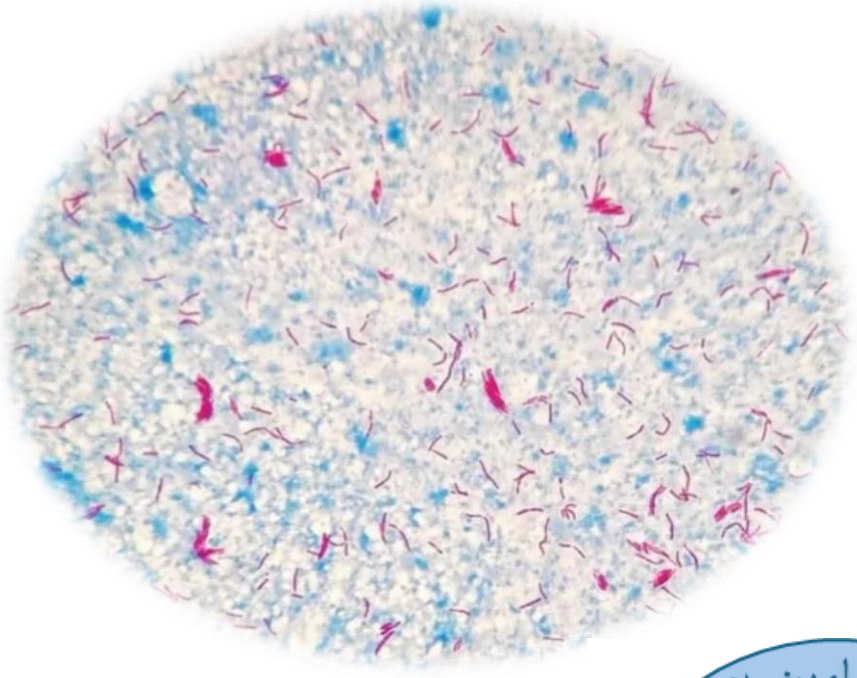
MYCOBACTERIA

Sheet 6



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Mycobacteria

Background and Taxonomy

The mycobacteria are rod-shaped, aerobic bacteria that do not form spores, they are neither G+ nor G-, they are **acid fast** stained.

Class actinomycetes, family mycobacteriaceae, genus mycobacterium, this genus has members of high medical importance and are classified as groups:

1. ***Mycobacterium tuberculosis complex* (MTC)** a genetically related group of 11 species of *Mycobacterium* that can cause tuberculosis in humans, they include:
   - ✓ ***Mycobacterium tuberculosis*** *(MTB),* the principal member and the cause of most tuberculosis cases in humans.
   - ✓ ***Mycobacterium bovis,*** found in cow's milk and it was associated with abdominal tuberculosis; it is now prevented through the process of pasteurization.
   - ✓ ***Mycobacterium africanum***
   - ✓ ***Mycobacterium microti***
   - ✓ ***Mycobacterium mungi***
   - ✓ ***Mycobacterium suricattae***
   - ✓ ***Mycobacterium caprae***
   - ✓ ***Mycobacterium pinnipedii***
   - ✓ ***Mycobacterium dassie***
   - ✓ ***Mycobacterium oryx***
   - ✓ ***Mycobacterium canetti***

It was not until the 19th century, when Robert Koch utilized a new staining method called the Ziehl-Neelsen stain (ZN stain), he applied it to a sputum taken from patients leading to the discovery of the causative agent of the disease Tuberculosis (TB), MTB or Koch bacillus.

2. ***Mycobacterium leprae*** causes leprosy or Hansen's disease
3. Non-tuberculosis mycobacterium (NTM):
    - ✓ Also called Mycobacteria other than tuberculosis
    - ✓ ***Mycobacterium avium-intracellulare*** (M avium complex, or MAC) and other *non-tuberculous (NTM) mycobacteria* frequently infect patients with AIDS, they are opportunistic pathogens immunocompromised persons, and occasionally cause disease in patients with normal immune systems.

Nocardia genus also belongs to the same order and class of mycobacterium, it is **partially acid fast** with branched morphology, an important species is ***Nocardia asteroids*** *the causative agent of nocardiosis*.

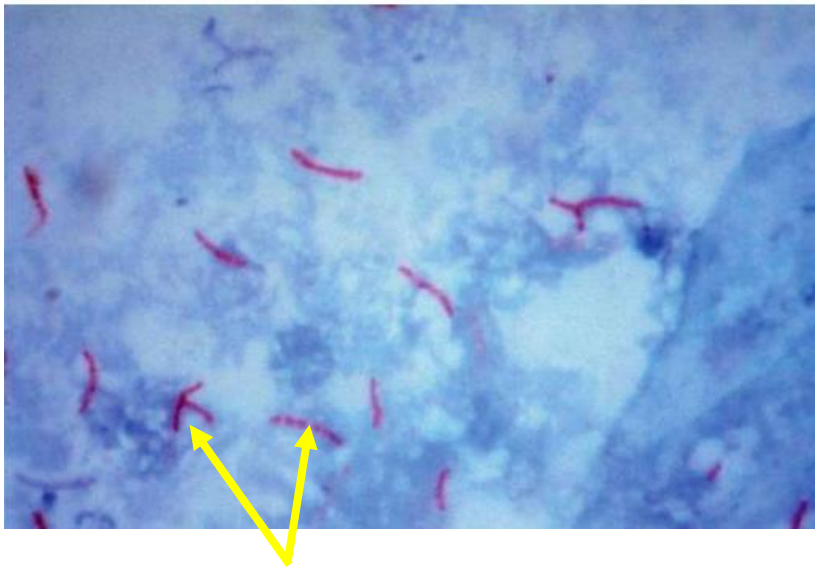
Mycobacterium Tuberculosis (MTB)

It is the principal member of MTC, the main causative agent of tubercolosis, the disease was called **consumption**; as one of the chief complaints of the disease is weight loss (consumes patients), the disease was also called **white plaque** (extreme pallor due to hypoxia).

The family mycobacterium tuberculosis complex (MTC) can cause Tuberculosis (TB) in humans and other livings; MTB is one of them.

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.
- They are, non-motile, non-capsulated, obligate aerobes and derive energy from the oxidation of many simple carbon compounds, they grow slowly (we need 8 weeks to notice growth in a culture; as the doubling time is about 18 hours, thus the diagnosis is more challenging), they are facultative intracellular, the main target of them is circulating monocytes and resident macrophages, this is noticed clearly in patients with pulmonary TB (most common type of TB in 80-90% of patients).
- 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.



Notice this high-power micrograph of a sputum sample, these red bacilli are the acid-fast bacilli stained with Ziehl-Neelsen (ZN) stain.

Steps of staining MTB with ZN stain

1. We stain the sample with carbol fuchsin stain that gives the red color, this step requires heating the sample to aid in the penetration of the stain as they are the only prokaryotes that contain a waxy lipid cell wall which contains mycolic acid.
2. Acid-alcohol decolorizers are used, these decolorizers erase the first stain. However, this is not the case in *mycobacteria* as they preserve the first stain without getting affected by the decolorizers, this is why they are called acid-fast, as they fasted (صاموا) on the first stain even with the use of acid decolorizer.
3. Using counterstains such as methylene blue won't change the color of the bacteria.

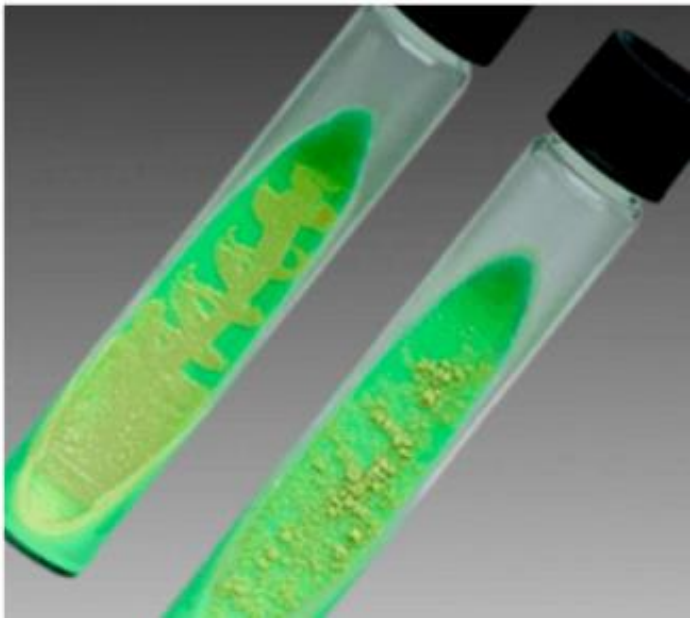
Staining **mycobacteria** with gram stain will give a weekly G+ result, but we consider them **neither G+ nor G-.**

Remember that the only bacteria that are **acid-fast are *mycobacteria* and *nocardia* (partially)**. Other bacteria are **not** acid fast. Some parasites are stained by **modified** acid fast stain.

MTB Culture

- The media for primary culture of mycobacteria should include a nonselective medium and a selective medium.
- Semisynthetic agar media e.g., Middlebrook 7H10 and 7H11 contain defined salts, vitamins, cofactors, oleic acid, albumin, catalase, and glycerol.
- Inspissated egg media, e.g., Löwenstein- Jensen medium that contains defined salts, glycerol, and complex organic substances e.g., fresh eggs or egg yolks, potato flour, and other ingredients in various combinations.
- Broth media, e.g., Middlebrook 7H9 and 7H12, support the proliferation of small inoculate.
- The **definite way** of diagnosis of tuberculosis is culturing.
- **Slowly grows**; it may take 3-4 weeks to grow noticeably.
- **Broth is faster**; 2-3 weeks, **but still slow**.
- Negative diagnosis can be made after 8 weeks of no growth.
- Negative results can be false; not all negatives reflect absence of TB.

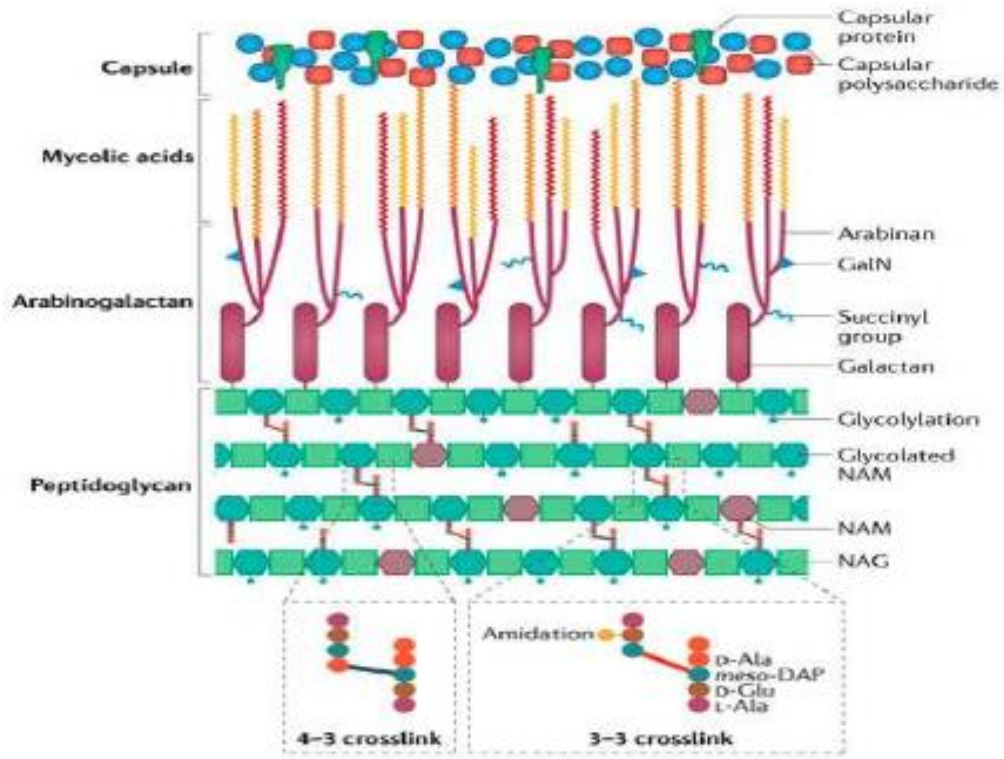
MTB Colonies



- This is Löwenstein-Jensen (LJ) medium.
- It contains malachite green → inhibits the growth of the normal flora, preventing the contamination of the sample.

Notice the rough raised wrinkled colonies

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 an inner layer and an outer layer that surrounds the plasma membrane. The inner layer is 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
- The peptidoglycan layer surrounds the plasma membrane and comprises long polymers of the repeating disaccharide N-acetyl glucosamine and N-acetyl muramic acid (NAG–NAM) that are 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 the 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.
- 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).

Virulence factors found on the outer layer

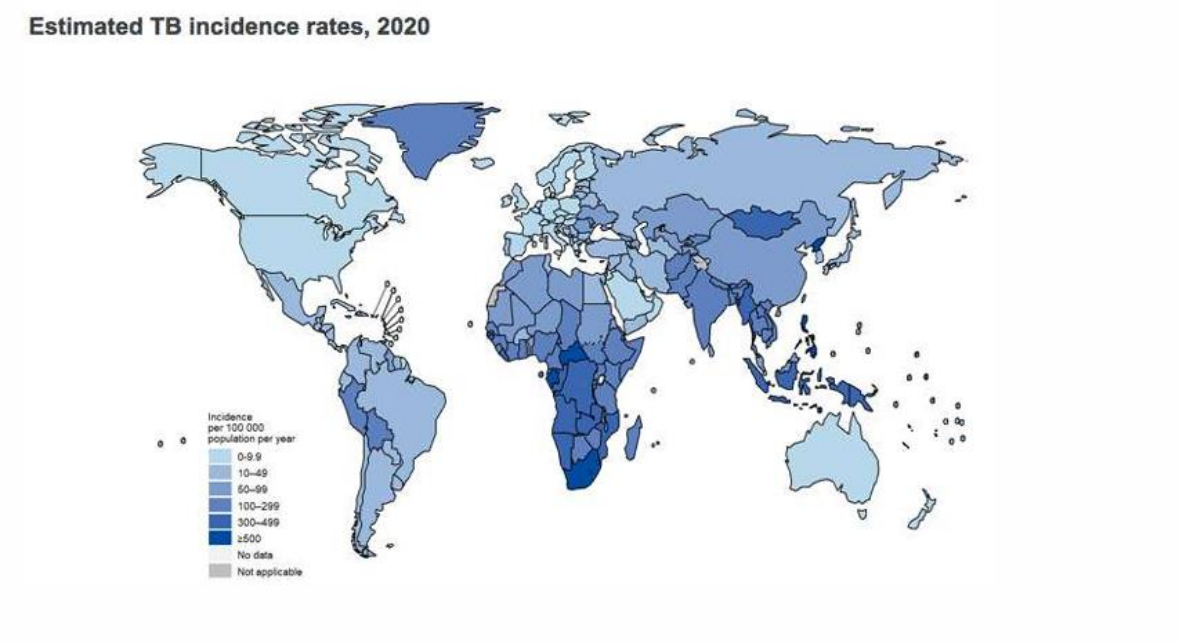
The outer layer isn't considered a capsule (even though it's mentioned in the figure on the last page).

The outer layer contains different virulence factors such as:

- **Lipomannan**
- **Lipoarabinomannan**
- **Sulfolipids** (sulfatides + lipids) inhibit the phagosome-lysosome fusion in macrophages, that's how they can infect the macrophages.
- **Trehalose dimycocerosate** causes the bacteria to grow in serpentine cords (مثل المسبحة), a special morphology of MTB.
- **Type 7 secretion system**
- MTB develops new virulence factors and antibiotic resistance through chromosomal mutations. It is not well known yet if MTB could get infected with plasmids.

Epidemiology

- Two TB-related conditions exist: latent TB infection (LTBI) and active TB disease. If active TB isn't treated properly, TB disease can be fatal, people who have latent TB infection don't feel sick, don't have any symptoms, and can't spread TB to others.
- About one third of the world's population is infected with TB bacteria (TB latency). However, only a 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 worldwide.
- TB is considered an airborne infectious disease although ***M. tuberculosis*** complex organisms can be spread through un-pasteurized milk, direct inoculation and other means.



In Jordan, the incidence of TB is about 15 cases per 100,000 people.

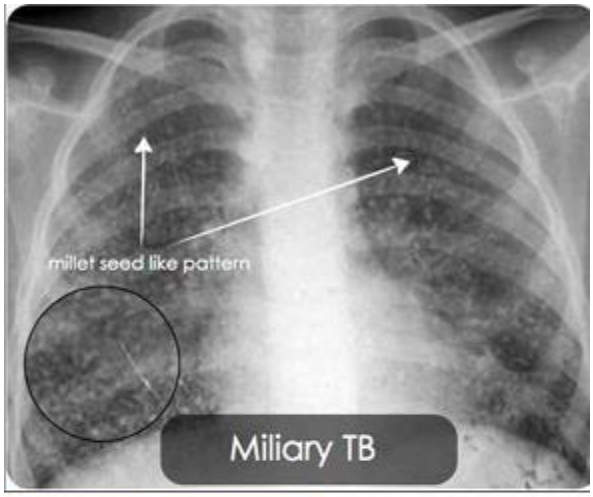
The formula: 10/3/1 means that, for every 10 people who get infected with MTB 3 will develop latent TB, 1 will develop active TB, the remaining 6 somehow cleared the infection through their innate or adaptive immunity, it's not well known yet. However, many immunologists believe that there is no sterilizing immunity for MTB in our bodies.

Patients with latent TB are not contagious, they may not face reactivation throughout their whole life. However, there are some risk factors associated with a higher incidence of reactivation such as age and immunocompromised states such in patients with AIDS. Patients with high risk of reactivation could receive preventive treatment of isoniazid for 9 months.

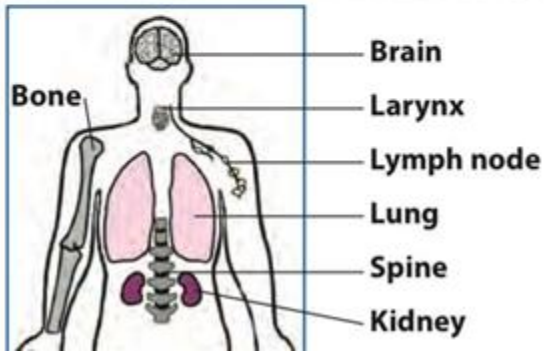
Tuberculosis TB

Primary infection: once patients are infected, they will develop active TB

Secondary infection: reactivation after latency.



- The primary site of TB is usually the lungs in 80-90% of patients, 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.



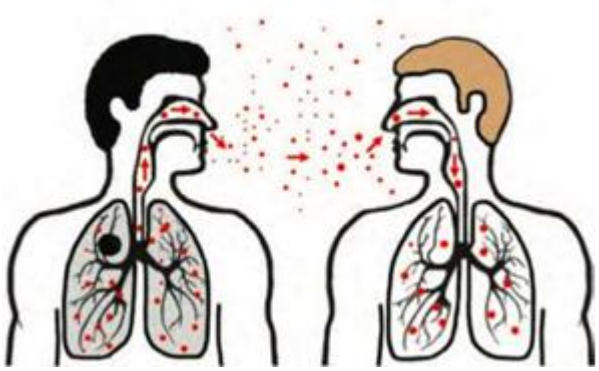
Once a patient has active tuberculosis, spread of infection could happen through **lymphatics** or **hematogenous (miliary)** spread or **direct erosion** from the granuloma to adjacent areas.

TB bacteria can attack any part of the body such as the pleura, lymph nodes, pericardium, kidney, spine, brain and abdomen (abdominal tuberculosis), these are collectively known as extrapulmonary TB.

Primary Infection (Active) and Reactivation Types of Tuberculosis.

One of the severe forms of pulmonary TB is miliary TB, chest X-ray will show millet seed like pattern of hematogenous spread. It could reach the brain causing tubercules meningitis (the most severe form of TB).

Transmission

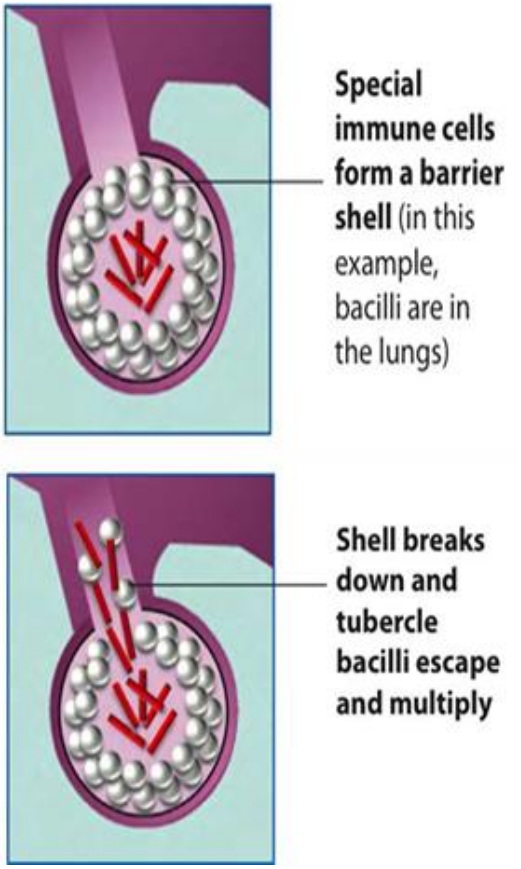


TB is considered an airborne infectious disease, could be transmitted through droplet nuclei and aerosols. Mycobacterium tuberculosis complex organisms can be spread through unpasteurized milk, direct inoculation and other means.

Mycobacterium tuberculosis is the second after spores in resistance to sterilization, they are also **resistant** to desiccation (dryness), they are **sensitive** to UV light.

The underlying pathophysiology of TB is the 10/3/1 formula.

Pathogenesis



Mycobacteria are in droplets when infected people cough, sneeze, or speak. 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 (fibrous ring) with giant cells, epithelioid cells and fibroblasts to contain and limit the spread of the bacteria. It is a hallmark of TB. This barrier shell is called granuloma that keeps the bacilli contained and under control (LTBI).

The type of granuloma is caseating granuloma as there will be caseous necrosis in the middle.

If the immune system cannot keep the tubercle bacilli under control, the bacilli begin to multiply rapidly (TB disease), erosion of the granuloma will lead to the spread of the bacilli hematogenously or through lymphatics. (TB active disease).

Healing could happen through fibrosis or calcification. This may be the case with the 6 people that didn't develop a latent or active infection (10:3:1).

The prolonged containment and control of the infection is the case in latent infections, any imbalance that happens with the immune system will lead to reactivation of TB.

Pathology

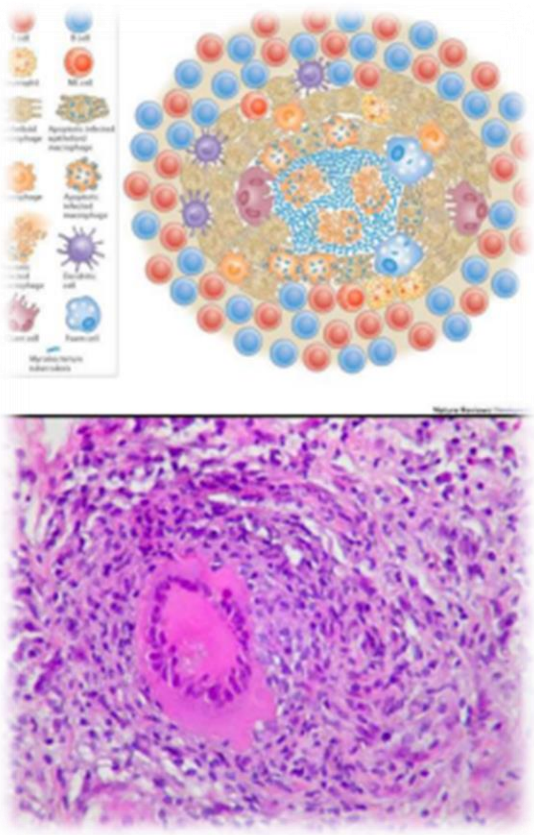
Exudative type — This consists of an acute inflammatory reaction with edema fluid; polymorphonuclear leukocytes; and, later, monocytes around the tubercle bacilli. This type is seen particularly in lung tissue, where it resembles bacterial pneumonia.

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;

(2) a mid-zone of pale epithelioid cells, often arranged radially; and

(3) a peripheral zone of fibroblasts, lymphocytes, and monocytes.



Primary Infection and Reactivation of TB

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. These types are usually in the **apexes** of the lungs not the bases, and this is mostly traced to the oxygen levels. TB is an obligate aerobe.

The reactivation type almost always begins at the apex of the lung, where the oxygen tension (PO_2) is highest.

Clinical Manifestation

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

Tuberculosis is usually a chronic disease; it presents slowly with weight loss, low-grade fever, and symptoms related to the organ system infected.

For example:

- If the kidney is involved → hematuria.
- If the vertebrae are involved → back pain.
- In scrofula (lymphadenitis) → enlarged lymph nodes

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 because its clinical manifestations vary widely depending on the organ system affected. This variability allows TB to resemble many other diseases, including cancer, autoimmune disorders, and other infections.

Laboratory Diagnostic Methods

❖ Smear microscopy (gold standard)

- 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 auramine.
- Smear-negative doesn't mean that the patient is free of TB, especially when the clinical picture implies, as the sputum sample (in pulmonary TB) may not include enough of the lower area of the lung where TB resides. We seek further diagnosis by culture for example.
- Smear-positive cases are for sure more contagious.

❖ Culture (definite diagnosis)

- 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.

❖ Nucleic acid amplification test (NAAT) includes PCR of samples; this test is specific but not sensitive, meaning that there is high false negative rate.

❖ Tuberculin skin tests (TSTs); purified protein derivatives (PPDs) are injected to the skin to measure the immune response, and then after 48 hours the thickness of the skin is measured (with a ruler), and specific cut-off values are interpreted differently. If the patient is immunocompromised or at high-risk, the cut-off value for positive is lower. **False positive** can occur in case of **vaccination** (prior exposure to TB antigens), especially in our area as vaccination against TB is still used. **NTMs** (environmental) can also cause false positive. False negative can also occur in this test as well.

❖ Interferon-gamma release assays (IGRAs) are commonly used as well.

- This test is more advanced with lower false positive rate.
- Blood sample is taken.
- Antigens, namely ESAT6 and CFP10 are added to the sample.
- INF-gamma release from WBCs in the patient's blood is observed.
- At a certain cut-off value, the patient is declared positive.
- The specificity (low false positive) of this test is traced to that the antigens used are exclusive to the TB of interest and does not exist in NTMs or vaccines.

Even if the test is positive, one cannot conclude the disease state (active, latent, immune), and this is the case for the other tests as well.

Tuberculosis is **difficult to diagnose (and to treat)** as it needs multiple tests, clinical examinations and X-rays to be able to reach the right diagnosis.

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 TB usually involves a drug cocktail, 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).

➢ Adverse effects of the drugs:

- Isoniazid → neuritis
- Rifampin → red color of body fluids (and urine)
- Pyrazinamide → hepatotoxicity & hyperuricemia
- Ethambutol → optic neuritis

- Long period → low patient compliance.
  - After 3 weeks the symptoms greatly diminish, and the patient is rendered non-infectious (quarantine terminated).
  - MDRs (multidrug-resistant) and XDRs (extremely drug-resistant) strains commonly emerge during the treatment if the course of the treatment is not continued to the end. Commonly observed in soviet nations.
  - DOT (directly observed treatment) is applied in some countries to ensure patient compliance in some countries.

- Isoniazid preventive therapy IPT (for 9 months) is the recommended treatment for LTBI, but the regimen's main drawback is the duration of therapy.

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.
- Mycobacterium bovis Bacillus Calmette–Guérin (BCG), an attenuated vaccine derived from M. bovis, is the only licensed vaccine against tuberculosis (TB).
- This vaccine leaves scars in place of injection (usually left shoulder in infants).
- The efficacy ranges from 0% to 80% (very wide range).
- In Jordan, we still give the BCG vaccine because it helps prevent **severe** forms of TB, namely **tuberculous meningitis** and **miliary tuberculosis.**
- In western nations, it is no longer used because they believe it has low to no protection against the **most frequent** form, namely **pulmonary TB**.

Other Mycobacteria

- The nontuberculous mycobacteria (NTM) are a diverse group of organisms commonly found in the **environment** **(soil and water → noncontagious)**, and the group includes both saprophytes and human pathogens.
- 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.
    - Photochromogens (produce pigment in the **presence** of light)
    - Scotochromogens (produce pigment in the **absence** of light)
    - Non-chromogens (**no pigment** production)
- Mycobacterium avium Complex (MAC or MAI).
- 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):
    - Photochromogens (slow growers):
        - *Mycobacterium kansasii* → TB-like pulmonary infections.
        - *Mycobacterium marinum* → aquarium granuloma.
        - *Mycobacterium ulcerans* → skin and soft tissue infections.
    - Scotochromogens (slow growers):
        - *Mycobacterium scrofulaceum* → TB-like scrofula (lymph nodes)
    - Non-chromogens (slow growers):
        - *Mycobacterium avium* complex, or (MAI).
        - *Mycobacterium tuberculosis* (not an NTM of course)
    - Fast growers (regardless of the pigment):
        - *Mycobacterium fortuitum* Complex → lung, skin and soft tissue
        - *Mycobacterium chelonae-abscessus* → skin and soft tissue

Mycobacterium leprae

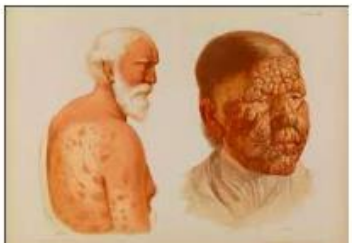
- Mycobacterium leprae is an acid-fast rod.
- It is impossible to grow this bacterium In vitro .
- It causes the famous disease **leprosy** (aka Hansen's disease).
- The bacteria appear to grow better in cooler body temperatures closer to the skin surface .
- Skin lesion consistent with leprosy and with definite sensory loss.
- The severity of the disease is dependent on the host's cell-mediated immune response to the bacilli (which live intracellular, like MTB).
- ***Mycobacterium leprae*** is intracellular with tropism to **Schwann cells**, **skin histiocytes** and **endothelial cells**.
- In contrast to **TB** which favors mainly **macrophages** and **monocytes**.
- 30-32 degrees Celsius is the optimal temperature, and this can provide some insight into the nerve and skin tropism which are cooler parts.
- Nerves are thickened, skin nodules appear, and eyebrows are typically lost.
- This presentation is commonly known as Leonine facies (lion face).
- Sensation is typically lost from affected areas.
- They are strictly in vivo organisms; they cannot be cultured on any medium.

Pathogenesis

- Lepromatous leprosy (LL)
  - very **weak** cell-mediated immunity → high bacillus count
  - **negative** lepromin test
- Tuberculoid leprosy (TL)
  - very **strong** cell-mediated immunity → low bacillus count
  - **positive** lepromin test
- Borderline lepromatous (BL)
  - Wait for it to enter one of the two extreme and then diagnose

Clinical Manifestation

- The onset of leprosy is insidious.
- Very long incubation period, slow symptoms and chronic nature.
- The lesions involve the cooler tissue of the body, including the skin, superficial nerves, nose, pharynx, larynx, eyes, and testicles.



Leonine facies

Lost eyebrows

Skin lesions

- The incidence of leprosy is much lower nowadays compared to TB.
- Transmission mainly occurs due prolonged contact with the affected skin legions or nasal discharge.

Diagnosis

- Skin or nasal mucosa or a biopsy of earlobe skin are smeared on a slide.
- Smears are stained by the Ziehl-Neelsen technique. Biopsy of skin or of a thickened nerve gives a typical histologic picture.
- No serologic tests are of value. Immunity is cellular and not humoral.

Treatment

- Sulfones such as dapsone (for long periods → years) are first-line therapy for both tuberculoid and lepromatous leprosy.
- RMP or clofazimine generally is included in the initial treatment Regimens.

سبحانك اللهم وبحمدك، نشهد ألا إله إلا أنت، نستغفرك ونتوب إليك

دعواتكم