

Tuberculosis

Color index:

- Important
- Doctor Notes
- Extra, TN



Objectives :

- **Recognize that tuberculosis as a chronic disease mainly affecting the respiratory system.**
- **Know the epidemiology of tuberculosis worldwide and in the kingdom of Saudi Arabia.**
- **Understand the methods of transmission of tuberculosis and the people at risk.**
- **Know the causative agents, their characteristic and staining methods.**
- **Understand the pathogenesis of tuberculosis.**
- **Differentiate between primary and secondary tuberculosis and the clinical features of each.**
- **Understand the method of tuberculin skin test and result interpretation.**
- **Know the laboratory diagnostic methods.**
- **Know the chemotherapeutic agents and other methods of prevention and control of tuberculosis.**



Overview by Dr. Alsomaily

- Tuberculosis (TB) Occurs in many places the most common & most important is:

- **Pulmonary TB**

- Is caused by the species *Mycobacterium Tuberculosis*
- More susceptible in HIV patients
- Symptoms can be Fever, cough.

- **Staining:**

- The organism: *Mycobacterium tuberculosis* Resist gram stain because of mycolic acid in their cell wall thus, they are not gram stainable,
- instead we use a different stain called Ziehl-Neelsen stain (ZN stain).
 - gram stain show gram+ve or -ve after decolorization of saffron,
 - ZN stain show +VE AFB OR -VE AFB after decolorization of carbon fusion so in ZN stain we don't say gram +ve or -ve, we say AFB +ve or -ve.
- Generally AFB can't be decolorized by its carbon fusicol and this indicate *Mycobacterium tuberculosis* (It appear Red when +VE)
- we also use other stain Auramine Rhodamine stain AR (Fluorescence stain) stain shows 3b's for TB;(bacilli, bright, bedell.)

- **More common in:**

- Developing countries E.g. India, south africa, pakistan ..
- HIV patients have higher mortality and incidences.

- It is important that *infection* differentiated from *disease*.

- **Primary TB:**

- TB disease from other patients that have active TB to someone who never had TB, Recover spontaneously.

- **Secondary TB:**

- It is reactivation, person Who had TB and was born in susceptible place and brought it to Clean environment and gave them Primary TB.
- Cough, fever, lose of weight → always think of secondary TB.






- **Mantoux skin test:**

- this test show the size of lesion on skin it indicate reaction. the larger the lesion the more possibility of being TB infection (it is not conclusive).
 - >5----> suspect TB if patient has other diseases Risks.
 - >10 -----> maybe TB so, give prophylactic if patient agrees.
 - >15 -----> high chance of TB infection patient must get prophylactic to prevent it from being active TB.

- after doing mantoux skin test: if the test showed more than 15mm, then this is TB infection and therefore, we want to see if it has become Active (secondary) TB. to test for active TB and severity we do:

- **Culture (gold standard very accurate) :**
 - LJ media alone takes weeks so we add to LJ Media MGIT media.
 - MGIT makes the culture faster from weeks to days.
- **Smear** (not as accurate as culture)
- **Biochemical**
- **Molecular testing (PCR)** used for rapid testing.
- **Measurement of interferon-gamma:**
 - Used to test for latent TB NOT active.

Introduction

- 1 Tuberculosis (TB) is an ancient, chronic disease affects humans, caused by **Mycobacterium tuberculosis complex**. 
- 2 A major cause of death worldwide resulting from a single infectious agent. 
- 3 **Usually** affects the lungs, but other organs **can be affected** in one third of cases.
- 4 If properly treated is **curable**, but fatal if untreated in most cases. 
- 5 TB affects 1/3 of human race (2 billions) **as a latent dormant tuberculosis**. 
- 6 Incidence: a worldwide disease, more common in developing countries (**India, china, indonesia, philippines, pakistan, nigeria, bangladesh, and South africa**) .
- 7 Affects all age groups who are subject to get the infection. 

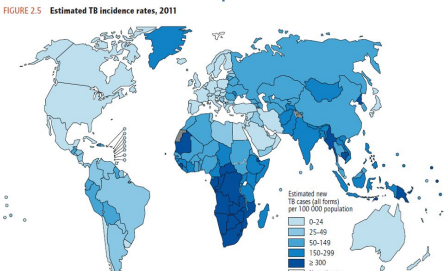
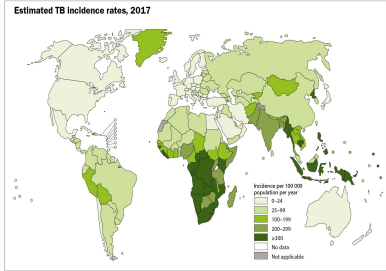
New cases and death

- The WHO estimated 8.9 million new cases in 2014 & 2 - 4 million death (1 million in boys slides).

Incidence

- KSA : 32-64 cases /100,000.
- [(KSA 2011 Data): 0-24 cases /100,000 population in girls slides].
- USA : 5.2 cases/100,000
- Southeast Africa : 290 cases /10,000 due to coupling with HIV infection.
- Incidence among HIV 20 times. 1.3million deaths from TB among HIV-negative people in 2017 and an additional 300 000 deaths From TB among HIV-positive people

Epidemiology



Characteristics of the Genus Mycobacteria

It's unusual Gram positive, slim, and **rod in shape (bacilli)**, non-motile, **non-spore forming**, and it's **strict aerobes (loves and need Oxygen)**.

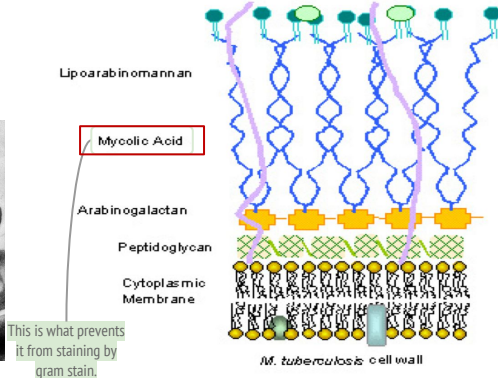
Do **not stain by Gram stain** because it contains **high lipid conc. (Mycolic acid)** in the cell wall which resist staining. (prevent crystal violet to reach Peptidoglycan)

Called **Acid- alcohol fast bacilli (AFB)**, because it resists decolorization with up to **3% HCL, 5% ethanol or both**. So, it is stained by **Ziehl-Neelsen (Z-N)** and **Auramine staining**

Mycobacterium species appear tiny red bacilli acid fast bacilli (AFB) by Z-N stain.



Mycobacterium under electron microscope (extra pic)



* Species of Mycobacteria:

Species of Mycobacteria

Mycobacterium tuberculosis complex

Cause tuberculosis

M. tuberculosis (human type)* very common

M. bovis (bovine type)
(rare because of pasteurization of milk)

M. Africanum

BCG strains (used for vaccination because it's a weak bacteria but in rare cases it can cause TB in immunocompromised children)

Mycobacterium leprae

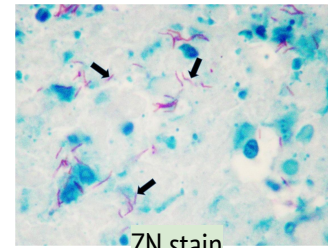
Causes leprosy.

Atypical Mycobacteria / Mycobacteria other than tuberculosis (MOTT)

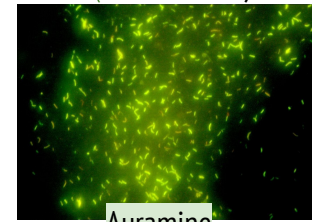
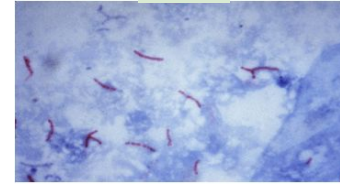
Cause infections in immunosuppressed patients.

Acid-Fast Bacilli (AFB)

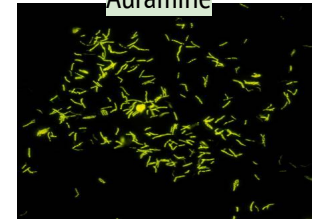
- 1 • Stains used : **Ziehl-Neelsen stain (ZN stain)** and **Auramine Rhodamine stain**.
(Auramine rhodamine is a **Fluorescence stain**).
- 2 • Strict aerobes
- 3 • **Multiply intracellularly** (inside the cells, macrophages, and other tissues)
- 4 • Cause delayed hypersensitivity reaction type 4 of immune response
• It cause **delayed** hypersensitivity because it multiplies intracellular
- 5 • **Slowly growing (between 2 - 8 weeks.) due to the thick layer of mycolic acid that surrounds the cell wall preventing nutritions to reach the cell**



ZN stain



Auramine



Ziehl-Neelson Stain Kinyoun Modification

Acid Fast Organisms



Red color
+VE AFB

Not Acid Fast Organisms



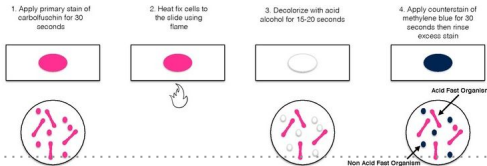
Blue color
-VE AFB
Other bacteria

A small amount of organism suspended in saline solution is fixed on a slide.

Slide is flooded with Carbol Fuchsin and phenol for 3 minutes, and gently rinsed with water.

Slide is decolorized with 3% HCl in 70% alcohol until color appears to be removed (approx. 2 mins), and rinsed with water.

Slide is flooded with methylene blue counterstain for 30 secs, rinsed with water and air-dried.

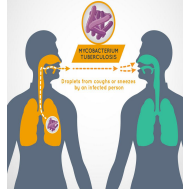


1. Staining by using carbon fusion stain (red color)
 2. Fixation (using the heat to allow the dye to go inside the wall)
 3. Decolorization by strong acid (methanol 3-5% or hydrochloric acid)
- We use a very powerful acid to make sure that the bacteria can handle the decolorization with acid that's way it was named Acid Fast Bacilli
So no matter how powerful is the acid, the bacteria will not lose the dye
In case of TB it will keep its red color and it won't change

Transmission + Epidemiology :

Mainly through

- Direct person-to-person transmission by inhalation of airborne **droplet** (tiny and wet) **nuclei** ($< 5 \mu\text{m}$) in pulmonary diseases case.



Rarely through

- GIT & skin

Reservoir: source

- patients with open TB. (when a person has TB with chronic cough).

Age:

- Young children
- Adults

People at risk:

- **lab Technicians** (risk of exposure)
- **workers in mines** (risk of developing)
- **immunosuppressed patients** (risk of developing as secondary)
- **contacts with index case.** (People around the infected person)

Pathogenesis of Tuberculosis:

1

Mycobacteria acquired by airborne Droplet reaches the alveolar macrophages **intracellular**, and are able to survive their' main virulence factor.

2

This starts cell mediated immune response; which controls the multiplication of the organism but does not kill it.

3

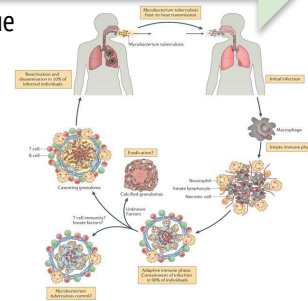
•Granuloma is formed, organism lives in dormant state (latent tuberculosis infection). The Person is infected but doesn't have disease

4

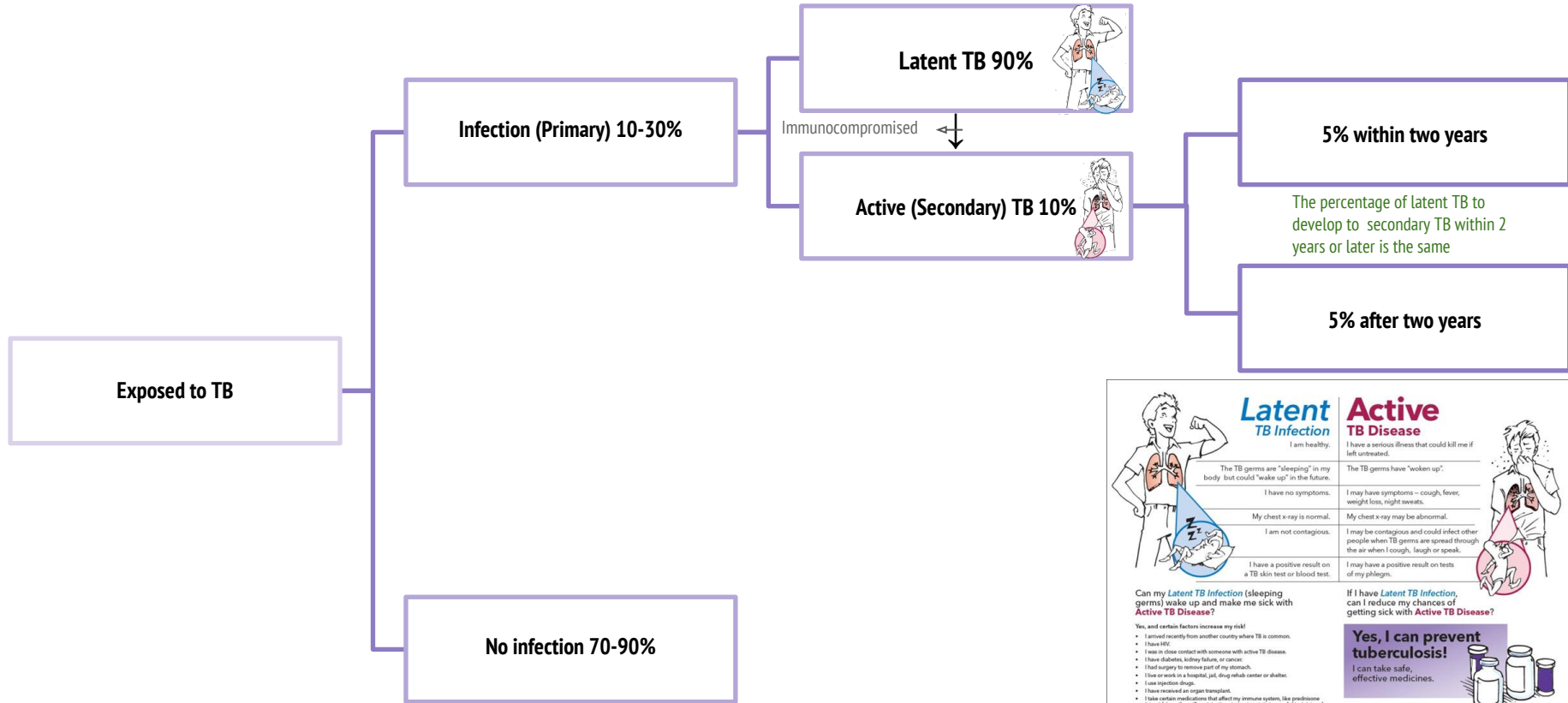
Patient show evidence of delayed cell mediated immunity (CMI). (hypersensitivity reaction)

5

Disease result due to destructive effect of CMI .



Natural History of TB Infection:



Latent TB Infection	Active TB Disease
<p>I am healthy.</p> <p>The TB germs are "sleeping" in my body but could "wake up" in the future.</p> <p>I have no symptoms.</p> <p>My chest x-ray is normal.</p> <p>I am not contagious.</p> <p>I may have a positive result on a TB skin test or blood test.</p>	<p>I have a serious illness that could kill me if left untreated.</p> <p>The TB germs have "woken up".</p> <p>I may have symptoms – cough, fever, weight loss, night sweats.</p> <p>My chest x-ray may be abnormal.</p> <p>I may be contagious and could infect other people when TB germs are spread through the air when I cough, laugh or speak.</p> <p>I may have a positive result on tests of my sputum.</p>
<p>Can my Latent TB Infection (sleeping germs) wake up and make me sick with Active TB Disease?</p> <p>Yes, and certain factors increase my risk!</p> <ul style="list-style-type: none"> I arrived recently from another country where TB is common. I have HIV. I was in close contact with someone with active TB disease. I have diabetes, kidney failure, or cancer. I had surgery to remove part of my stomach. I live or work in a hospital, jail, drug rehab center or shelter. I use injection drugs. I have received an organ transplant. I take certain medications that affect my immune system, like prednisone (steroids) or other pills or injections to treat certain types of skin, joint and gastrointestinal conditions. 	<p>If I have Latent TB Infection, can I reduce my chances of getting sick with Active TB Disease?</p> <p>Yes, I can prevent tuberculosis!</p> <p>I can take safe, effective medicines.</p>

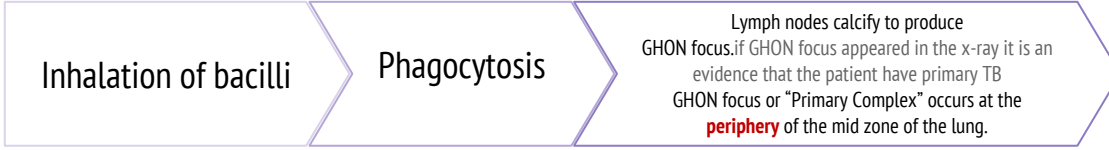
Published 2012

HMC Bellevue
NYU School of Medicine
UP FLORIDA
Southwestern Medical Center

Primary Tuberculosis

(occurs in patients not previously infected).

Pathogenesis:

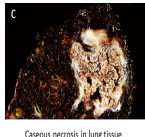
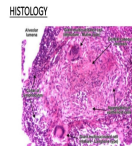
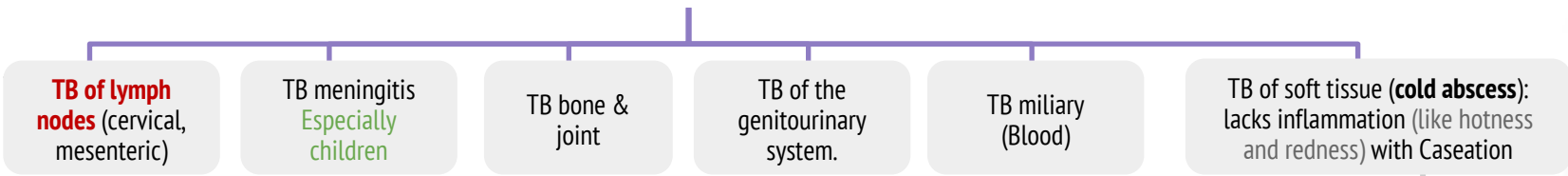


- Microscopy of lesion shows → **Granuloma.**
- Clinically → primary TB usually **asymptomatic** or minor illness (Flu Like symptoms) . (rarely transmitted)

Other sites beside the lung:

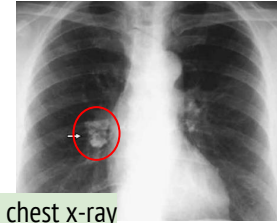
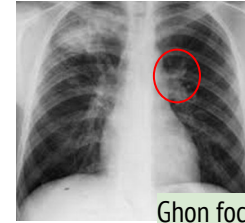
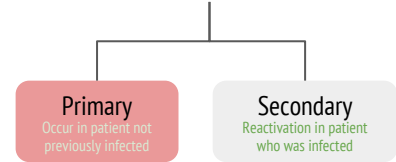
Non-pulmonary TB :

may spreads from **pulmonary infections** to other organs eg.:

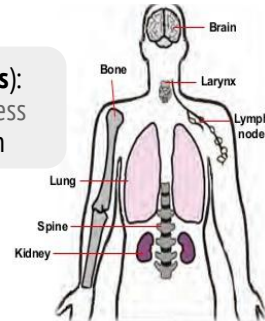


Caseation: due to delayed hypersensitivity reaction. Contains many bacilli ,enzymes, O₂,N₂ intermediates → necrotic centre of granuloma (cheesy material). No inflammation this is usually Cold abscess TB , if inflammatory infiltrate present the this is other type of TB.

Clinically the TB is divided into:



Ghon focus in chest x-ray



Secondary TB (reactivation)

Occurs Later in life

Most common site:

Lung.

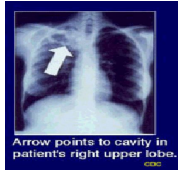
Infect:

- Immunocompromised patients.
- Old people.
- Diabetic patients.
- sometime renal diseases.
- Alcoholism.
- chronic lung disease (Silicosis).

Lesion:

Localized in **apices**.

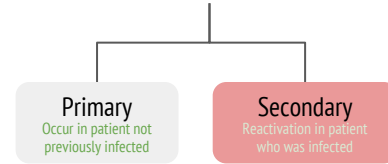
Because apices are rich with O₂ and MTB is aerobe.



Divided into:

Infectious & Symptomatic.

Clinically the TB is divided into:



Microscopy:

many bacilli, large area of caseous necrosis → cavity (open TB) with granuloma and caseation.

(sometimes the cavity will spread into the bloodstream of the lung and once its spread the coughing of patient will contain blood which cause the cavity to be mixed with the blood)

Clinical symptoms:

Fever, chough, hemoptysis, **weight loss, night sweats & weakness.**

(chronic cough and fever that lasts for months **NOT DAYS** and the patient isn't responding to treatment)

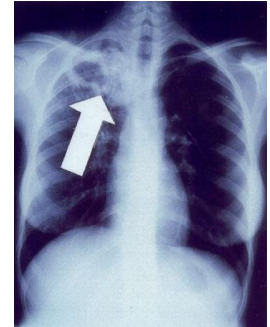
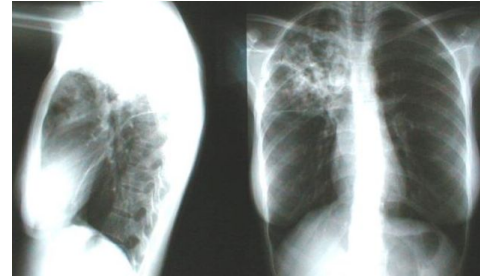


Sources:

- **Endogenous:** Reactivation of an old TB (latent → active).
- **Exogenous:** Re-infection with new strain. (new infection).

Chest radiology:

- No chest X-ray pattern is absolutely typical of TB
- 10-15% of culture-positive TB patients not diagnosed by X-ray
- 40% of patients diagnosed as having TB on the basis of x-ray alone do not have active TB
- Chest x-Ray can be anything even nothing (normal X-Ray), but that doesn't rule out TB.



Classical Radiographic Appearance:

- Infiltration
- Cavitation
- Fibrosis with traction
- Enlargement of hilar and mediastinal lymph node
- Pleural thickening

In reactivation TB:

- Classically fibrocavitary apical disease

Primary TB:

- Middle or lower lobe consolidation

Immunity to Tuberculosis

- Cell-mediated immunity associated with delayed hypersensitivity reaction.
- Detected by **tuberculin skin test**.
- Tuberculin test takes 2-10 weeks to react to tuberculin and becomes positive.



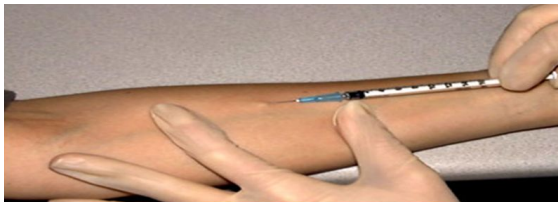
* Tuberculin Skin Test

Methods of Tuberculin Skin Test:

1

Mantoux Test:

Intradermal inoculation of 0.1 ml of PPD, (purified protein derivative) 5 tuberculin units.
Read after 48-72 hrs.



1

Uses purified protein derivative (PPD).

2

Activity expressed by Tuberculin unit.

3

Activates synthesized lymphocytes to produce CMI which appear as skin induration(toughness).

4

May not distinguish between **active and past infection** except in an individual with recent contact with infected case.

5

Low level activity induced by environmental mycobacteria, previous vaccination. (cross reaction with the mycobacteria in the environment might cause the TB skin test to be false positive)

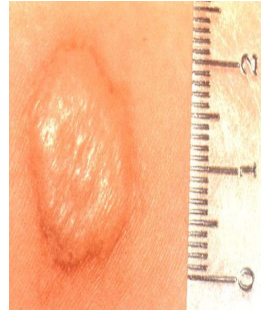
*Tuberculin Skin Test



POSITIVE

(the patient has or had TB infection)

Mantoux Test:



NEGATIVE

Mantoux Test:

1) >5mm induration positive in:

- 1-Recent contact with active TB
- 2-**HIV or high risk of HIV**
- 3-Chest X-ray consistent with healed TB
- 4-Organ transplant

2) >10mm induration positive in:

- 1- IV drugs user
- 2- HIV seronegative* patient
- 3- **medical conditions eg: diabetes, malignancy**
- 4- Children < 4 years or exposed to adult high risk group
- 5-Patients from country with high incidence.
- 6- mycobacteriology Lab personnel.
might use prophylactic

3) >15mm induration positive in:

- **considered positive Any persons including those with no risk of TB**
must use prophylactic

A) No Induration, Either due to:

- 1.No previous infection
- 2.Pre-hypersensitivity stage
- 3.Lost TB sensitivity with loss of antigen (the antigen was lost because the last exposure was from a very long time)

B) AIDS patients are anergic and susceptible to infection.

**because they have very few T helper cells to react to this antigen.*

seronegative*: The lab result is negative for HIV although the person have it. If the patient has TB we have to suspect HIV because it's always associated with it

★ Laboratory Diagnosis of TB



1

Specimens

• Pulmonary TB:

- **Three** early mornings **sputum samples** (immediately after waking up before eating or washing the mouth)
- or **bronchial lavage***
- or **gastric washing(infants)***

• TB Meningitis:

Cerebrospinal fluid (CSF).

• TB of the genitourinary system:

Three early morning urine.

• TB bone & joint:

Bone, joint aspirate (never swap)

• TB of lymph nodes:

pus or tissues, NOT swab.
Repeat the sample

2

Direct microscopy of specimen

• Z-N or (Auramine) stain.

*We use bronchial lavage for patient who can't give sputum (المنومين)
*And gastric washing because children swallow their sputum

3

Culture

• Culture is the gold standard.

Important for:
- identification
- sensitivity.

Media used:

Lowenstein-Jensen media (LJ)*

Media contains:

asparagine, glycerol, pyruvate/ malachite green.
- Colonies appear in LJ media after **2-8 weeks** as eugenic, raised, buff, adherent growth - enhanced by glycerol: (MTB)
- enhanced by pyruvate: (M.bovis).

4

Other media could be used in addition to LJ media may be used:

• Automated methods : using Bactec MGIT (Mycobacteria Growth Indicator Test):

- Liquid media
- Faster growth of mycobacterium 4-14 days
If it is used with LJ culture 4-14 days

• Measurement Interferon –gamma release assay (IGARAs):

positive in latent TB..
(increased risk of developing TB), More specific than TST

- Molecular method: to detects the DNA of the bacteria
1- ProbTech ; **detects nucleic acid** directly from respiratory samples.
2- Xpert MTB/RIF **detect nucleic acid and resistance** to rifampicin .
3- **PCR***(polymerase chain reaction): molecular test directly from specimen (CSF).

Mycobacterium



Fig. 5.3: Myc. TB in Sputum, Z.N stain
(few thin pink bacilli with blue background)



Fig. 5.1: Selective media for Myc. T.B.



Fig. 5.2: Culture of Myc. TB on L.J. media
- Grow after 6-8 week

Growth of MTB on LJ media

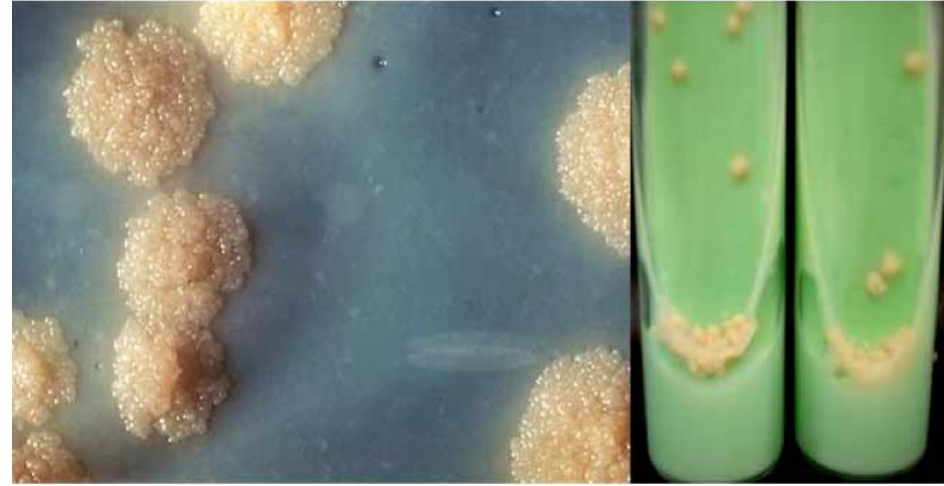


Fig: Cultural Characteristics of *Mycobacterium tuberculosis*

Identification & Management & prevention of TB

-Isolation for **10-14** days after this period the patient can't infect others and can complete the treatment at home (for smear positive cases i.e. > 1000 organisms / ml of sputum considered infectious case).

-Triple regimen of therapy combination of three antimicrobial agents at once. **Why?**

1. To prevent resistant mutants
2. To cover strains located at different sites of the lung
3. To prevent relapse

Treatment must be guided by sensitivity testing.

Management of a TB case

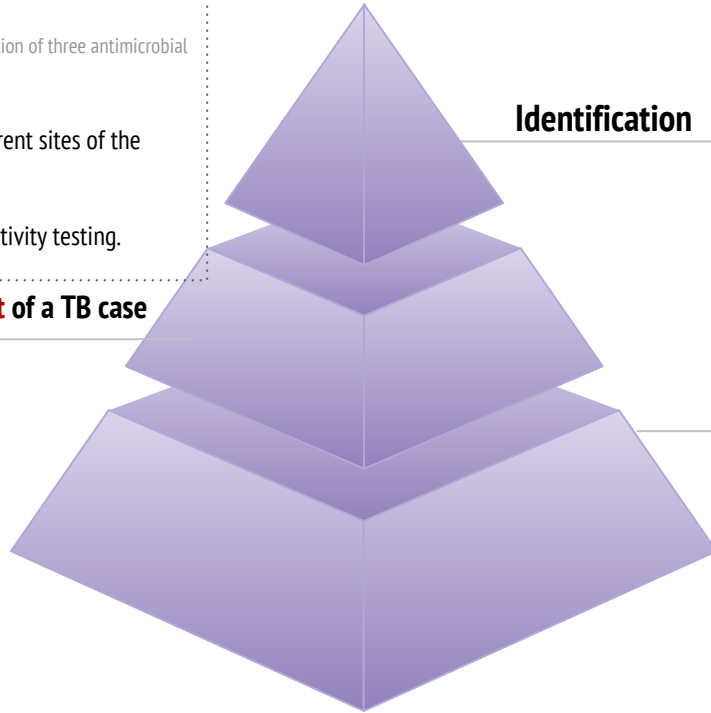
Identification

Morphological characteristics, growth at 37 c + 5 -10 % CO₂

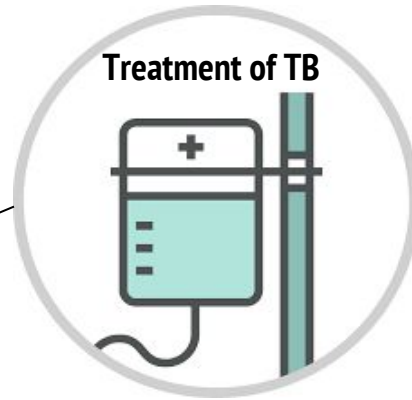
- **Biochemical tests:** Niacin production & Nitrate test.
- **Susceptibility testing** to detect resistance to anti-TB agents

Prevention of TB

- Tuberculin testing of herds.
- Slaughter of infected animals.
- Pasteurization of milk to prevent bovine TB.
- Recognition of new cases.
- Prophylaxis with INH of contacts. People who are in contact with the patient
- Follow up cases.
- **Immunization with BCG (live attenuated) to all newborns**



Treatment of TB



Why do we use this large amount of drugs ?
Because some “multidrug resistant TB” is resistant to INH and RIF.

First Line Treatment*

- **Isoniazid (INH)**
 - **Rifampicin (RIF)**
 - **Pyrazinamide (P)**
 - **Ethambutol (E)**
 - **Streptomycin (S)** (Ethanbutol As effective twice or trice weekly)
- Directly Observed Therapy (DOT).

- Combination therapy:
- For the first 2 months → INH + RIF + P or E + S
- For the next 4--6 months → INH + RIF

Second Line Treatment

- **PASA (Para-Aminosalicylic acid).**
- **Ethionamide.**
- **Cycloserine.**
- **Kanamycin.**
- **Fluoroquinolones.**

- Used if the bacteria was resistant to first line drugs.
- **More toxic** than the first line drugs.

SUMMARY of the IMPORTANT POINTS

Mycobacterium Tuberculosis Complex Causes Tuberculosis	Diagnosis of AFB (TB) (By clinical order)	Treatment & Prevention & Management OF TB
Cause tuberculosis	1-Tuberculin Skin Test Mantoux Test: (PPD) Positive in: >5: RECENT CONTACT WITH TB, OR HAS HIV >10: Diabetes , country with high incidence. >15: positive : in any person post-Mantoux test: for active TB	First line treatment: Isoniazid Pyrazinamide Rifampicin Ethambutol
M. tuberculosis (human type) Causes pulmonary TB	2-Specimens of pulmonary TB (an active TB) -sputum samples -or bronchial lavage -or gastric washing(infants)	Prevention: - Vaccination with BCG - prophylaxis with INH
M. tuberculosis bovis (rare because of pasteurization of milk)	3- Direct microscopy of specimen: 1-Ziehl-Neelsen stain (ZN stain) 2-Auramine Rhodamine stain. (not the gold standard = not accurate)	Management: -Triple regimen of therapy -Isolation
M. Africanum	4- culture (Gold standard) Lowenstein-Jensen media (LJ)	
BCG strains	5- LJ media + other media -MGIT (FASTER GROWTH 4-14 DAYS)	

We left this space for you so you can breath in this block :-)
 Many Thanks.

Quiz :



1) How do you get pulmonary TB ?

- | | | | |
|--|---------------------------|--|------------------|
| A. Through the air via droplets nuclei | B. Through sexual contact | C. Through Air by inhalation of spores | D. Through blood |
|--|---------------------------|--|------------------|

1)A
2)D
3)D

2) How is TB diagnosed?

- | | | | |
|----------------|---------------------|--------------|--------------------|
| A. Chest X-ray | B. Sample of sputum | C. Skin test | D. All are correct |
|----------------|---------------------|--------------|--------------------|

3) to diagnose acid fast bacilli AFP what is the proper microscopy technique ?

- | | | | |
|---------------------------------|-----------------------------------|-----------------------------|-----------------|
| A. Lowenstein-Jensen media (LJ) | B. Ziehl-Neelsen stain (ZN stain) | C. Auramine rhodamine stain | D. Both B and C |
|---------------------------------|-----------------------------------|-----------------------------|-----------------|

1— A 65 year old male came to the hospital complaining of blood when coughing, fatigue, and weight loss. The nurse checked his temperature and it was high. The doctor requested to do some tests and x-ray, the x-ray showed a lesion in the apices of the right lung and the doctor immediately started the management and treatment of this case

A) What is the diagnosis of this situation? Secondary Tuberculosis

B) What is the bacteria that causes this disease ? Mycobacterium Tuberculosis

C) What is the management of this case? Isolation for 10-14 days and Triple regimen of therapy

D) Patients was in a critical situation The physician needed a quick, fast Diagnostic method what is the type of method should the physician follow ? Culture is the gold standard , For fast diagnosis we use; **Lowenstein-Jensen media + MGIT**



Team Leaders

Badr AlQarni

Renad AlMutawa

Team Sub-Leader

Abdullah Alassaf

 **This lecture is done by:**

Team Members

Boys

-  Faisal Alkoblan
- ★ Faris Almubarak
- ★ Alwaleed Alazmi
- ★ Mohammed Alshoieer
- ★ abdullah Alothman
- ★ Faisal Alzahrani
- ★ Abdullah Alzamil

Girls

-  Noura Almazrou
- ★ Rema Almutawa
- ★ Elaf Almusahel
- ★ Lina Alosaimi
- ★ Ghada Alsadhan
- ★ Sarah Alhelal
- ★ Amerah Alzahrani
- ★ Rawan Alzayed
- ★ Sarah Alkhalife

Contact us:



Editing
file



Microbiologyteam438@gmail.com

