Sheet n. 11

Doctor 22

فريق طوفان الاقصى

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Mycobacteria

"بكتيريا غلّبت البشرية على مدى عقود"

It's an acid-fast bacteria.

There's also another bacteria of the same class & order as Mycobacteria; which is called Nocardia (causes nocardiosis). Both are acid-fast bacteria

BACKGROUND

- **The mycobacteria are rod-shaped (bacilli), aerobic bacteria that do not form spores.**
- **Taxonomy: class: Actinomycetes. Family: Mycobacteriaceae, this family includes several members of high medical importance, most importantly [1] Mycobacteria Tuberculosis Complex which consists of 11 members. (MTC) is a genetically related group of Mycobacterium species in which each member can cause tuberculosis in humans and other livings.**
- **Another member of mycobacteriaceae is [2] Mycobacterium leprae which causes leprosy الجذام.**

A third member of Mycobacteriaceae is [3] **nontuberculous mycobacteria (NTMs)** which cause tuberculosis-like diseases. Another name for them: "MOTs" which is short for "Mycobacteria other than tuberculosis". They are collectively known as environmental mycobacteria (their source is the environment).

- **Most popular member of NTMs is *Mycobacterium avium-intracellulare* (Mycobacterium avium complex, or MAC). MAC and other nontuberculous mycobacteria (NTMs) frequently infect patients with AIDS (No.1 killing in AIDS patients), are opportunistic pathogens in other immunocompromised persons, and occasionally cause disease in patients with normal immune systems.**

هذول الثلاث بنسميهم Mycobacteria لأنهم من نفس العائلة Mycobacteriacea

So, in this lecture we'll talk briefly about 3 small topics, starting with:

MYCOBACTERIUM TUBERCULOSIS (MTB)

بيعملوا مرض السُّل أو التدرُّن

- **It was not until the 19th century, when Robert Koch utilized a new staining method (ZN stain) and applied it to sputum from patients discovering the causal agent of the disease Tuberculosis (TB) which is MTB or sometimes referred to as: Koch bacillus.**

Koch was the first to do isolation and identification to Mycobacteria, (another name of MTB is Koch bacillius) he modified the gram-stain technique to fast-acid stain, then ziehl-neelsen stain appeared afterwards.

- **Other names for Tuberculosis: consumption (consume patients, significant weight loss in a short duration), white plaque (extreme pallor seen among patients because of hypoxemia and chronic productive cough), phthisis.**
- **Any member of family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis (TB) in humans and other livings.**

Remember that the MTC family is of 11 members, the principle member of which is MTB. But the other 10 can also cause tuberculosis. They vary in geographical distribution, virulence, severity of disease

- **It includes M. tuberculosis (Mtb), Mycobacterium africanum, Mycobacterium bovis, Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium suricatte, Mycobacterium mungi, Mycobacterium dassie, Mycobacterium oryx and Mycobacterium canetti.**

-Mycobacterium africanum ⟶ high prevalence in Subsaharan Africa.

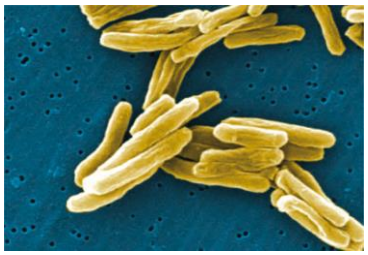
-Mycobacterium bovis ⟶ found especially in unpasteurized milk. وهو النوع الوحيد الي سوينا منه مطعوم

NOTE: classification of gram +ve and gram –ve doesn't apply on Mycobacteria, it is neither of them. It's an acid-fact bacilli.

MORPHOLOGY

- **In tissue, tubercle bacilli are thin, straight rods measuring about 0.3 ~ 3 µm.**
- **True tubercle bacilli are characterized by "acid fastness"—that is, 95% ethyl alcohol containing 3% hydrochloric acid (acid-alcohol) quickly decolorizes all bacteria except the mycobacteria.**
- **Mycobacteria are obligate aerobes and derive energy from the oxidation of many simple carbon compounds.**

  ⟶ And that's why the most clinical form of tuberculosis is called "pulmonary tuberculosis", happens in the lungs. Another clinical entity of tuberculosis is collectively known as "extra pulmonary TB".



They are also facultative intracellular, their preferred habitat is professional phagocytes (macrophages) even though they are antigen-presenting cells.

So, they (MTB) hijack the immune system for their benefit.

- **The growth rate is much slower than that of most bacteria. The doubling time of tubercle bacilli is about 18 hours.**
- **Mycobacteria tend to be highly resistant to the commonly used disinfectants, sterilizing methods and chemical agents than other bacteria because of the hydrophobic nature of the cell surface and their clumped growth.**

*Order of resistant to disinfectants: prions > endospores > mycobacteria.

*Phenolic compounds might work to sterilize them.

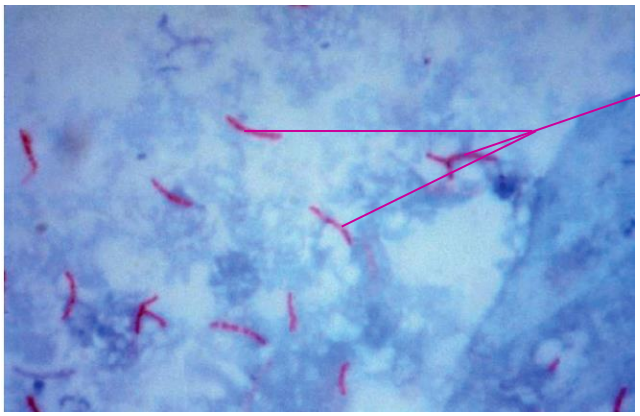
Also, mycobacteria is highly resistant to dryness and desiccation التجفيف.

One of the very famous experiments:

If you take a sputum بلغم sample from a TB patient and put in a dark and humid room. MTB will stay viable up to 3 months.

أصلًا فكرة ال quarantine طلعت مع مرضى السل، لأنه معدي جدًّا.

*TB is a highly contagious airborne infection, transmitted through droplet nuclei (details later on).



The sticks represent MTB (bacilli)

(This is a hypermicrograph for a sputum sample of a pulmonary TB patient)

Here in ZN stain or Acid fast stain, we use a red or pink pigment called carbonfyoxin stain, then we heat the sample to aid the penetration of the dye through the cell wall of MTB, which is of a high level content of lipid. Then we treat it with acid alcohol which will remove ANY dye from the bacteria UNLESS it's acid-fast. So if we put methylene blue for example, it won't dye the sample since we have already used the the red and the acid-fast stain on the sample.

بنستنتج انه اسم الصبغة acid-**FAST** لأنه بتخلي العيّنة تصوم عن الصبغات الثانية (بتمنعها).

يعني العيّنة انصبغت باللون الأحمر أو الزهري وطالما استخدمنا ال acid-fast بعد هيك، رح يثبت اللون الزهري ولو حطينا صبغة تانية بعد هيك ما رح تشتغل (بتصير زي مانع أو درع).

*Mycobacteria mainly affects the lower respiratory tract.

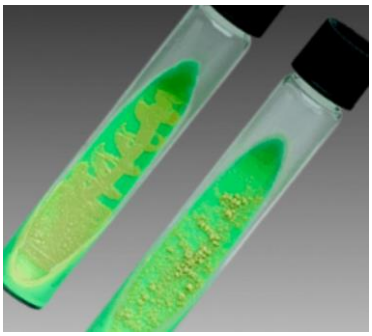
*مافي بكتيريا بينطبق عليها ال fast-acid stain غير الMycobacteria

وبتشمل ال environmental, leprae, nocardia اللي حكينا عنهم بأول المحاضرة

MTB CULTURE

- **The media for primary culture of mycobacteria should include a nonselective medium and a selective medium.**
- **Semisynthetic agar media (semi-solid)— These media (eg, Middlebrook 7H10 and 7H11) contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol .**
- **Inspissated egg media (solid)— These media (eg, Löwenstein- Jensen) contain defined salts, glycerol, and complex organic substances (eg, fresh eggs or egg yolks, potato flour, and other ingredients in various combinations.**
- **Broth media (fluid)— (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inoculate.**

MTB COLONIES



(Stain: malachite green, to inhibit growth of other contaminant)

- Most specific method for diagnosis of MTB is culture
- This is how the MTB colonies appear on the agar-based media
- Dry, rough, wrinkled colonies
- Growth is slow, starts after 4th week. to discard it as –ve, you're going to have to wait 8 weeks to say it's –ve for MTB.
- Of course, the patient can't wait for 4 weeks. So, in case of high index of suspicion, they should start with the treatment immediately before the diagnosis takes place

- Highly specific but the sensitivity is low,

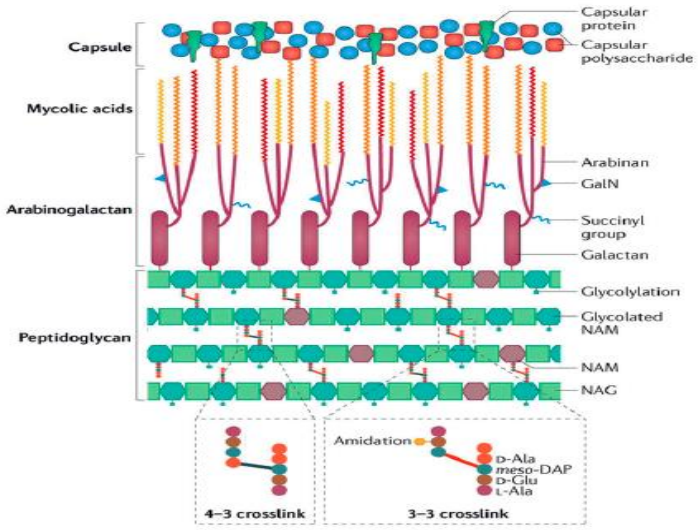
يعني اذا ما طلع معك بال culture هذا لا يعني انه المريض ما عنده.

- Doubling time is very slow. If you compare it to E-coli, its population doubles every 20 minutes. Meanwhile in MTB it need 24 hours for doubling. Which reflects on the treatment duration (6-12 months).

MTB CELL WALL

- **The mycobacterial cell wall is a complex structure that is required for cell growth, resistance to antibiotics and virulence. The complexity of mycobacteria is attributable to the high lipid content in the cell wall.**

Mycobacteria is non-capsulated, non-motile, non-spore-former



- **The cell wall of MTB consists of an inner layer and an outer layer that surrounds the plasma membrane. The inner compartment is composed of three distinct macromolecules — peptidoglycans (PG), arabinogalactans (AG), mycolic acids (MA) — covalently linked together to form a complex known as the MA-AG-PG complex.**
- **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 cross-linked via peptide bridges.**
- **Most of the arabinan is ligated with long-carbon-chain mycolic acids, which form the characteristic thick waxy lipid coat of mycobacteria and are major contributors to the impermeability of the cell wall and to virulence.**
- **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.**

  The outer layer (cell wall) consists of lipids and polysaccharides, increasing the complexity. 50-60% of dry weight of MTB is just lipids. And this is where the idea of acid-fastness came from.

- **These mycolic acids are esterified to glycerol and trehalose where trehalose can contain one or two molecules of mycolic acids forming trehalose dimycolates (TDM) (Cord Factor) and trehalose monomycolates (TMM).**

There are many virulence factors present in MTB. The most well-studied one is Cord Factor or TDM which is responsible for 2 things: In vivo, it prevents the migration of leukocytes towards the infected area, giving a chance for mycobacteria to multiply. In vitro, when we do culture, this cord factor is responsible for “serpentine growth” of mycobacteria. Making the bacteria grow in parallel to each other (clumping). بتجمعوا وبينموا جنب بعض

Sulfolipids live inside macrophages, prevent phagosome-lysosome fusion, shutting off the oxidative burst inside the antigen-presenting cells.

Phosphatidyl monocytes & Type-7 secretion system are also virulence factors present in MTB.

EPIDEMIOLOGY

There 2 clinical entities after the infection of MTB:

1. Active TB: acute inflammation, development of signs and symptoms
2. Latent TB: they have it but they don't feel sick or show symptoms or signs, **NOT CONTAGIOUS.**
   but once the host becomes immunocompromised, they might develop secondary reactivation for TB.

يعني الناس اللي انصابوا أول مرة ← primary TB ، وثاني مرة ← secondary TB

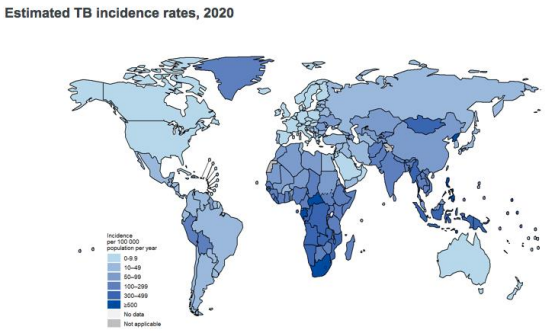
- **Two TB-related conditions exist; latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal. People who have latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB to others**
- **About 1/3 of the worlds population is infected with TB bacteria (TB latency).**
- **However, only small proportion of those infected will become sick with TB.**
- **TB remains a leading cause of infectious diseases morbidity and mortality. In 2015, an estimated 10.4 million new TB cases were seen world wide.**
- **TB is considered an airborne (main route: respiratory, inhalation) infectious disease although M. tuberculosis complex organisms can be spread through un-pasteurised milk, direct inoculation and other means.**

The most common form of TB active disease is **pulmonary TB**

-MTB is the no.1 killer of all bacterial diseases, single caused

Also the no.1 killer in patients with AIDS

أكثر دول بيعانوا منه جنوب أفريقيا(الصحراء الكبرى)، جنوب شرق آسيا، أمريكا الجنوبية، جرينلاند ودول الاتحاد السوفييتي سابقًا.



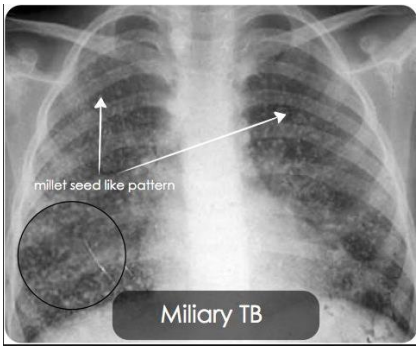
-MTB has **no temperature preference**, unlike Mycobacterium leprae (optimal growth at 30°C) which usually infects patients with leprosy in relatively cold parts of the body like the extremities and face.

TUBERCULOSIS TB

- **The primary site of TB is usually lung (pulmonary TB), 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.**

Inhalation, then they picked up by monocytes (remember they're not motile), arrive to the alveoli and they called alveolar macrophages. So once they arrive, they do the inflammatory process

- **Spread – Lymphatic vs hematogenous (Miliary).**
- **TB bacteria can attack any part of the body such as the pleura , L.N. , pericardium, kidney, spine, brain and abdomen (abdominal Tuberculosis) collectively known as extrapulmonary TB.**
- **Primary Infection (Active) and Reactivation Types of Tuberculosis.**

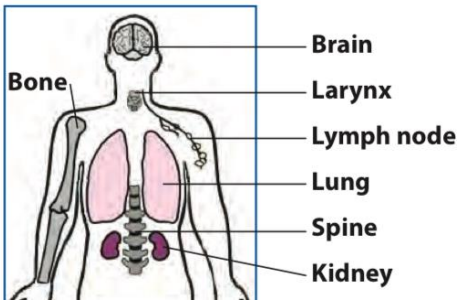


The white dots "millets", each one represents "gohn focus" that has inflammation around multiplying MTB. They might infect adjacent structures or spread hematogenuously throughout all the body by blood, which is called **miliary TB.** One of the severest forms of TB, which infects a lot of children. Leading to **tuberculous meningitis**.

In addition to adjacent structures and hematogenuous routes(blood), it could also go through the lymphatics, If there's an involvement of regional lymph node, each ghon focus becomes ghon complex.

ممكن كمان يتوزع على lymph nodes و lymphoid organs أكثر من الregional lymph

*If the the body contained this inflammation (granuloma), then the granuloma becomes sterilized (complete resolution) or it gets developed to caseous necrosis, cavitary lesions.



Pulmonary TB makes up about 80-85% of the cases of active TB

The rest 15% are collectively known as **extrapulmonary TB,** which can infect any part of the body outside the lungs. Like: bones, kidney, spine (PoT disease), abdomen (abdominal TB), pericardium, pleura, larynx, cervical lymph node (Scrofula disease).

Transmission

- **TB is considered an airborne infectious disease 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**.

- The "10/3/1" formula has high consensus and is widely accepted, as it means that: Out of 10 people exposed to mycobacterium tuberculosis ➔ 3 of them will go through LTBI (Latent Tuberculosis Infection) ➔ and 1 out of 10 exposed people is going to develop active tuberculosis from the first exposure.
  Notice that there are 6 people left —out of the 10 exposed people— who somehow cleared the infection by their innate or adaptive immunity. However, there is a big debate regarding mycobacterium tuberculosis that our body cannot get rid of it and it remains present in our body as we don't have a sterilizing immunity against it.

- Despite the researches and the studies on mycobacterium tuberculosis, we still use the same drugs, vaccine and treatment procedures from 70 years!
  Maybe one day in the future you'd come up with new treatment methods and win the noble prize□□♂.

- Remember that the body of the people with LTBI contains mycobacterium tuberculosis; but those people are not contagious to others and they don't show any symptoms or signs of disease. However, when these people become immunocompromised ➔ the latent mycobacteria might be reactivated.

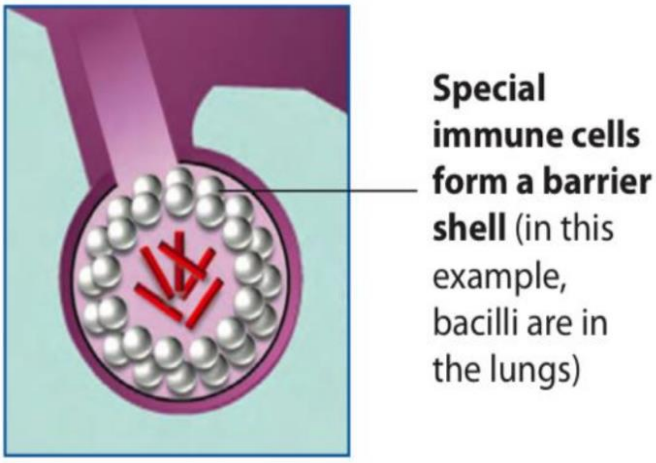
Pathogenesis

- **Mycobacteria are in droplets** (airborne route through infectious aersols and droplet nuclei, as the most common clinical form of tuberculosis is pulmonary tuberculosis) **when infected persons cough, sneeze, or speak (or shout or sing). The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli**.

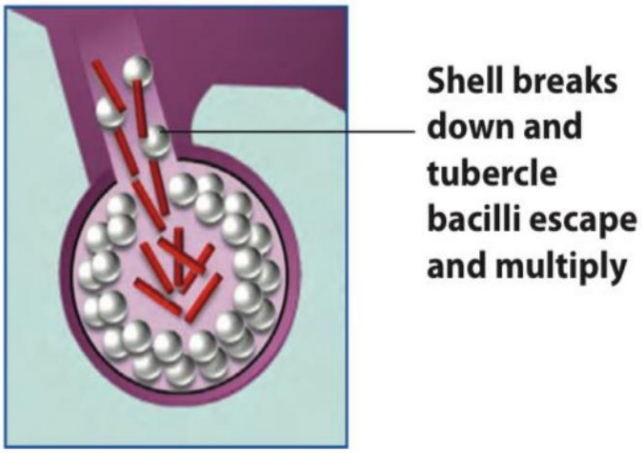
- **Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages.**

- **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 cells form a barrier shell, called granuloma ,that keeps the bacilli contained and under control (LTBI**).



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



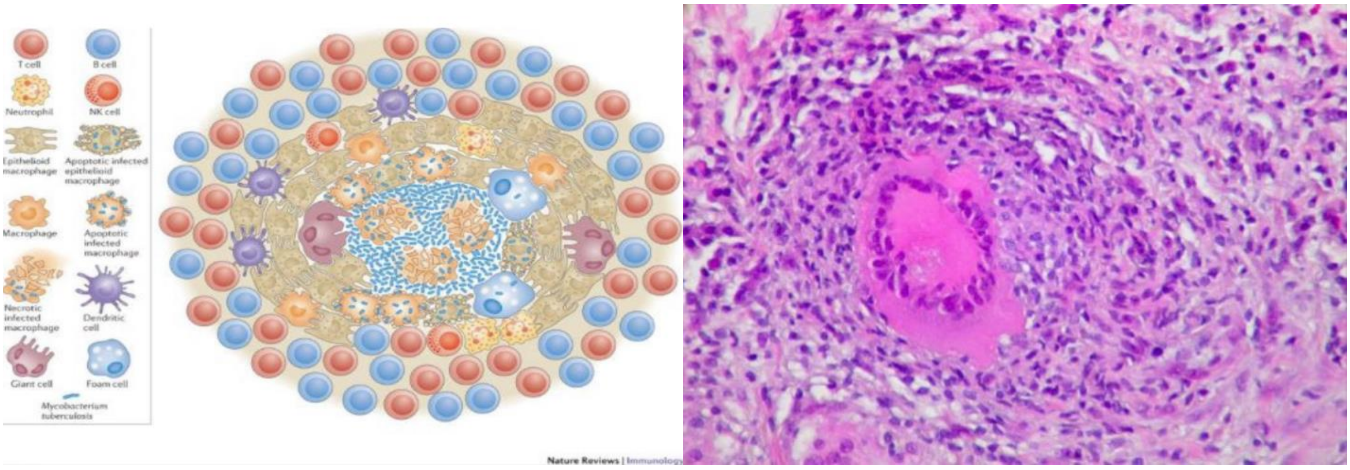
Pathology

- Inflammation-wise, when the mycobacterium tuberculosis first enters the body, it gets phagocytosed by alveolar macrophages which produce an exudative lesion (edema), in which the majority of inflammatory cells there are polymorphonuclear cells (PMN's).

- **Exudative type—This consists of an acute inflammatory reaction with edema fluid; polymorphonuclear leukocytes; and, later, monocytes around the tubercle bacilli. This type is seen particularly in lung tissue, where it resembles bacterial pneumonia.**

- The edematous fluid may stay as it is, or it may be absorbed and the tissue be healed, or it may develop into chronic productive type (granuloma formation).

- **Productive type—When fully developed, this lesion, a chronic granuloma, consists of three zones: (1) a central area of large, multinucleated giant cells containing tubercle bacilli** (and foamy cells could be present, which are lipid-containing macrophages)**; (2) a mid zone of pale epithelioid cells** (macrophages with abundant cytoplasm which resemble epithelial cells)**, often arranged radially; and (3) a peripheral zone of (fibrous ring) fibroblasts, lymphocytes, and monocytes**.



Histological section from lung biopsy

- The whole pathology of mycobacterium tuberculosis infection is the granuloma formation.

- Granuloma is a dynamic structure which is formed as a response of the immune system to contain intracellular infection, which might heal spontaneously or the condition might elevate, and caseous necrosis might occur through which immune cells in the granuloma rupture and the mycobacteria leave the granuloma and spread to invade adjacent structures.

- There are many factors involved in whether the mycobacteria remains latent or gets reactivated, but most importantly, remember that people who are immunocompromised develop reactivated latent mycobacterium tuberculosis or primary acute tuberculosis.

- There are many immunosuppressive conditions nowadays, for example: cancer patients, AIDS patients, malnutrition (which is number 1 cause of immune deficiency worldwide) and people who take steroids.

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** (in both lungs).

- **The reactivation type is usually caused by tubercle bacilli that have survived in the primary lesion.**

- **The reactivation type** (secondary reactivation) **almost always begins at the apex of the lung** (in both lungs), **where the oxygen tension (PO2) is highest** (highest partial pressure of oxygen).

Clinical manifestation

- It is not easy to diagnose someone with tuberculosis, as there is a lot of dilemma in the diagnosis, but usually, the patients share a few symptoms or signs.

- **Classic clinical features associated with active pulmonary TB are coughing** (a chronic productive cough, which is the most common symptom and it is called productive because it produces sputum), **weight loss/anorexia** (significant weight loss over a short period of time), **fever, night sweats, haemoptysis (coughing blood), dyspnea (chest pain) and malaise/fatigue**. اذا بتفكر حالك رح تشخص مريض سُلّ من اول مرة، لازم يعطوك جائزة نوبل =)

- The symptoms mentioned above are associated with pulmonary tuberculosis; which is the most common clinical form and occurs in 85% of the cases of tuberculosis.
If the tuberculosis is extrapulmonary; then the symptoms of the disease depend on the infected organ.

- **Tuberculosis is usually a chronic disease; it presents slowly with weight loss, 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**, as it imitates cancer because one of its symptoms is significant weight loss in a short period of time which also occurs in cancer.

- That's why people who are suspected to have lung cancer try differential diagnostic methods because pulmonary tuberculosis is also suspected as it shares common symptoms with cancer.

Laboratory diagnostic methods

❖ **Smear microscopy:**

- **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.**

- Smear microscopy as a diagnostic method is cheap and quite easy to process. However, it has very low sensitivity. For example, if you take sputum from the patient and put it under the microscope after acid-fast (Ziehl-Neelsen) staining, the test could be negative without showing any bacilli when the patient actually has tuberculosis (false negative), so the negative test is not a conclusive result in this technique.
- Since babies (<1 year) don't have sputum, we use BAL (bronchoalveolar lavage) زي الغسول

❖ **Culture:**

- **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.**

- Culturing is the gold standard diagnostic method, as it is highly specific. وممكن يطلع غلط برضو

- The issue with culturing is that it takes quite a long time.
- Broth culture is the quickest culturing method as it takes from 2 to 3 weeks.
- Custamized broth cultures for mycobacteria: BACTEC radiometric system and MGIT.

❖ **A nucleic acid amplification test (NAAT)** (also PCR with specific probes for mycobacteria could be used for diagnosis, but it is not highly specific)**, Tuberculin skin tests (TSTs), Interferon-gamma release assays (IGRAs) are commonly used as well.**

- Imaging could also be used in the diagnosis of tuberculosis, such as CT or X-ray imaging which allow you to observe cavitary lung lesions in the cases of tuberculosis.
- We use multiple methods instead of 1 in the diagnosis of tuberculosis + high index of clinical suspicion.

❖ Tuberculin Skin Test (TST):

- Tuberculin skin test is quite famous and was used a lot in the diagnosis of tuberculosis, but it turned out to have many constraints in interpreting it.
- Another name for TST is mantoux test.
- The principle of this test is type-4 hypersensitivity reaction (cell-mediated immunity).
- We take virulence factors from the mycobacteria, which are callled PPD's (Purified Protein Derivatives) that are derived from the mycobacteria's cell wall.
- Then, we inject them intradermally and we ask the patient to come back after 48 hours to measure the induration
- After that time period, there are two possible outcomes:

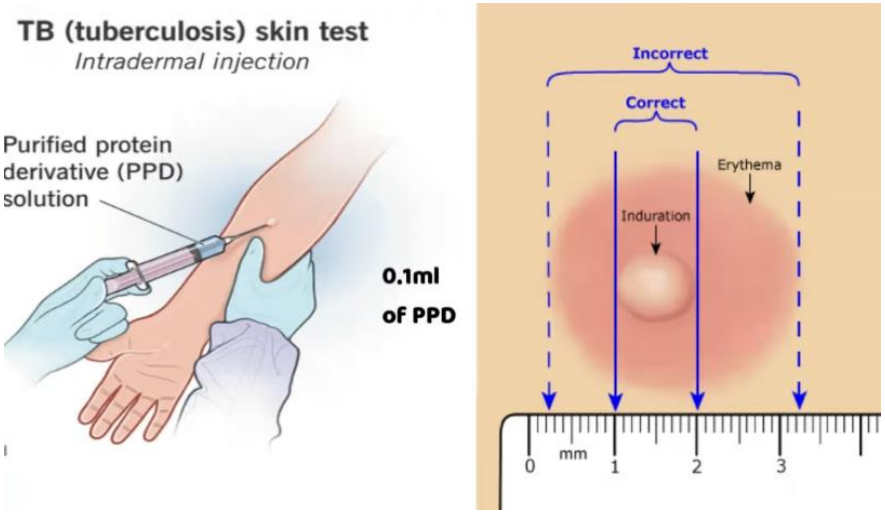
1) Either there will be an induration at the site of injection (induration is a hardened edema which we can sense or examine by palpation, not the area covered by erythema or redness).
2) Or there won't be an induration and the skin is going to be flat.

- Presence of an induration indicates an inflammatory reaction at the site of injection, which also means that the body's immune system was exposed before to Mycobacterium tuberculosis or another mycobacteria and recognized tuberculin PPD (which is a Mycobacterium-derived PPD) when it was injected ➔ the test is positive.

- The absence of an induration and the flatness of the skin indicates that the body got rid of the PPD's without a hypersensitivity reaction, which means that the body was not exposed to Mycobacterium tuberculosis before and its immune system didn't recognize tuberculin PPD when it was injected ➔ the test is <u>negative</u>.

- Remember, after the 48 hours we will look for an induration (a thickening of the skin) and measure its diameter if we find it.

- We do not look for erythema (redness) and we certainly don't measure the diameter of the area of redness.



Extra image for further understanding

- induration is a hardened edema which we can sense or examine by palpation, not the area covered by erythema.

- Then, we use a ruler to measure the diameter of the induration (which is approximately circular in shape) after the 48 hours.

- There are certain cutoffs in the measurement of the induration's diameter:

1) More than 5 millimeters: positive test and indicates high risk in the tested people, for example: immunocompromised people and AIDS patients.
2) More than 10 millimeters: positive test as it occurs in people with intermediate risk, such as homeless people, drug abusers and people who live in prisons or in overcrowded places.
3) More than 15 millimeters: positive test for the general population who don't know if they have previously been exposed to Mycobacterium tuberculosis.

- The TST gives an answer to one question: can the tested-person's body recognize tuberculin PPD? (Is his immune system sensitized to it?)

- TST only tells if the tested person is exposed or has been previously exposed to Mycobacterium tuberculosis.

- In the case of Mycobacterium tuberculosis infection, the test can't tell you if the tested person is in the active or latent phase, it only tells you if the tested-person's immune system recognizes Mycobacterium tuberculosis or not. But we can know if the infection is in the latent or active phase via the clinical signs.

- The issue with the TST is that it gives false positive results many times, as it may result in a positive result without the tested person being actually infected, and that is because:

1) The tested person is vaccinated with BCG (the vaccine for tuberculosis which is given in the left upper arm and it leaves a keloid scar + neonatals are given BCG within 1 month after their birth), which means that his body will recognize tuberculin PPD when it is injected and the TST will give a positive result.
2) Environmental Mycobacteria (NTM's) contain mycobacterial antigens which could also lead to false positive TST.

- BCG stopped being a part from the national vaccination program in Europe and the USA in the last 2 or 3 decades ➔ so that the interpretation of TST results would be more clear.

- Due to these issues and confounding factors in the results of TST; an alternative test has been found, which is the IGRA test.

❖ Interferon Gamma Release Assay (IGRA):

- Firstly, we take a blood sample from the patient. &for sure this sample has monocytes

- In the laboratory, there are antigens specific only for Mycobacterium tuberculosis and mainly there is two of them found in the cell wall of Mycobacterium tuberculosis:

1) Culture filtrate protein (CFP-10/10KDa).
2) Early secretory antigenic target (ESAT-6/6Kda).

- Secondly, we put the antigen in the blood sample.

- If the monocytes in the blood sample recognize the Mycobacterium tuberculosis-specific antigens, they will starting pouring huge amounts of interferon-gamma (IFN-gamma).

- We have a certain cutoff for IFN-gamma levels,
  if the IFN-gamma levels are below it ➔ negative results
  if the IFN-gamma levels are above it ➔ positive results

- It is very Important to know that whether the test is TST or IGRA, they both only tell us if there was a past exposure to Mycobacterium tuberculosis.

- Through TST and IGRA, we can't know if the infection occurred before or it is occurring now and in the case of current infection we can't know if it is in the latent or active phase.

- IGRA only tells us if the tested person's immune system recognizes Mycobacterium tuberculosis or not.

- That's why the diagnosis of tuberculosis is not easy and requires a combination of methods.

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**.

- Treatment of tuberculosis in Jordan takes about 6 months and in Europe and USA it takes about 12 months.

- **Treatment of TB usually involves a drug cocktail (according to an accepted regimen), or a mixture of multiple drugs, with an intensive initial 2-month 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).**

- In the intensive phase ➔ the patient takes 4 drugs
  In the continuation phase ➔ the patient takes 2 drugs

- The two drugs which the patient takes in the continuation phase are: isoniazid (INH) and rifampin (RIF).

- **Isoniazid preventive therapy (IPT) is the recommended** (for 9 months) **treatment for LTBI** (when the patient is imprisoned or a drug abuser or generally at high risk of the disease) **but the regimen's main drawback is the duration of therapy.**

- Drugs involved in tuberculosis treatment have many side effects, as they are nephrotoxic, hepatotoxic and optic neuritis.
  Rifampin forms red body fluid, such as in saliva and urine.

- Because of the long duration of the treatment, the side effects of the drugs and the feeling of getting better after after 2-3 weeks, the compliance of the patient to keep on taking the drugs decreases (through which resistant bacteria develop).

- And to prevent this from happening, WHO (World Health Organization) in south africa seek to contain the infection in the presence of HIV; so they treat people with tuberculosis with the DOT treatment (directly observed treatment) in which the patient must take the drugs in-front of a healthcare professional and sign a paper saying that he took the drug, or else, the police will interfere; and that is because of the very low compliance.

Prevention

- **The best way to prevent TB is to diagnose and isolate infectious cases rapidly and to administer appropriate treatment until patients are rendered noninfectious (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.**

- BCG vaccination is the same vaccine since the isolation and identification of tubercle bacilli.

- **Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine derived from M. bovis** (it is propagated in the laboratory and they lost their virulence factor, so they lose their ability to cause disease but they retained their immunogenicity) **, is the only licensed vaccine against tuberculosis (TB).**

- "Calmette" and "Guérin" are two French scientists who studied and worked on Mycobacterium bovis which causes bovine tuberculosis and it used to be transmitted to humans via milk before pasteurization.

- The issue with the BCG is its low efficacy. Furthermore, It is mentioned in the books & articles that the efficacy of BCG is from 0% to 80% ➔ which means that out of 100 vaccinated people, the worst chance is that no one out of them is actually going to be protected, and the best chance is that 80 people out of the 100 vaccinated people are going to be actually protected. And that is why they stopped giving it in Europe and the USA. ممكن ٨٠ واحد يستفيد وممكن ولا واحد

- However, BCG is still given in developing countries; because we believe that this way, we are protecting the children from the most severe forms of tuberculosis, which are tuberculous meningitis and miliary tuberculosis.

OTHER MYCOBACTERIA

- **The nontuberculous mycobacteria (NTM)** (which cause tuberculosis-like diseases that differ depending on the species of the bacteria) **is a diverse group of organisms commonly found in the environment, and the group includes both saprophytes and human pathogens.**

- Important note!: NTM are also called environmental bacteria ➔ Because they are not contagious as they don't get transmitted from an infective person to a susceptible person ➔ instead, they are transmitted from the surrounding environment to the susceptible person.

- **The NTM can be further classified into 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** (the colonies of these NTM may produce pigment in the presence of light or in the absence of light or they might not produce pigment at all):

- Regarding pigment production, NTM are divided into 3 groups:

1) Photochromogens: they produce pigment in the presence of light.
2) Scotochromogens: they produce pigment in the absence of light (in the dark).
3) Nonchromogens: they do not produce pigment at all.

- **Mycobacterium avium Complex (MAC or MAI)** (Mycobacterium avium intracellulare)) ➔ which is the number 1 killer in AIDS patients.

- **MAC organisms infrequently cause disease in immuoncompetent humans.**

- **MAC infection is one of the most common opportunistic infections of bacterial origin in patients with AIDS.**

- 90% of the isolates in the USA are: Mycobacterium avium complex (MAC), Mycobacterium kansasii and Mycobacterium gordonae.
  The rest 10% of the isolates are from the 11 member of Mycobacterium tuberculosis complex (MTC).
  But in South Africa for example, the major Mycobacteria there are from the MTC not from MAC.

The nontuberculous mycobacteria (NTM)

- **Mycobacterium kansasii** (causes tuberculosis-like pulmonary disease) ,**Mycobacterium marinum** (causes aquarium granuloma which is also a tuberculosis-like disease and it infects aquarists (people who work with aquariums)(احواض السمك)) and **Mycobacterium ulcerans** (causes soft tissue infection).
  ⇨ They are slow-growers and photochromogens.

- **Mycobacterium scrofulaceum** (causes cervical lymphadenitis (lymph node infection) and we call this condition scrofula. Also, remember that Mycobacterium tuberculosis could cause scrofula but Mycobacterium scrofulaceum is the most common cause for scrofula in children)
  ⇨ They are slow-growers and scotochromogens.

- **Mycobacterium avium complex, or (MAI)** (famous opportunistic bacteria which commonly infects immunocompromised people like AIDS patients and patients who take immunosuppressants like cancer patients or recipient patients in organ transplantation operations)
  ⇨ They are slow-growers and nonchromogens.

- **Mycobacterium fortuitum Complex ,Mycobacterium chelonae-abscessus** (and Mycobacterium smegmatis, they all cause subcutaneous and soft tissue infection)
  ⇨ They are fast growers and nonchromogens.

- Mycobacterium tuberculosis (the principle member of tuberculosis disease)
  ⇨ is a slow-grower and a nonchromogen.

Mycobacterium leprae

- **Mycobacterium leprae is an acid-fast rod.**

- **It is impossible to grow this bacterium In vitro** (ExVivo) (impossible to culture).

- The only option for diagnosis was via an animal model because Mycobacterium leprae can't be isolated on an artificial media.

- We can see them under the microscope as acid-fast bacilli.
- The specimen for diagnosis is taken from a skin lesion.
- The infected skin is mainly the skin of the face or of the extremities. (Cooler body parts
- **It causes the famous disease leprosy.**
- Fortunately, leprosy is so rare nowadays, especially in the region where we live.
- **The bacteria appear to grow better in cooler body temperatures** (optimal temperature for Mycobacterium leprae to grow is around 30°C) **closer to the skin surface** ➔ that's why they infect and invade mainly the skin of the face and the extremities.
- **Skin lesion** (nodular lesion) **consistent with leprosy and with definite sensory loss** (complete anesthesia).
- Important! In medical licensing examinations such as USMLE or PLAB they might use another name for leprosy, which is Hansen's disease.
- **The severity of the disease is dependent on the host's cell-mediated immune response to the bacilli (which live intracellular, like Mtb)** (generally,the immune system tries to get rid of intracellular infections by cell-mediated immunity)

Pathogenesis

- There are three main stages of leprosy:

❖ **Lepromatous leprosy (LL)** ➔ The most severe form of leprosy

❖ **Borderline lepromatous (BL)** ➔ A form with intermediate severity

❖ **Tuberculoid leprosy (TL)** ➔ The least severe (mildest) form of leprosy

- Hansen's disease always starts with borderline lepromatous leprosy.
  Then, in some patients, borderline lepromatous leprosy develops to Lepramatous leprosy (the most severe form).
  And in other patients, borderline lepromatous leprosy may change to tuberculoid leprosy (the mildest form).
- The development of the lepromatous leprosy or the tuberculoid leprosy from borderline lepromatous leprosy depends on the cell-mediated immunity of the host

(immune status of the host), because it is for the strong cell-mediated immunity to contain (trap) the multiplying bacteria.

- People with strong cell-mediated immunity ➔ strong T-helper 1 response ➔ high levels of IFN-gamma ➔ if these people were to be exposed to Mycobacterium leprae; they would develop tuberculoid leprosy.

- People with weak cell-mediated immunity ➔ weak T-helper 1 response ➔ low levels of IFN-gamma ➔ if these people were to be exposed to Mycobacterium leprae; they would develop lepromatous leprosy.

- There is a diagnostic skin test for leprosy which is like TST,
  it is called lepromin skin test, through which an antigen is injected under the skin, and after a certain period of time, we observe if there is an induration.

- Remember that the formation of an induration means that the body recognizes the antigen and has been exposed to it before (Mycobacterium leprae in the case of leprosy). Also, the formation of an induration also means that the tested patient's body has a relatively strong cell-mediated immunity (the principle of the test).

- The lepromin skin test is used to determine what type of leprosy a person has, so we already know that he has leprosy.

- If the lepromin test was positive for a tested leper ➔ this means that he has strong cell-mediated immunity ➔ which means that the leper has tuberculoid leprosy

- If the lepromin test was negative for a tested leper ➔ this means that he has weak cell-mediated immunity ➔ which means that the leper has lepromatous leprosy

Clinical manifestation

- **The onset of leprosy is insidious.**

- Leprosy is a chronic disease, in which the incubation period of the disease takes years and the treatment takes at least 2 years.

- **The lesions involve the cooler tissue of the body, including the skin, superficial nerves, nose, pharynx, larynx, eyes, and testicles.**

- The patient loses sensation at the nodular lesions (complete anasthesia and paresthesia)

- When the face of a leper is filled with nodules, it is then typically called a leonine facies. (اسد)

- Anesthesia is definite sensory loss, while paresthesia is the prickling feeling as if there were needles on the skin.

- Every nodule represents multiplying Mycobacterium leprae intracellularly and the body tries to contain it.

- Mycobacterium leprae isn't transmitted from the respiratory route like Mycobacterium tuberculosis. Mycobacterium leprae can be transmitted by prolonged direct contact with the skin lesions, or through the nasal secretions of a leper.

Diagnosis

- **skin or nasal mucosa or a biopsy of earlobe skin are smeared on a slide** (we look for acid-fast bacilli). Doesn't have relation to lungs like TP

- **Smears are stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened nerve gives a typical histologic picture**.

- Remember, culturing of Mycobacterium leprae is not an option.

- **No serologic tests are of value** (they are of limited value in intracellular infections, even in tuberculosis).

Treatment

- **Sulfones such as dapsone are first-line therapy for both tuberculoid and lepromatous leprosy.**
- **RMP** (rifampicin) **or clofazimine generally is included in the initial treatment Regimens**.
- Treatment and using drugs for leprosy takes at least 2 years.
- There is no vaccine for leprosy.