TUBERCULOSIS

This year I decided to start from TB. I know that you've studied TB in microbiology and heard something about it in the respiratory course but I want to explain to you something different such as for example TB in immunosuppressed patients or extrapulmonary complications. What I want you to know is first of all that diagnosis of TB is based on serology.

In this lesson we will talk about:
1. Epidemiology
2. Microbiology
3. Transmission
4. Pathogenesis
5. Clinical presentation (pulmonary and extrapulmonary)
6. IRIS
7. Diagnosis
8. Latent TB
9. Treatment
10. MDR and XDR TB

I want to go through a little comparison between TB and Pneumonia (fig.1). If you are a doctor in an ER, which patient do you think is the one with the most serious clinical presentation? CAP
We usually imagine TB patients always with hemoptysis and severe clinical presentation immediately as acute onset, but this is not true.
If you think about CAP the patient will come with high grade fever, acute onset, possible dyspnea and lymphocytosis. While in TB low grade fever, subacute onset, weight loss, night sweat and WBC are usually normal. So the presentation is quite different and you might see hemoptysis in TB but in really advanced stages. Hemoptysis means blood in the sputum, and it is usually when there is a cavity and the cavity starts to involve blood vessels so bleeding is starting. Both patients can have thoracic pain and pleural effusion. They may have something common so there might be pain due to pleural involvement because in the lung we do not have neural processes.
Of course there are chest x-rays and CT-scans that appear very similar.

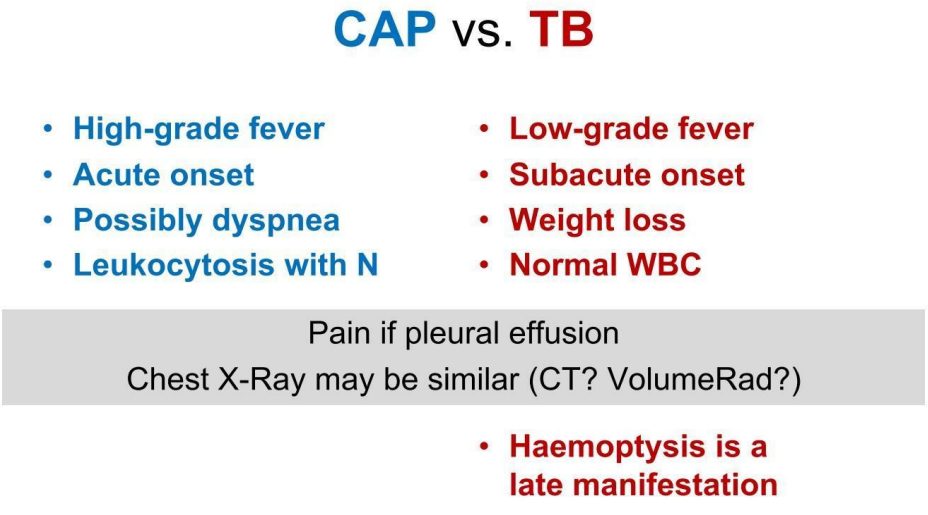

fig.1

This (fig.2) is called a tree in buddy. It looks like a tree that has fruits on the branches. It is caused by end bronchial dilation that is due to bacterial or fungal infection. This is very typical of bacterial pneumonia. Consolidation in one area of the lung can be appreciated (fig. 3)

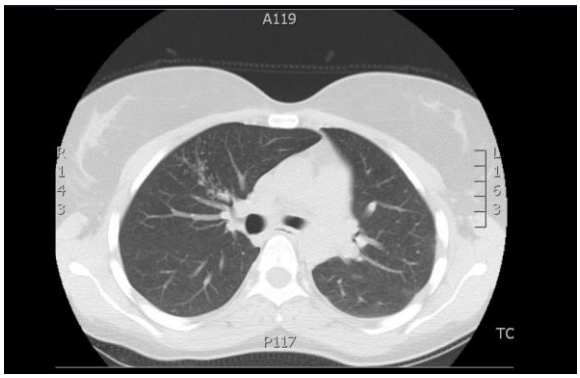

fig.2

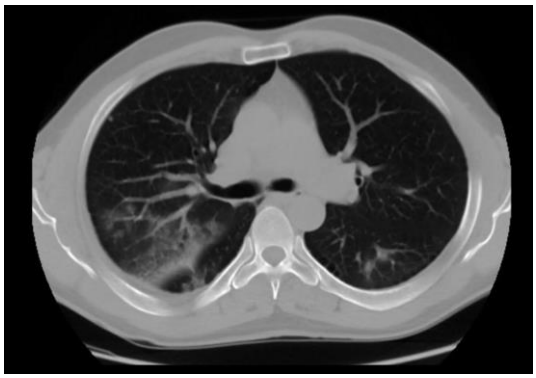

fig.3

While if you see a cavity like this it can be TB but also cancer. Or it can be a previous TB and now we have the granuloma. (fig. 4)

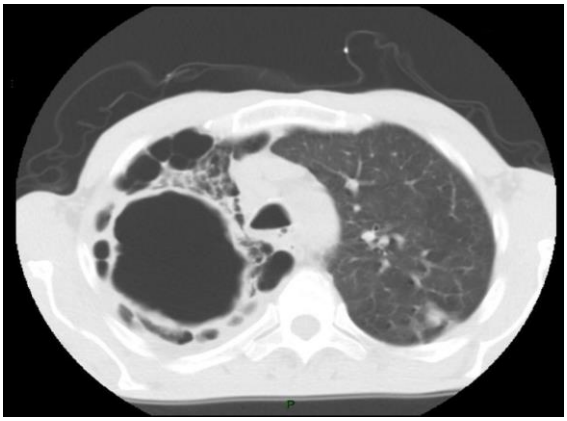

fig.4

EPIDEMIOLOGY

Remember that we think one quarter of the human population can be infected by mycobacteria. So the estimate is that 1.6 billion people have mycobacteria in their lungs. Our task is to identify the people at risk of reactivation. So we will talk about latent and active infections and there is a very important difference.
How many people have an active TB disease manifesting respiratory symptoms?
It is around 10.6 million, so the numbers are really high. 10% are children. HIV, TB and malaria are the most important worldwide killers (fig.5) with the highest rate of mortality (6 million people dying every year).By the way, it is difficult to say that you died because of TB so we rather say that you died with TB, we focus on people that die with an TB infection ongoing. Men have higher incidence and worst prognosis while in most of the infectious diseases females have higher incidence and worst prognosis. Every year the incidence is falling by 2%. Of course there are 30 countries with very high endemic TB. Together they have 80% of all the cases. India, China, Indonesia, Nigeria and South Africa are the most important ones. (fig. 6)

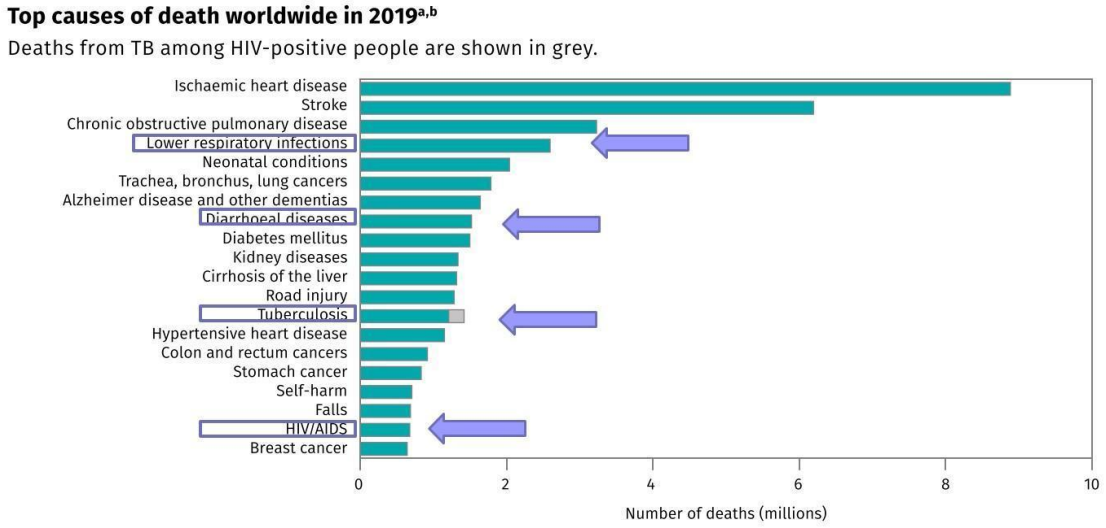

fig.5

TB is the number 13 cause of death. If you go to endemic countries, it is number two. With the maps you have an idea of the incidence. Countries with the darker colors, depicted in fig.6, are those with the higher incidence. Dark blue is more than 500 cases per 100.000.

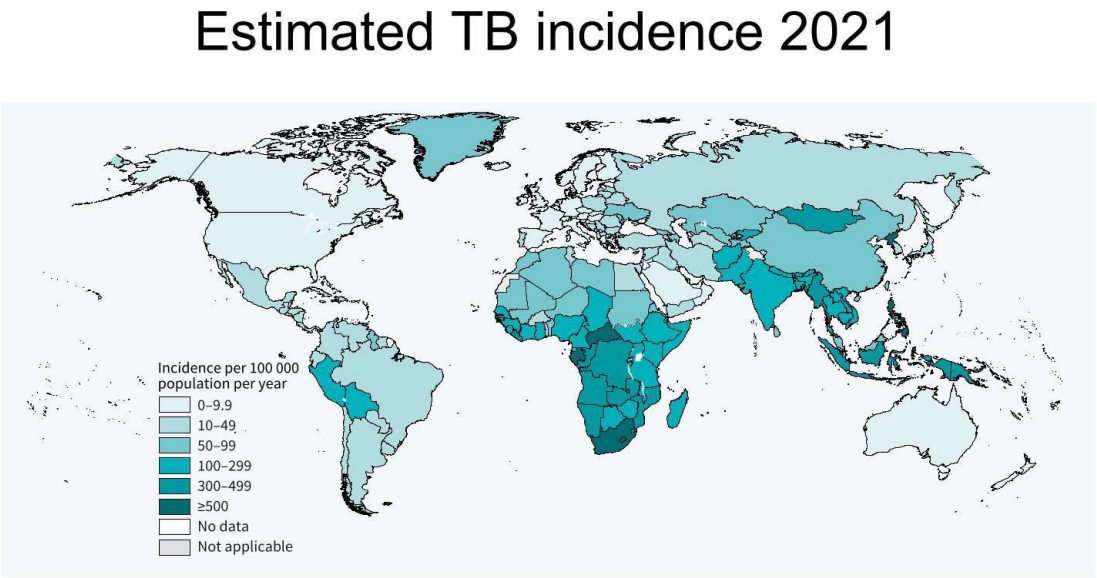

fig.6

In Europe the incidence is between 6 and 10. In Italy it is 6. In Sub-Saharan Africa and South Asia it is 100 times higher. This data is for active TB for latent TB we do not have clear data, we just have the estimates.
We will discuss HIV in depth, because it can accelerate lots of other infections and diseases.
HIV is a strong factor for TB activation and TB accelerates HIV. Since you have both it is harder to treat, and the mortality is higher. Of course every HIV patient should be tested for TB. Just to give you an example, more than 50% of TB patients in South Africa have HIV. For this reason, it is very important to test for HIV.
Q: Why is there a so strict correlation between HIV and TB, is this because of immunosuppression ?
A: It is. HIV can increase the TB risk of reactivation.

In 2020, due to COVID, there was a reduction in notification rates for TB and in specific areas this decrease was really steep, especially in notifications by hospitals. This means that in these countries they were not

able to notify TB patients. For example, Angola in 2020-2021 did not receive TB medication for months, without any medication at all. Half of TB cases now have resistance. As we can appreciate in fig.7 less notifications correlate with higher mortality. A paradox is that patients with HIV receive better care in certain countries than patients without HIV, thus focusing on HIV is important. As you can see here mortality related to HIV has decreased. Covid had an important impact on TB.

Global trends in the estimated number of TB deaths (left) and the mortality rate (right), 2000–2021

The horizontal dashed line shows the 2020 milestone of the End TB Strategy, which was a 35% reduction in the total number of TB deaths between 2015 and 2020. Shaded areas represent 95% uncertainty intervals.

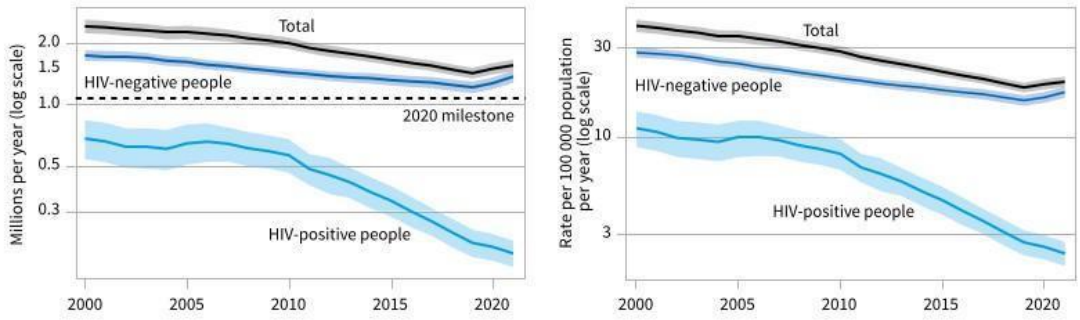

fig.7

In Europe last year we had 33.000 cases and the countries with the highest numbers are in East Europe. The number in Europe has slightly decreased. In Italy, according to the latest data, we have a low incidence country with 5 cases per 100000 people. It has prevalence in two populations: young patients that are born abroad since **migration is a risk factor**, and then we have people that were born in Italy above the age of 65, because age is an important immune suppressor factor. In Piedmont we have 400 cases per year. People that were born abroad have an average age of 30, while those that borned in Italy have reactivation at the age of 65.

We can appreciate that there is a correlation with TB and the hours of sunlight (fig.8). In areas with lower sunlight, we have higher incidence of TB. That is because of the **strong correlation of vitamin D and TB.** Vitamin D is one of the hormones that controls T Cells response. So the lowest vitamin D, the higher the risk for tuberculosis. Overall all those factors that are able to reduce the immune system function can induce TB reactivation passing from a latent and silent infection to the clinical onset of symptoms.

Tuberculosis Incidence Correlates with Sunshine: An Ecological 28-Year Time Series Study

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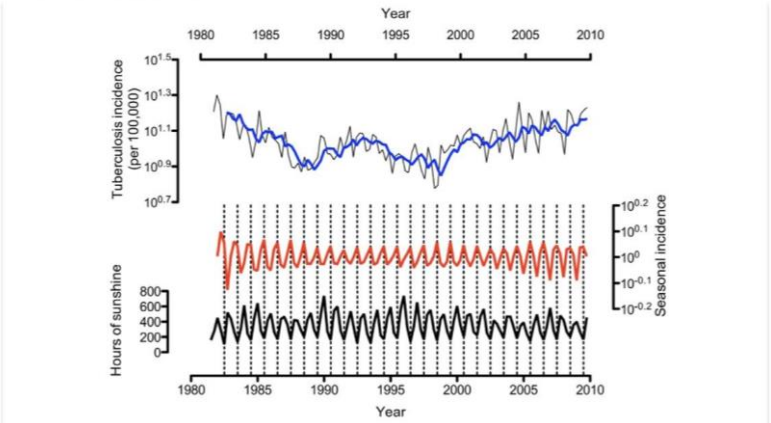

fig.8

MICROBIOLOGY

I do not want to go back to what you studied in microbiology. I just want to remind you of some things. First, mycobacterium tuberculosis is a complex of mycobacteria: M. tuberculosis, M. bovis, M. africanum, M. canetti and others.

M. bovis is the only exception because the spread is not from person to person but from contact with cows, so direct contact or ingesting dairy products that derive from cows infected with M. bovis.

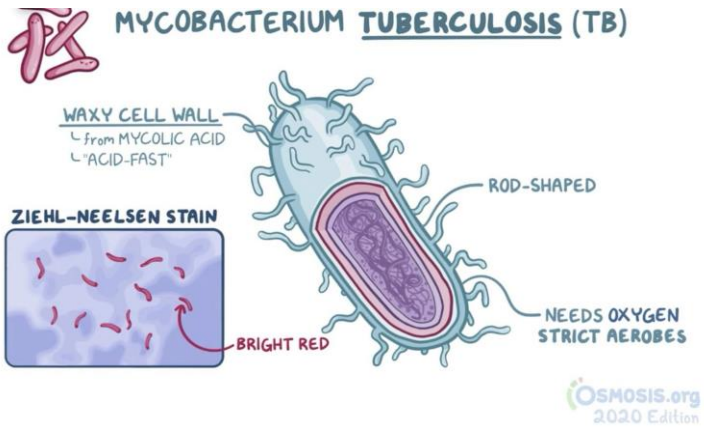

fig.9

For these reasons, we commonly have intestinal TB due to the ingestion of milk. So less respiratory much more intestinal. M. bovis causes 3% of TB overall, while in India it is 15%.

Rod shaped bacillum, not mobile, not sporogenous and very small (fig.9). They are strict aerobes and can survive where there is a high concentration of O2. They can survive and replicate both extracellularly and intracellularly, in fact they are able to infect macrophages and survive inside of them. Normally macrophages can survive for years so that's why M.tuberculosis infection can persist for so long.

I also want to remind you that the cell wall of mycobacteria is really complex. It is made of several waxy layers composed of mycolic acid and this guarantees resistance against the immune system.

This is why the replication cycle is so long. If you do a culture you have to wait 30-70 days, so we usually perform a test in order to detect LAM (lipoarabinomannan). This is enough concerning microbiology, I don't want to go into details.

TRANSMISSION

The transmission is airborne, but you have to remember three different ways of airborne transmission (depending on the weight of the particles). Fig.10

1. Big droplets that can travel up to 1 meter. (e.g, If I have meningitis from neisseria I will transmit it to people one meter away from me). They are very big particles.
2. Small droplets can travel more, up to 3 meters. (e.g. TB)
3. Infectious droplets that can be viruses (e.g influenza). It can affect anyone in the room.

The transmission of TB depends also on the environment. For example, twenty people living together in a small room without windows, fresh air and sunlight, those are the cases where the transmission is higher. People migrating to other countries in the first years live in conditions like these and this causes reactivation of TB.

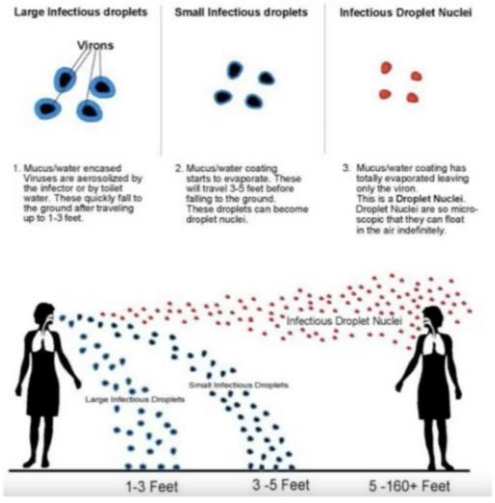

fig.10

PATHOGENESIS

If one of you has tuberculosis, 90% of droplet nuclei are withheld and expelled by the ciliary system; the smallest particles (<2 µm) can reach distal alveolar spaces which are full of alveolar macrophages. The first step is basically phagocytosis, where mycobacteria are eaten by macrophages. Macrophages try to use lysosome and reactive oxygen species in order to get rid of those bacteria entered in the airways. When macrophages are not able to get rid of the mycobacteria, they start producing a lot of chemokines that attract other cells and specifically other macrophages. At the end of this process of recruitment we observe the formation of the granuloma. In the middle of TB granuloma, we have caseous necrosis because macrophages, inflammatory cells and bacteria themselves remain without oxygen. This is specific for TB because we also have granuloma in other diseases (specifically in chronic conditions) but not caseous necrosis as: NTM, Brucella, Sarcoidosis, fungal infections and lymphomas (fig.11)

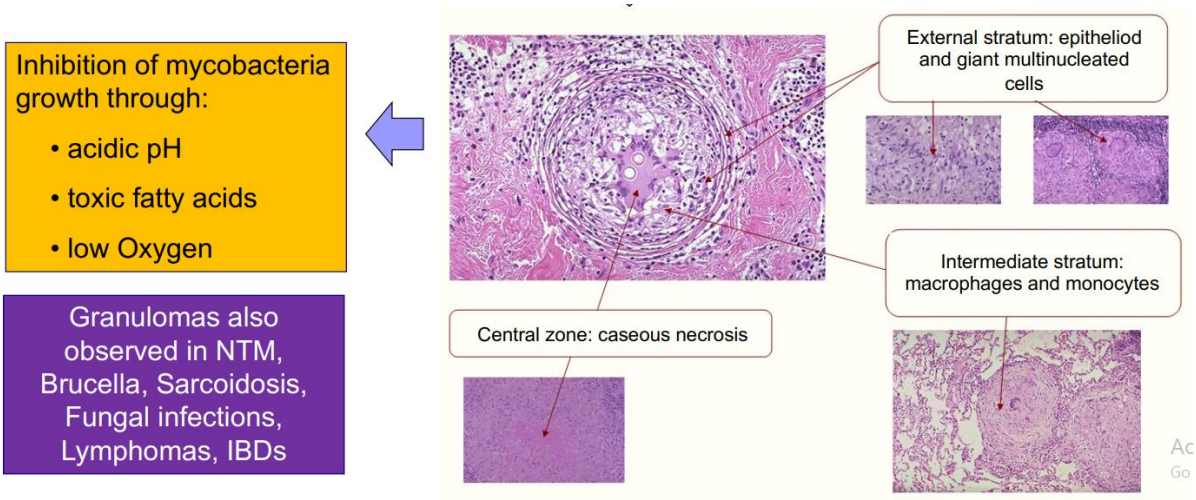

fig.11

Then we have an intermediate stratum of macrophages and monocytes and then the external stratum that is made of epithelioid and giant nucleated cells. One thing that I want you to remember is that this structure is not stable. You constantly renovate the wall with the aim to block whatever is inside. This process is very sensitive to immune depression. Even if years after I acquire HIV or if I do extensive use of corticosteroid I am not able to replicate the wall and mycobacteria slowly replicate and I will have secondary TB. The immune system is required to replace the cells in the "protective wall".
Before giving immune suppressor drugs, I have to test the patient for HIV, TB and syphilis. If I have a TB patient, I do not initiate the treatment otherwise the risk of reactivation is very high.

In the majority of people infected with mycobacteria the response of the macrophages is very effective and they form the granuloma which protects them. However, 5-10% of the people have a weak macrophage response. Who are those? Immunosuppressed people and kids below the age of 5. They receive tuberculosis and the symptoms start within the next few days. The fact is that if I am not able to control TB in my lungs, mycobacteria will spread all over the body (primary TB). What is important to underlie about primary TB is the high amount of extrapulmonary manifestations (e.g. TB meningitis that has a very high mortality)
BCG vaccine is an attenuated mycobacterium. It is used for many years as a vaccination for TB. The problem with the vaccine is that it does not prevent secondary tuberculosis. It only prevents primary tuberculosis. This vaccination is used nowadays only in children in order to prevent TB meningitis. As you get the granuloma, the vaccine is not useful anymore.

The other 90-95% of the population have a good and efficient response with the formation of the granulomas. This is the reason why 95% of the people have latent tuberculosis without any type of symptoms so basically the infection remains but is controlled and confined. The risk of reactivation is 5-10% (secondary TB). Going back to numbers if we have 1.5 billion people with tuberculosis we will have 150 million reactivations. In HIV patients without treatment the risk of reactivation increases 10% every year. In ten years all of them will have TB.

CLINICAL PRESENTATION

As we said there is the classical TB presentation:

- Insidious onset and chronic course
- Chest symptoms: cough (usually productive), hemoptysis (late) and chest pain (usually pleuritic)
- Nonspecific constitutional symptoms (more common in children and HIV)
- Extrapulmonary symptoms (if involved)

But we also have some not specific ones. Often we see patients with:

- Fever in 65-80% of cases
- Chills/night sweats
- Fatigue/malaise
- Anorexia/weight loss

However, 10-20% of TB cases have no symptoms at the moment of diagnosis.
Probably you should understand that it is chronic, subacute and also you have to consider if someone was born in a country with high incidence of TB. This is why the diagnosis of people born in Italy is much more delayed compared to those born abroad.

The appearance of TB in x rays can be different as we mentioned in the beginning of the lecture. It can appear as a buddy tree or as consolidation. It can be a cavity as you can see in fig.12, or multiple as in fig.13. Whenever you see pulmonary cavitation, you must think about NTM, fungi, cancer or pulmonary vasculitis but you should never exclude tuberculosis too. But overall do not think about TB only as people with cavities and hemoptysis because the spectrum is really wide. This is how TB is represented in lungs but what about TB outside the respiratory system?

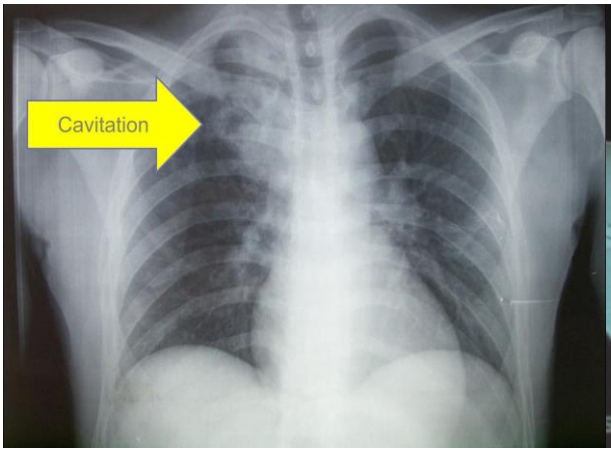

fig.12

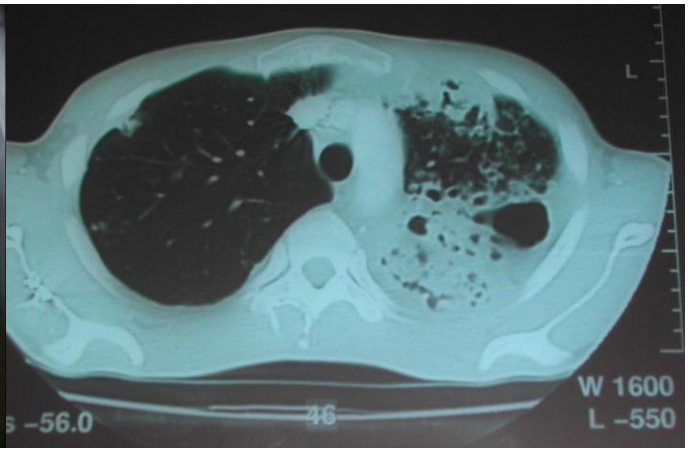

fig.13

Now we move to extrapulmonary disease.

- Miliary or disseminated TB is like sepsis but with TB. It's rare but is dangerous. We are not able to control TB and it spreads everywhere lungs, blood and lymph nodes. This condition is characterized by a lot of small nodules spread everywhere (reticulonodular pattern). This is considered an emergency condition because as well as for sepsis, is an acute and rapidly evolving condition with fever and dyspnea. So what you see again here is the reticular aspects with hundreds of thousands very small nodular lesions in the interstitium. These all are granulomas that have been spread everywhere. The granuloma are not only in the lungs they are also in the larynx and in the mouth that's why people presenting with miliary tuberculosis are very contagious.

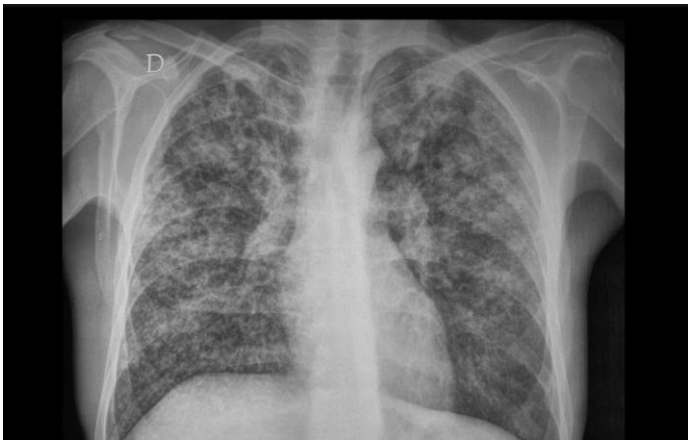

fig.14

We do not need to remember all the TB diseases but to remember that it can occur everywhere. So in the differential diagnosis of whatever, you need to think about TB.

- **TB lymphadenitis.** Mainly affects children and usually is infraclavicular, cervical or submandibular. TB from lungs spread into mediastinal lymph nodes. Usually is monolateral, if you have bilateral means that is something systemic (remember that when you observe fever and bilateral cervical lymph nodes swelling you must think about something viral). The lymph nodes are soft, non-painful and they are cold. Remember that this is an important characteristic: when you observe cold lymph nodes you must think about TB. All abscess and bacterial lymphadenoma are all warm (sign of inflammation: tumor, rubor, calor, dolor).
  If you have a mouth abscess or a dental one the lymph nodes in the neck are swollen, painful and hot. So every time that you see TB lymphadenitis the lymph nodes are cold. One of our patients was a young man from Somalia and he got spondylitis, an infection in the spine and an abscess in his back. There was a huge lymph node and it was cold. For diagnosis you can perform some biopsies through needles inserted in the abscess, cultivation and detection of TB. By the way lymphadenitis is not so dangerous and is considered a benign form, mortality in this case is really low.

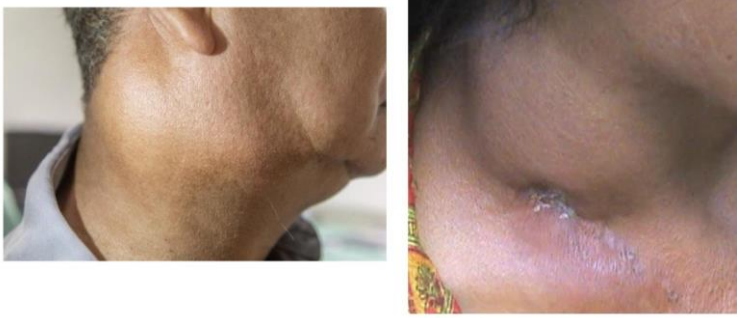

fig.15

- **TB serositis (pericarditis, peritonitis or pleuritis).** Is uncommon but all serosa can be affected. Of course among the three, pleuritis is the most common because the lungs are the primary site of

infection. Pericarditis is rare and has a lot of complications as for peritonitis. Remember that when you see a patient with unexplained serositis, always think about TB. For diagnosis you can cultivate serosal fluid in order to detect TB.

- **Laryngeal TB** is highly contagious (fig.16)
- **Skin TB.** This one is a patient from South America that presents with these lesions similar to eczema but our suspicion of TB is very high so we did a biopsy of one of the lesions. (fig.18)

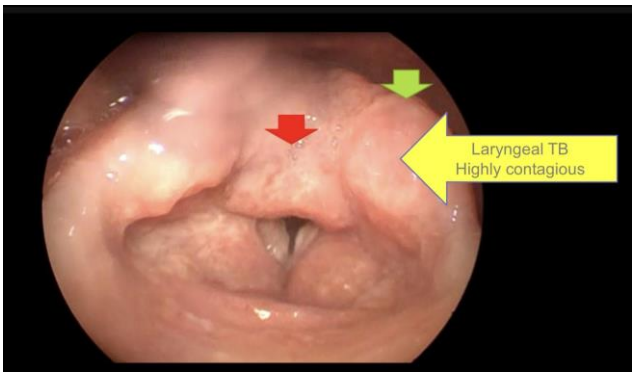

fig.16

fig.17

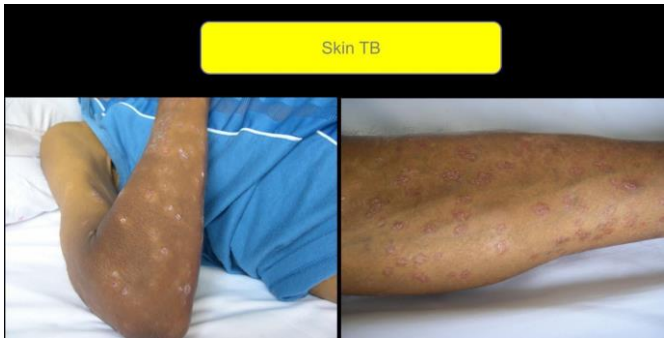

fig.18

- CNS TB. As we said before this is the condition that presents the highest mortality rate. TB meningitis is a very atypical form. It presents a slow onset with weight loss and cranial nerves paralysis. Hydrocephalus is very common due to altered CSF circulation). CSF is clear with lymphocytes, elevated proteins and very low glucose content. TB can also generate tuberculomas. The patient will present with CNS symptoms and we do a CT scan or an MRI.
- You can also have TB in the spine. It is usually in the thoracic spine. This is the difference between TB and acute bacterial spondylitis-Pott's disease-(more common in the lumbar part). TB is usually in the thoracic part because it is in contact with the lungs. It is a very slow process.
- We can also have TB in genital and urinary tract.

IRIS

IRIS is an acronym that stands for **Immune reconstitution inflammatory syndrome.** Is something that we discovered when people with HIV were treated with antivirals and the immune system increased (T cells) and after that patients felt worse.

It is defined as "a paradoxical inflammatory reaction against a foreign antigen (alive or dead) in patients who have started antiretroviral therapy and who have undergone a reconstitution of their immune responses against this antigen".

IRIS Pathogenesis

- Increased lymphoproliferative response to mycobacterium antigens in vitro
- Restoration of cutaneous response to Tuberculin

- Increased [Il-6], activation markers (CD38)
- Associated with TNFA-308*1, IL6-174*G

I had a patient present with very low CD4 cell count. Sputum was TB negative. We treated the immunosuppression. After two months the patient presented with fever and weight loss. Basically TB was there but the immune system was so weak that it was not able to recognize it. Just right after iatrogenic stimulation, an inflammatory reaction occurred. The immune system was cured and was strong enough to recognize TB.

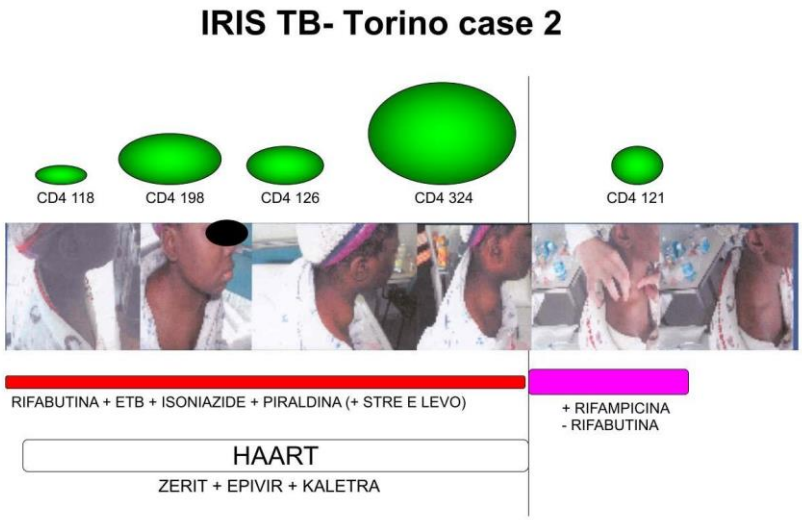

fig.19

IRIS Treatment

«Unmasking IRIS»

- Absent before starting antiretroviral therapy
- Need diagnosis and treatment of the opportunistic infection

«Worsening» IRIS

- Management of the infection including treatment and source control
- NSAIDs if mild
- Corticosteroids (Prednisone 1 mg/kg)
- Antiretroviral therapy interruption in case of life-threatening or
- serious disorders (CNS or eye involvement)

DIAGNOSIS

Remember that latent and active TB are two different things. We need some epidemiological features and some clinical features. How do we diagnose active tuberculosis ? Sputum, serum or urine samples (in order to look for TB bacteria directly or some parts of them as LAM) and culture (14-15 days) represent the gold standard in microbiology. What we can do to save more time is stain to see TB bacteria directly through a microscope (Ziehl-Neelsen staining) and then culture is performed later for confirmation. Another option is PCR (everywhere in the world nowadays) that is very fast (6hr), the machine does everything. Remember that there is a very wide spectrum in TB from active, subclinical and latent. So it is really important to diagnose the infection in time in order to avoid spread among other individuals.

When, instead, we want to diagnose latent tuberculosis we have two tests. (we performed those in migrants or immunosuppressed people in order to exclude a possible TB latent infection).

One is the **Mantoux test** (fig.20). We just inject intradermally a protein from Mycobacterium TB and we see in three days how big the reaction is. We have to measure the papula. If the papula is above 1 cm it is positive. There are a lot of false positives due to the bcg vaccination (tuberculosis vaccine). The problem is that it is not so sensitive and we need the people to come back in 3 days and someone will forget. The cost is one euro per test.
The other test is a group of tests called Interferon gamma release assays (IGA) and **QuantiFERON** (fig.21) is the most used one. I take blood from a patient and I put it in four tubes, each of them with different things.

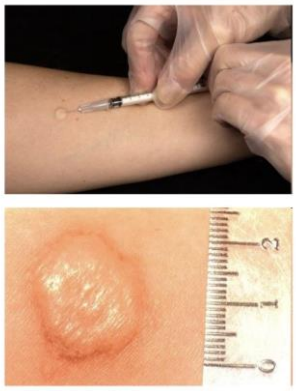

fig.20

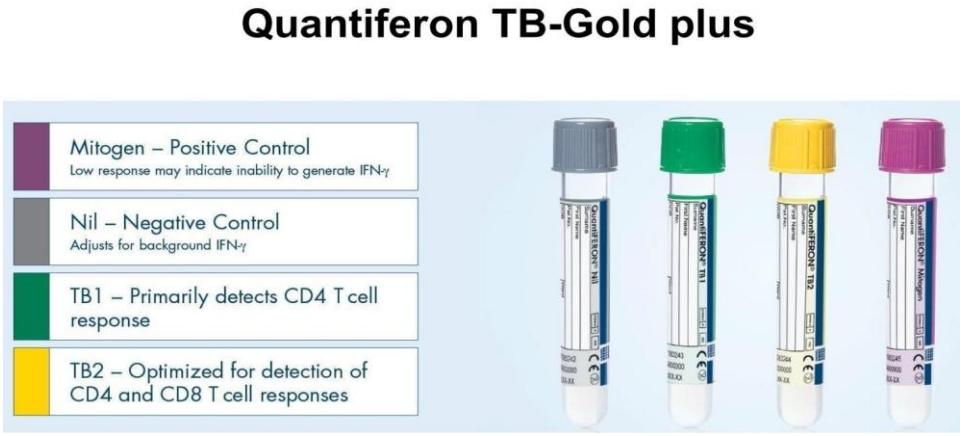

fig.21

In one tube I got nothing, in one tube I put mitogen which increases the reactivity of T cells and then I have 2 tubes with MTB antigens. I measure how much interferon γ is produced in each tube. If I see higher production in the tubes with MTB antigens means that T cells have been previously sensitized to TB, so that's a latent TB. I also measure mitogen response because if the patient's T cells are not reacting it means the immune system is not functioning properly. T cells react to TB antigens with very high sensitivity. Just one time the patient does not have to come back. It is expensive so we use it in immunocompromised patients or to confirm the Mantoux test. Remember that some NTM can lead to false positives even in QuantiFERON tests.

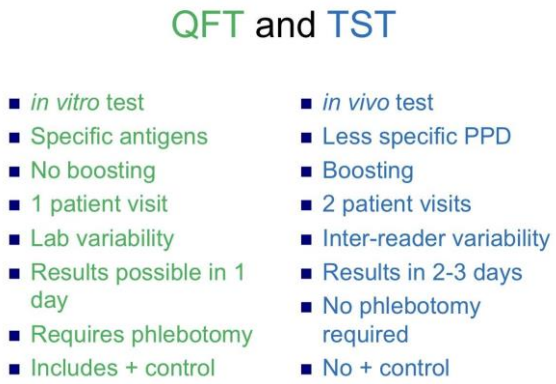

fig.22

LATENT TB TREATMENT

Let's say that one of you is tested positive. Would you receive treatment or not?
The risk of activation is 5-10% so in 90% of people treatments are not necessary. The treatment is cheap but with a lot of side effects. This is the list that CDC suggests to treat:

• People living with HIV (PLWH)

• Recent contacts of a TB case if you are exposed to someone with TB they will give you a Mantoux test if it is negative it will be repeated in six months. If in six months it is positive it means that you recently got infected by TB and you will receive the treatment because in the first 2 years the granuloma is very unstable, so the risk of reactivation is higher during this time. By the way latent TB is not infectious at all.
• Persons with fibrotic changes on chest x-ray consistent with old TB
• Organ transplant recipients
• Persons who are immunosuppressed for other reasons (e.g., taking the equivalent of >15 mg/day of prednisone for 1 month or longer, taking TNF-α antagonists)
• Persons from high-prevalence countries
• Injection drug users • Residents and employees of high-risk congregate settings (e.g., correctional facilities, nursing homes, homeless shelters, hospitals, and other health care facilities)
• Mycobacteriology laboratory personnel
• Children under 4 years of age, or children and adolescents exposed to adults in high-risk categories

We give Isoniazid for 6 months and in pregnant women we also give vitamin B6. Or we give rifampin for 4 months. The treatment is shorter and with less drugs compared to the one of the active TB.

ACTIVE TB TREATMENT

Remember that during bacterial replication we observe an heterogeneous colony (fig.23), they are all different. Is not true to say that they are all the same copy of the first bacterium. So we observe in some of the newly formed bacteria some drug resistance. That's why it is important to use different drugs simultaneously. Is important to eradicate all the different colonies that are forming all with different characteristics: rapidly growing, slowly growing or with sporadic replication (TB in macrophages replicate once every two weeks).

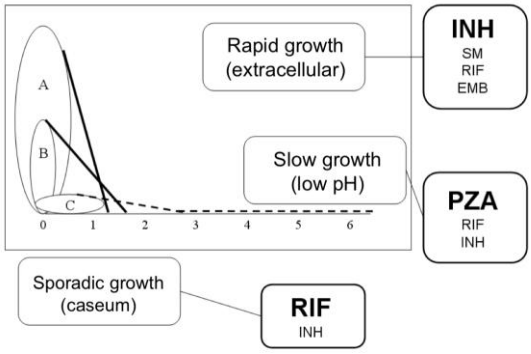

fig.23

Drugs have different effects on those populations. Isoniazid is more efficient on rapidly growing bacterias, Pyrazinamide on slowly growing and Rifampicin os sporadically growing. So for those replicating quickly, isoniazid is the best drug. For those replicating in caseous necrosis only Pyrazinamide works because it is activated by low ph. Rifampicin is the most important drug to eradicate MTB. After 2 weeks of treatment contagiousness is 5%.

We use four drugs: Rifampicin, Pyrazinamide, Ethambutol, Isoniazid. We give all 4 together for 2 months and then we give Rifampicin and Isoniazid for 4 months. We give four drugs to avoid resistance and we have to consider that every population of mycobacteria is not homogenous. So if I combine 4 drugs the probability of resistance to all of them is very low. Another reason is that mycobacteria are not homogenous in terms of growth.

Doses and side effects

	mg/Kg	
Rifampicin	10*	• Orange urines • Hepatotoxicity • Flu-like • CYP e PgP induction → drug to drug interactions
Isoniazid + piridoxine (vit B6)	5	• Hepatotoxicity • Peripheral neuropathy
Ethambutol	15-20	• Uveitis, color blindness
Pyrazinamide	20-25	• Hepatotoxicity • Hyperuricemia

fig.24

We have at the beginning a lot of mycobacteria replicating extracellularly, then we have some bacteria replicating in caseous necrosis and very few of them inside macrophages. I will just give an example of leprosy. We give one dose every six months because they replicate very slowly.
What about side effects? (fig.24). Rifampicin can cause the excretion of orange urine and all other body fluids. Three of them are hepatotoxic (no ethambutol) so we need to check liver enzymes to prevent severe hepatic failure. Ethambutol is also associated with color blindness and it is irreversible in some patients.
Remember that isoniazid must be given with Vit.B6 otherwise you will have peripheral neuropathy. In certain scenarios we have to go beyond 6 months as in TB meningitis or bone disease. Theoretically the efficacy of the 4 drugs is 95% but according to the data the real efficacy is around 85% because some patients will die, some patients will stop taking the drugs and some of them will have severe side effects. So the real efficacy is 85%. But in HIV patients the efficacy is 75%. It is hard to treat because the immune system is not functioning properly. In MDR-TB the efficacy is 50%.

MDR AND XDR TB

The difficulty is in resistant tuberculosis. This is something that I ask in the exams. This is the definition of Multidrug resistant tuberculosis (MDR TB) and Extensively Drug Resistant Tuberculosis (XDR TB). **MDR TB is resistant to isoniazid and rifampicin.** We have 450.000 cases in the world so 3% of all the TB cases. While **XDR TB is extremely resistant to fluoroquinolones and one group A drug (bedaquiline)**. XDR TB is 6% of the 3% (MDR TB). In Russia more than 50% of TB relapse are drug resistant. Also in Somalia, East Europe and South Asia. Estonia has the highest proportion of MDR cases in europe. (fig.26)
Before the new guideline, the treatment for resistant strains was 5 to 6 drugs for 2 years with a lot of side effects (not well tolerated by patients). The new guideline consists of 3 to 4 drugs (bedaquiline, pretomanid,linezolid and moxifloxacin) for 6 months, some of them are also intravenous. It is effective, short and well tolerated. (fig.25)

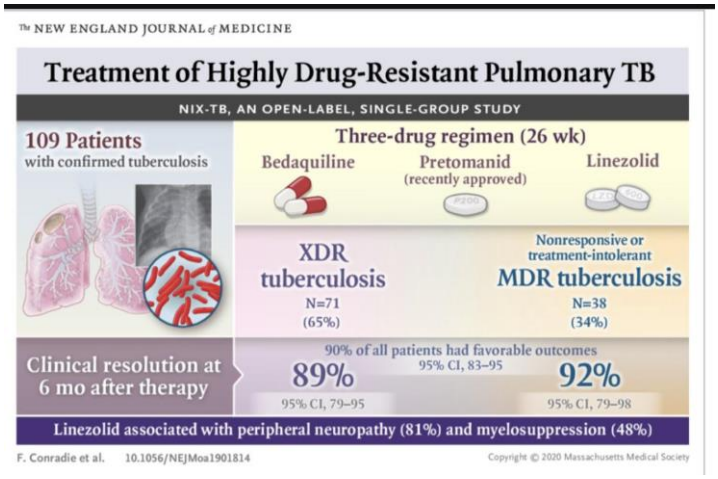

fig.25

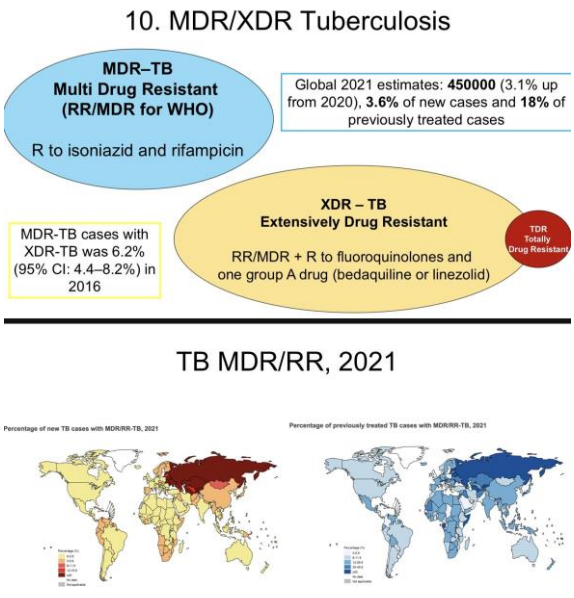

fig.26