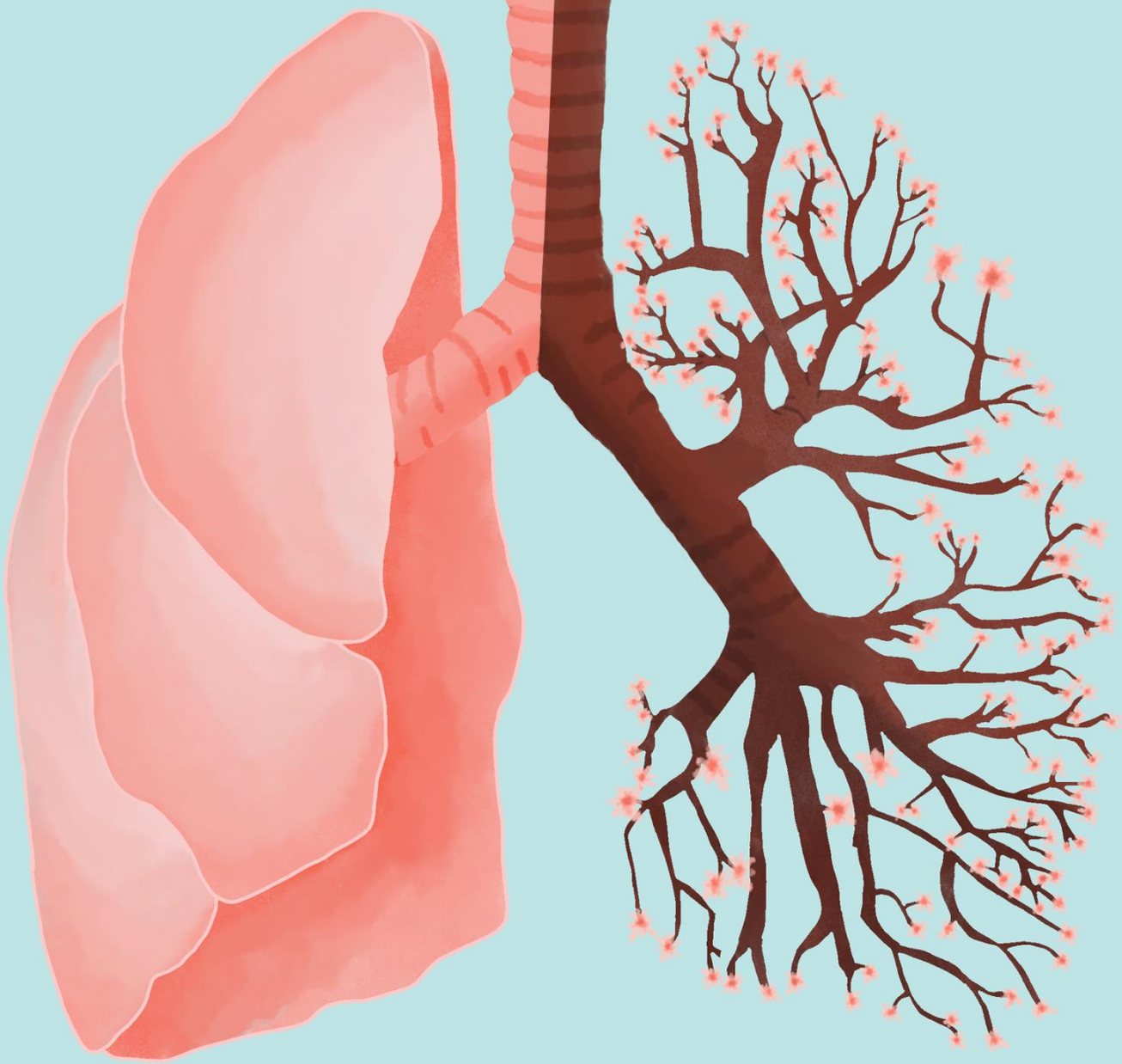
RESPIRATORY SYSTEM

MICROBIOLOGY



Title: Sheet 5 – Tuberculosis

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Science: The chipmunks

Final: The chipmunks

Doctor: Dr. Nader Alaridah

Today we are going to talk about Mycobacteria -The causative agent of tuberculosis.
~The underlined sentences were not mentioned by the doctor~

Enjoy <3

Background

Mycobacterium is difficult to stain (because of mycolic acid & waxy lipids), but once stained it's difficult to decolorize even in the presence of strong acid

We don't use the gram description anymore (gram positive previously), we use acid fast stain instead.

• The mycobacteria are rod-shaped, obligate aerobe, facultative intracellular bacteria that do not form spores.

- 3 types of species that cause diseases in humans:

1. Mycobacterium tuberculosis complex (MTC): a genetically related group (they are 11 members) of Mycobacterium species that can cause tuberculosis in humans.
2. Mycobacterium leprae: a causative agent of leprosy (This is outside the scope of the lecture)

lepromatous leprosy
Tuberculoid leprosy
depending on the arm on the arm of defense against them

environmental mycobacteria

3. Non-Tuberculous (NTM) Mycobacteria: The most known type is Mycobacterium avium-intracellulare (M. avium complex, or MAC) They are opportunistic infections and mostly infect AIDs patients. (We will talk about this briefly, at the end of this lecture)

non-contagious mycobacteria
main route of entry (ingestion)

NTM & AIDS = Fatal

1/3 of the world population have latent Tb and at high risk to develop reactivation if the immunity is decreased and then latent tb will be secondary active Tb

Mycobacterium Tuberculosis (Mtb)

M.bovis was the causative agent of tuberculosis (live in chattels), transmitted through non-pastoralized milk

- It was not until the 19th century, when Robert Koch utilized new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB); Mtb or Koch bacillus
- This is a very old disease which humans were never able to eradicate. Now, incidence rate in western countries is very low but it is still present in developing countries, so they call it "Disease of the Poor".
- It is also called: called consumption- it causes weight loss (used in diagnosis of the disease for cancer) and White plaque – patients become pale. But now, it's mostly called Tuberculous Bacillus, TB.

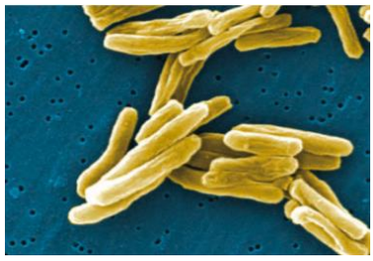
white plaque = extreme pallor seen among patients

• The family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis(TB) in humans and other livings.

• It includes: M. tuberculosis (Mtb) (the principle pathogen), Mycobacterium africanum, Mycobacterium bovis (previously more important, comes from cows before milk pasteurisation begins), Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium canetti. (others differ depending on geography) - 11 in total.

Morphology non-spore forming, non-motile, non-encapsulated

• In tissue, tubercle bacilli are thin, straight rods measuring about 0.3 ~ 3 µ m.



• True tubercle bacilli are characterized by "acid fastness"—that is 95% ethyl alcohol containing 3% hydrochloricacid (acid-alcohol) quickly which decolorizes all bacteria except the mycobacteria's primary staining- Carbon fuschin- a red dye.

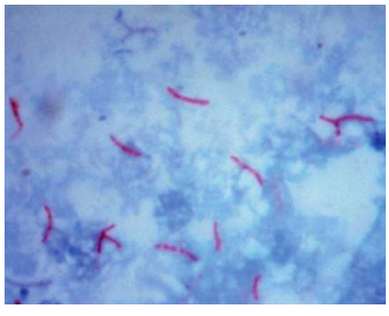
~Hence, mycobacteria are acid fast bacilli~

This technique is called The Ziehl-Neelsen technique

• Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds. Its main living micro-environment are macrophages, but sometimes it is extracellular- hence it's a facultative intracellular.

• The growth rate is much slower than that of most bacteria. The Doubling time of tubercle bacilli is about 18 hours. (We wait up to 8 weeks in labs, when diagnosing, to find colonies, and takes 6 to 12 months for treatment). This has to do with the complexity of the cell wall. E.coli duplicate every 20 minutes

- We call the bacteria waxy because more than 60% of the bacteria's weight is lipids. virulence of the bacteria, low-multiplication rate, acid fastness: all of these features are related to high lipid contents of the bacterial cell wall



As see in the diagram, Acid fast staining- carbon fuschin stain still retains its red colour even after decolourisers. So, it appears this way. This is called smear positive- sometimes found- found in the sputum. Smear negatives are less infectious, but the patients might still have tb.

They are resistant to disinfectants, so if the sputum that goes out of a TB patient lands in a cold-dark place, Mtb will keep viable in the sputum for up to 6 months and TB patients should be isolated - they become non-infectious after the 2-3 weeks after the beginning of the treatment

Acid-fast staining:

1. application of carbolfuchsin (primary stain)-Red
2.application of heat to aid dye penetration
3. application of acid alcohol (decolorizer)
4. application of Methylene blue (counter stain)
Acid fast bacteria will maintain the primary stain (red) while the other types of bacteria will acquire the counter stain (blue) after losing the primary one with the decolorizer

The Gold standard diagnosis is culture. But if there is a strong suspicion that the mycobacterium is Tb, immediately start the treatment and isolate the patient because he is infectious (The multiplicity of infection here is less than 10 mycobacteria).

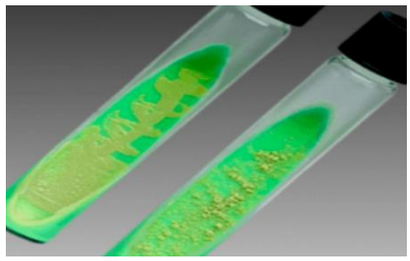
• Mycobacteria tend to be more resistant to chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth,

Mtb Culture

There are 3 options for culturing in labs:

1) Löwenstein- Jensen (oldest, most known): contain defined salts, glycerol, and complex organic substances (e.g., fresh eggs or egg yolks, potato, flour, and other ingredients in various combinations.

   Inspissated egg media and malachite green dye is added- which inhibits the growth of most contaminants but permit only Mtb. semi-solid media



2) Semisynthetic agar media— These media (e.g., Middlebrook 7H10 and 7H11): contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol.
   Selective medium- colonies that are white, creamy, fuzzy. solid-media

dry rough colonies

3) Broth media— (e.g., Middlebrook 7H9 and 7H12): less sensitive and specific than agar culture, but faster. fluid media

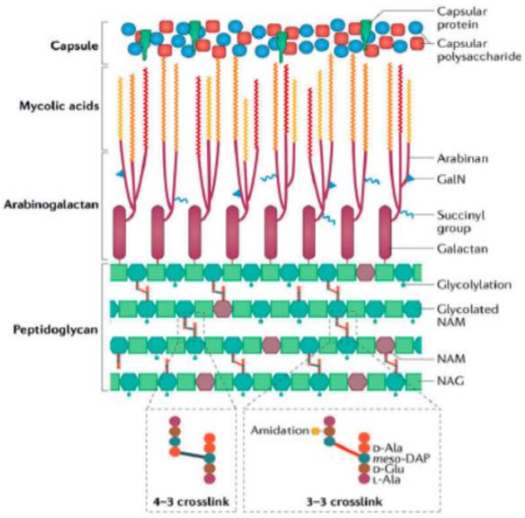
Mtb Cell wall

• The mycobacterial cell wall is a complex structure that is required for cell growth, resistance to antibiotics and virulence.

• It consists of 2 layers:

- An inner layer: composed of three distinct macromolecules— peptidoglycans (PG), arabinogalactans (AG) and mycolic acids (MA)

covalently linked together to form a complex known as the MA-AG-PG complex. These add the complexity of the cell wall. The peptidoglycan layer surrounds the plasma membrane and comprises long polymers of the repeating disaccharide N-acetyl glucosamine–N-acetyl muramic acid (NAG–NAM) that are linked via peptide bridges.



- An outer layer that surrounds the plasma membrane: other lipids and other polysaccharides, not required for exam purposes. (we took in 2nd year)

sulfatides are sulfur lipids present in the outer layer, they inhibit the phagosome-lysosome fusion and thus they evade the phagocytosis once they get inside the body, they will ingulfed by alveolar macrophages, inside these professional phagocytes they will evade the phagocytosis by blocking early autosomal autoantigen-1

Virulence factors:

- Lipoarabinomannan (LAM) and lipomannan

-trehalose dimycolates- Mycolic acids (long-chain fatty acids C78–C90), waxes, and phosphatides, can be found in Mtb cell wall and make up 50% of the dry weight of the mycobacterial Cell envelope, also called Cord Factor. It causes clumping of the bacteria, which deprives the bacteria from nutrients and allows it to enter the dormancy stage inside the human body.

they also have type-7 secretion system

Epidemiology:

• Two TB-related conditions exist:

-latent TB infection (LTBI): The MTB is present in the body but it's not infectious and shows no signs or symptoms, occurs in 2/3 of the world's population.

when they become immunocompromised, they will develop secondary reactive TB

***1/3 of the world's population

-Active TB disease. If not treated properly, TB disease can be fatal.

Only small proportion of those infected will become sick with TB.

In 2015, an estimated 10.4 million new, active TB cases were seen worldwide, of which 1.5 million died. It is the single most killer infecting agent in humans and the number one killer in AIDS patients (because it's an intracellular bacterium, so its main defence is the cell mediated immunity which is low in HIV patients) This is a deadly combination.

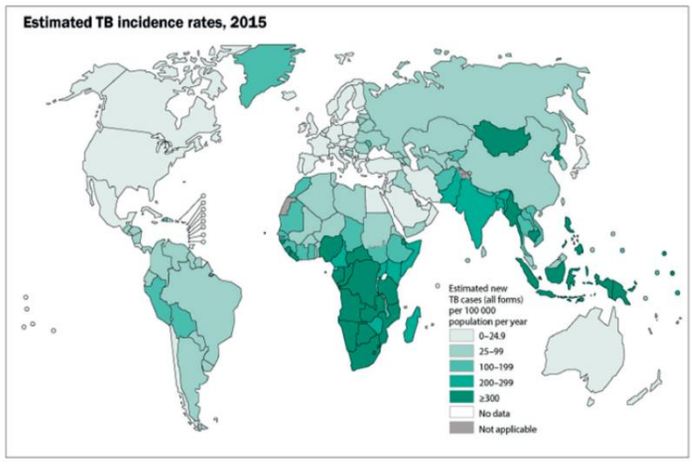
not everyone gets infected by Mtb will develop TB, that depends on the infectious dose, the main status of the patient and the environment

- 3 bacilli of Mtb are enough sometimes to establish the infection
- immunocompromised patients (AIDS or immunosuppressive agents) are at higher risk to develop active TB and it is the leading cause of death in AIDS patients

in 2020 statistics, 10 million TB cases were detected & 1.5 million deaths

In Jordan, in every 100,000 person there are 25-35 cases yearly, which increased later with the presence of more refugees, which is a good incidence rate in a developing country.

• TB is considered an airborne infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurised milk, and direct inoculation.



- As we can see in the picture , the countries with a dark color have TB incidence rates that reach hundreds.

Examples on countries with high rates : South Africa, Switezerland and the Soviet Union countries.

People who came from the Soviet Union countries were at the top in regarding to TB diagnosis. These people suffered from an issue called MDR (multi drug resistance TB) and also suffered from extensively drug resistance TB which will be talked about later on.

- INFO : No antibiotic works on extensively drug resistance

Tuberculosis TB

In pulmonary TB infiltration occurs allover both lungs: Miliary TB (millet seed-sized) and those lesions could erode to a blood vessel or lymphatics and access the circulation

As a disease entity we have 2 kinds of TB : Active TB and Latent TB
But depending on sites we have Pulmonary TB and Extra Pulmonary TB , Pulmonary TB is the most common form(more than 90% of the cases) and it can develop to become Extra Pulmonary TB. The Extra Pulmonary can take place anywhere in the body.

- The primary site of TB is usually lung, from which it can get disseminated into other parts of the body. The other routes of spread can be contiguous involvement from adjacent tuberculous lymphadenopathy or primary involvement of extrapulmonary organ.

• Spread – Lymphatic vs hematogenous (Miliary).

scrofula = cervical lymphadenitis

• TB bacteria can attack any part of the body such as the pleura ,L.N.(when TB happens here we call it Scrofula) ,pericardium, kidney, spine (we call it Pott's disease) , brain (TB here can happen in meninges , it happens in children and is

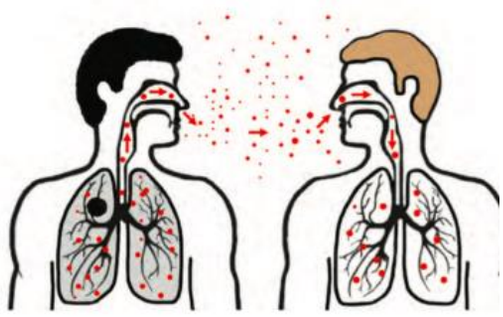
usually, extra-pulmonary TB occurs post to Pulmonary TB (but it could occur independently)

considered the most serious one) and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.

• Primary Infection(Active) and Reactivation Types (Secondary) of Tuberculosis.

- If someone is infected with the mycobacterium of TB and containment of it happens , it doesn't develop into an active disease and if the immune status got compromised for some reason after a year or 10 years , Reactivation happens to the dormant bacilli.
- There are clues to know which type of Tb it is , for example : Primary(active) happens in the middle and lower lobes while Reactivation happens in the apex of the lobe.
- Mycobacterium TB dissemination can be direct for example in abdominal TB : the TB in lungs can be disseminated through the diaphragm and reach the abdomen.

Transmission



• TB is considered an airborne infectious disease(means even when the droplets evaporate the bacteria is still alive which makes it dangerous), heating and ultra violet light are used to disinfect the mycobacterium although M. tuberculosis complex organisms can be spread through unpasteurised milk, direct inoculation and other means.

• The underlying pathophysiology of TB is the "10/3/1 formula : every 10 people exposed to mycobacterium TB , 3 of them will develop latent TB and 1 will develop Active TB which means that 6 people got rid from the mycobacterium through innate immune system or adaptive or both.

the risk of Reactivation of LTBI is high in the first 2 years (in 50% of cases)

Pathogenesis

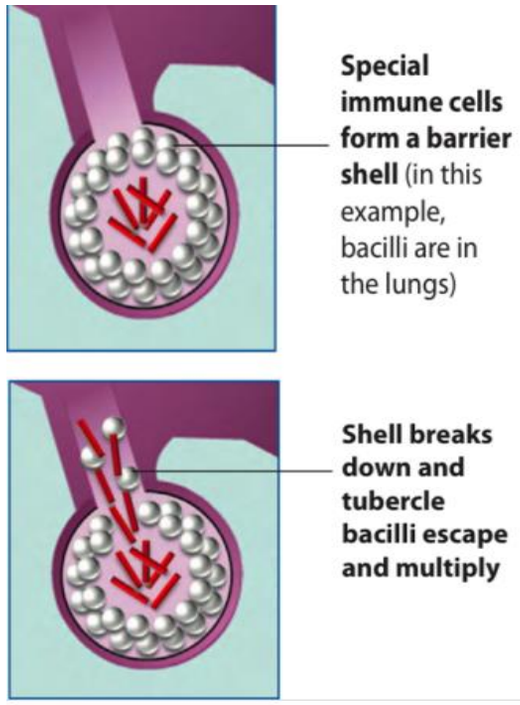
• Mycobacteria are in droplets when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli then they get internalized into macrophages

• Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages.

in the site of Mtb there will be formation of acute exudative lesion called Ghon focus if that ghon focus gets access to draining lymph nodes there will be formation of Ghon complex, resolution of the focus or complex could occur where the body absorbs the exudative lesion then fibrosis and calcification occur, and active TB will not occur
when dissemination happens, active TB occurs wither pulmonary or extrapulmonary

Mycobacteria they get inside endosome then phagosome ,it inhibits the fusion of phagosome and lysosome and inhibits the acidification of phagolysosome.

• Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli.



The thing that is considered a defense mechanism done by our bodies and the hallmark of pathogenesis of mycobacterium is granuloma formation which happens when an infected macrophages and another recruited macrophages are differentiated into epithelioid cells and then the recruitment of lymphocytes fibroblasts.

• The cells form a barrier shell, called a granuloma, that keeps the bacilli contained and under control (LTBI). the acute exudative lesions will develop into chronic productive type (granuloma), the hall mark of pathogenesis

• If the immune system cannot keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (TB disease).

Primary Infection and Reactivation Types of Tuberculosis

• An acute exudative lesion develops and rapidly spreads to the lymphatics and regional lymph nodes. The exudative lesion in tissue often heals rapidly.

• In primary infections, the involvement may be in any part of the lung but is most often at the base. • The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion

• The reactivation type almost always begins at the apex of the lung, where the oxygen tension (PO2) is highest.

Clinical manifestation the progress is very slow, and symptoms takes months to develop

• Classic clinical features associated with active pulmonary TB are coughing, weight loss/anorexia, fever, night sweats, haemoptysis (coughing blood), dyspnea (chest pain) and malaise/fatigue.

• Tuberculosis is usually a chronic disease; it presents slowly with weight loss(significant weight loss is seen), low-grade fever, and symptoms related to the organ system infected. Because of its slow course, it may be confused with cancer. Whenever you have an infection of any organ system, tuberculosis will be somewhere on your differential diagnosis list.

• It is one of the great imitators one of the differential diagnoses of weight loss

To diagnose Mtb there 2 not definitive and 1 definitive **diagnostic test** :

-**Both not definitive**

Look at the next page

1) complete Blood count ➔ Rise on WBCs
2) X-ray ➔ You can see the Ghori complex Hilar lymph node are commonly involved and Miliary TB if present of course you will see it

-**And Definitive ➔** through culture the most specific one

We can also use 2 tests (screening tests, not diagnostic): Tuberculin skin test (TSTs) and Interferon-gamma release assays (IGRAs).

- TSTs test => purified protein derivative that is taken from Mtb and given to patients intradermally. The patient is asked to come back after 48 hours , if he was sensitized to the mycobacterial antigen, he will develop erythema and raised skin.

But we have to know that this test has a lot of false positive results, especially in the vaccinated people and the people who were already infected with one of the environmental mycobacteria.

- IGRA test => we take a blood sample from the patient, we mix this blood with mycobacterial antigen, which we already have in the lab, and we measure the Interferon-gamma release and based on certain cut of values, we can determine if this patient has been expose to mycobacterium before or not.

no false positive ←

one tube contains Early secreted protein-6 and another one with culture filtrate protein-10 → Mtb antigens only

Note: these 2 tests don't tell us if the infection is active or not. They just tell us if this patient has a history with this disease by recognizing the mycobacterium antigen.

Laboratory diagnostic methods

highly specific but with low sensitivity, if no acid-fast baccili were found in the sputum this doesn't exclude that the patient has the infection

❖Smear microscopy — sputum sample

- Three specimens from each patient with suspected TB should be examined microscopically for Acid Fast Bacilli AFB (classically Ziehl-Neelsen) or mycobacteria can be demonstrated by yellow fluorescence after staining with auramin. → After auramin staining we do Ziehl-Neelsen stain

❖Culture (most specific diagnostic test)

- Both liquid and solid mycobacterial cultures should be performed for every specimen, and recovered isolates should be according to standard criteria (Lowenstein-Jensen or Middlebrook 7H10), Radiometric broth culture (BACTEC radiometric system) and mycobacterial growth indicator tube (MGIT)
- Culture for acid fast bacilli is the most specific test for TB and allows direct identification and determination of susceptibility of the causative organism

❖A nucleic acid amplification test (NAAT), Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well.

- smear microscopy usually correlates with disease severity as well as the infectiousness.

Treatment

The course of TB treatment depends on whether the individual is in the latent or active stage, and on his or her probability of risk.

The standard regimen treatment of TB is 6 months and usually involves a drug cocktail, or a mixture of multiple drugs. We start with 4 drugs during a 2-month intensive phase, followed by a slower 4- to 6-month continuation phase. The main anti-tuberculosis drugs used in the chemotherapy of TB are: isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM)

These drugs have a lots of side effect and a very low compliance. The patient starts to feel good during the first 3 to 4 week, then the bacteria will develop a selective resistance. That is why we give the patient multiple drugs.

Some countries treat it in 9 months and others in 12, and some countries use a type of treatment called DOT treatment (directly observed treatment), which makes the patient the medication in front of a medical person to that he takes it.

If the patient has a resistance for isoniazid and rifampin, we call this case a multi-drug resistance. In this case, we use the second line of treatment: fluoroquinolones and injectable anti-tuberculosis drugs.

If the bacteria develops resistance for isoniazid and rifampin and one of the injectable drugs, this is called extensive drug resistance, which is most likely not treatable .

Isoniazid preventive therapy(IPT) is the recommended treatment for latent TB but the regimen's main drawback is the duration of therapy (but if you have a suspicion of tb, initiate the treatment immediately and isolate him).

Prevention

As we know, prevention is better than cure and the best way to prevent TB is to diagnose and isolate infectious cases rapidly, and to administer appropriate

treatment until patients are rendered non-infectious (usually 2–4 weeks after the start of proper treatment) and the disease is cured.

Additional strategies include BCG vaccination and treatment of persons with LTBI who are at high risk of developing active disease.

أول مطعوم بناخده و بترك ندبة

Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine, derived from M. bovis, is the only licensed vaccine against tuberculosis (TB), but this vaccine can be effective from 0% to 70%. Scientists don't now why this happen exactly but we still take this vaccine because its effective to protect from the most serious one,TB meningitis, which occurs in young children. The low efficiency vaccine occurs in the most common form, pulmonary TB, on adults.

and military TB

0%

OTHER MYCOBACTERIA

They cause mild disease in normal individuals, but severe ones in the immunocompromised patients, mainly HIV.

The nontuberculous mycobacteria (NTM) is a diverse group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens

The NTM can be further classified into 2 groups: the rapid growers (grow in < 7 days) and slow growers (grow in > 7 days). Each group can be subdivided on the basis of pigment production to (photochromogens (produce pigment in light) , scotochromogens (produce pigment in darkness) and nonchromogens (don't produce pigment)))

Note :The most common type of **nonchromogens** is mycobacterium tuberculosis

Now , we will talk briefly about Mycobacterium avium Complex (MAC or MAI) :

route of transmission, inhalation or ingestion

- MAC organisms infrequently cause disease in immunocompetent humans. MAC infection is one of the MOST COMMON opportunistic infections of bacterial origin in patients with AIDS.

The nontuberculous mycobacteria (NTM) more than 100 type but the most important ones are :

1-Mycobacterium kansasii ➔ Cause Pulmonary disease (TB-like)

2-Mycobacterium marinum ➔ Cause Aquatic Granuloma

3-Mycobacterium ulcerans ➔ Cause skin and soft tissue infection

(1 and 2 and 3 are slow growing and Photochromogen)

4-Mycobacterium scrofulaceum ➔ cause lymph node inflammation (slow growing and Scotochromogen)

Scrofula
↳ Most common cause of scrofula in children;

5-Mycobacterium avium complex, or (MAI). ➔ Most common in AIDS Most one isolated

nonchromogen, slowly grower

6-Mycobacterium fortuitum Complex ➔ Causes Pulmonary infection

7- Mycobacterium chelonae-abscessus ➔ Causes skin infection

(6 and 7 are fast growing), nonchromogen

"The Internet has a lot of solutions. but there's no insane way to trap chipmunks"