5. Gentle chest physiotherapy may be carried out during the procedure.
6. The procedure should be stopped when:
   - patient has produced 1 – 2 ml of sputum for each specimen collected
   - 15 minutes of nebulisation is reached
   - patient complains of dyspnoea, chest tightness or wheeze
7. Transport the specimen (in a cool box) to the laboratory for processing as soon as possible (within 4 hours).
8. If it is likely to take >4 hours for the specimens to be transported, place them in the refrigerator (4 – 8 °C) and stored until transported.
9. The specimen should be labelled as induced-sputum sample.
Gastric aspiration should be performed on three consecutive mornings for each patient. The child must be fasted for at least 4 hours (3 hours for infants) prior to the procedure and children with a low platelet count or bleeding tendency should not undergo the procedure.

Gastric aspiration is generally not an aerosol-generating procedure, hence considered a low risk procedure for TB transmission and can safely be performed at the child’s bedside or in a routine procedure room.

1. Prepare the child and equipments as standard requirement for nasogastric tube insertion.
2. Attach a syringe to the nasogastric tube.
3. Aspirate gastric contents (2 – 5 ml) using the syringe attached to the nasogastric tube.
4. If no fluid is aspirated, insert 5 – 10 ml sterile water or normal saline and attempt to aspirate again.
5. Transfer gastric fluid from the syringe into a sterile sputum container.