NON-TUBERCULOUS MYCOBACTERIA (NTM)

NTM is an important topic because of two reasons: the first is that in recent years this problem has been overlooked. The second reason is that in certain countries (USA included) the incidence of NTM pulmonary disease is higher than the incidence of pulmonary tuberculosis. In Italy we don't have epidemiological data to compare the two diseases, but it's probably the same.
The definition of NTM is a negative one: NTM comprehend all the species other than: Mycobacterium tuberculosis, M. africanum, M. bovis, M. caprae, M. microti, M. leprae (etiological agent of leprosy), M. pinnipedii, M. canettii.

There are more than 150 species, of which 60 are considered pathological for humans. Most of them to be pathological require a very strong immune depression, this is why they are common in ospital. The severity of the pathogen can also be imagined by the velocity of growth. how quickly they grow tells us about how quickly they can create issues in the organisms.

CLASSIFICATION

There are two kind of classification for NTM. The first one classifies them into:

- **Rapid growing mycobacteria**: grow in less than 7 days. Further divided in:
    1. pigmented;
    2. non pigmented, which are very important, because M. abscessus, M. chelone, M. fortuitum, M. septicum, and M. smegmatis are part of this classification. M. abscessus was very common in cystic fibrosis patient in which it gave chronic nodular cavities.
- **Slow growing mycobacteria**: include M. avium Complex (avium, intracellulare and chimera) and also M. xenopi. They usually grow in 40-50 days.
- **Intermediate growing**: grow in 7 to 10 days, M. marinum (causes skin lesions) and M. gordonae are part of this group.

There is not a direct PCR, so it is not possible to use just the sputum, but there the physician need to wait for the culture. In some cases the PCR may result negative. There are some specific types of PCR for NTM but are not always specific.

Apart from their ability to grow in standard culture for Mycobacteria, there is also a classification that relies on their pathogenicity (fig.1).

Pathogenicity		
--	+/-	++
M. chelonae	*M. xenopi*	*M. szulgai*
M. fortuitum	*M. simiae*	*M. shimoidei*
M. scrofulaceum	*M. celatum*	*M. kansasi*
	MAC	*M. malmoense*

Pathogens even if moderate immune-depression

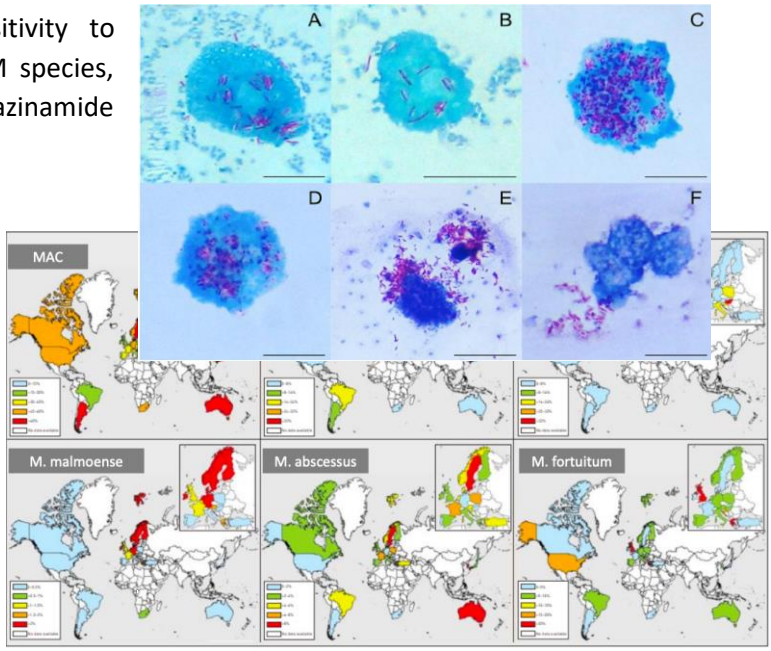
Fig. 1

This classification is relevant because if you find a Mycobacterium fortuitum, which is usually not a pathogen, it means that there might have been contamination from the environment. While the Mycobacterium in the middle column of fig.1 may be pathogenic in hosts that are immunosuppressed. Others like M. kansasi or M. malmoense, instead, can cause diseases to non-immunosuppressed patients.
There was a case of a young man in the field last week, which endured a Neurosurgery due to spinal injury. He got infected by M. fortuitum, probably due to infected water during surgery that was directly injected in the spine.

Fig. 2

DIFFERENCE BETWEEN NTM AND MT

- Habitat: NTM are ubiquitous, they are found everywhere in the environment (like soil and water).
- Virulence: NTM are much less virulent, they are opportunistic microorganism and require an acceptable host, while MT is always a pathogen.
- Transmission: NTM transmission comes from the environment, exception made for M. abscessus has a potential human-to-human spread in patients with CF, and even from contact with animals (rare). MT has human-to-human spread (however M. bovis can spread from an animal but is a rare situation).
- Sensitivity to antibiotics: the sensitivity to antibiotics is different for every NTM species, but globally there is resistance to pyrazinamide and most of them to isoniazid.



TRANSMISSION AND EPIDEMIOLOGY OF NTM

Fig. 3

NTM, as previously said, are ubiquitous and present in household, hospital water, bathrooms, potting soil, garden soil and household dust. Infection sources were identified matching genotypic profiles of clinical and environmental isolates. The transmission of mycobacteria is probably related to the aerosolized particles from water. Showering instead of bathing increases the probability to be infected, as we inhale this aerosol that might be contaminated. Gardening is also not suggested to immunosuppressed patient because of NTM presence in soil. Moreover, cough is a route of transmission. The recent global increase in NTM suggest that its transmission is linked to human activities like trade.
The majority of NTM has been found in different environmental sources, the only exception is M. ulcerans that is highly pathogenic *(will be dealt with later during the lesson)*.
NTM has also the ability to create biofilms, therefore can stay in ice machine, heater-cooler machine for cardiac surgery (M.chimera), seat dusts, vacuum machines and cobwebs[1].
Transmission occurs by:

- inhalation (water/soil) ;
- ingestion (water/soil and food);
- contact (cutaneous lesions).

[1] spider web.

However, M.abscessus can be transmitted by human-to-human interaction which is usually rare[2]. Tap water is considered the major sources of human pathogens such as MAC, M. kansasii, M. xenopi, M. simiae and RGM.

Incidence is poorly defined, varies between 1 and 1.8 cases per 100000 inhabitants, can be as high as 200-400 per 100000 patients with COPD in certain areas of USA, in patients with chronic lung problem or in cystic fibrosis, which is the biggest risk factor and presents the highest incidence. That's the reason why patients with CF are screened every year for the colonization of their upper and lower respiratory tract.
What is possible to understand from this slide is that according to the climate and the environment, there are different bacteria present in different countries. In high income country is usually more common to see NTM that TB, and it is believed that it may be due to:

- higher screening for NTM, so an higher diagnostic rate
- higher frequency in showering rather than bathing. if you bath you do not inhale a lot of water, while in the shower it directly get into yout cavities and increases the probability to get an NTM.

NTM AND AMEBAS[3]

An important aspect of NTM is that they are able to survive in amebas.
Amebas are able to phagocytize free-living bacteria and feed on them, but NTM can survive inside them, creating a sort of a niche in them that provides protection from external agents. Probably 88% of amebas in drinking water contain NTM[4]. These NTM found inside amebas showed an increased resistance to antibiotics, they probably underwent modifications in order to survive inside amebas.

AEROSOL

There is a hypersensitivity pneumonitis, linked to the use of hot tubs. So, patients using hot tubs, lifeguards and pool attendants, those working in indoor swimming pools with sprites and ionized aerosols are at risk for this disease. Such a pneumonitis in a clinical case might be misleading but it is most probably an immune reaction to aerosolized hot water containing NTM rather than a spread infection.

PERSON-TO-PERSON TRANSMISSION

Person to person transmission is a very debatable topic that was raised by a paper written in 2012 by Aitken. He reported a case of a 22 years old man having M. abscessus for 7 years. He transferred to Seattle and 8 months later 4 additional patients with the same species in sputum and same genetic background of this M. abscessus were found (although they had been negative to M. abscessus for years). None of these 5 people had any social interaction, however 4 of them had an overlapping clinic visit the same day. Therefore, following this and due to the potential transmission of M.abscessus in a setting of CF, there is an indication to deal with them as they are contagious: wearing a surgical mask and opening windows at the end of the day after visiting these patients.
This is the only reported case of person-to-person transmission.

PREDISPOSING FACTORS

Some predisposing and risk factors have been identified:

- Bacterial load: how much NTM is inhaled or ingested;

[2] Integrated with the slides
[3] Amoeba is a free-living, freshwater-associated protozoans that are ubiquitously found in water systems - From the slides
[4] the data derive from an year-long sampling study.

- Long-term colonization: if you leave them for long time, they may cause disease;
- Immune response: immuno-depressed patients (HIV+, rheumatological disorders);
- Previous state of organ and tissue: patients with chronic lung diseases have a microenvironment that favors infection of NTM;
- Concomitant systemic disease: for example, ciliary clearance[5] or cystic fibrosis;
- Morphotype: it includes low BMI, scoliosis, "pectus excavatum", mitral prolapse. These conditions may indicate a genetic predisposition associated with a higher risk of infection.

Fig.4 shows how the incidence of NTM changes in different cases.

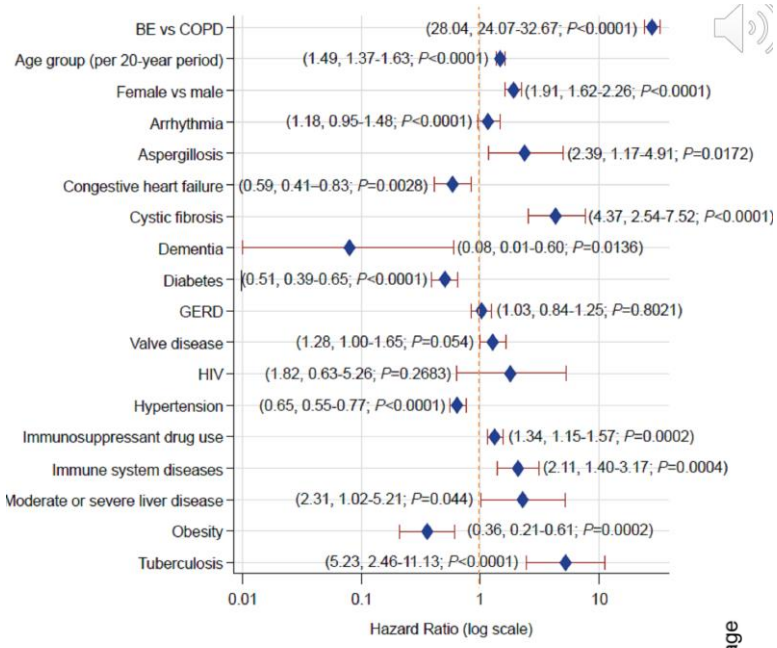
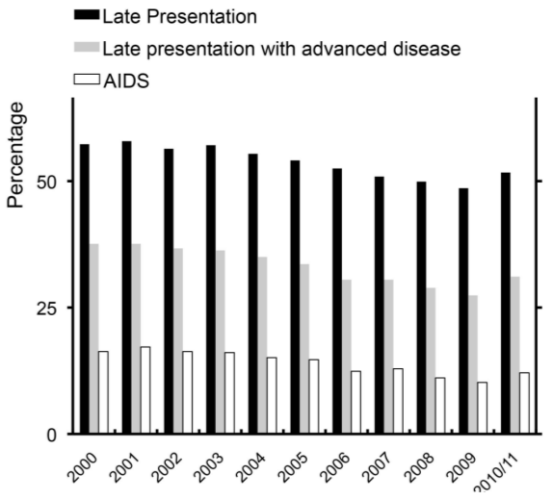


Fig. 5

In cases of cystic fibrosis, NTM incidence is 4/5 times higher; in immune system diseases, it is 2 times higher; in tuberculosis it is 5 times higher; Aspergillus 3 times; in COPD 28 times higher; and lastly, as shown on the graph, higher incidence is observed in elderly and females.

Fig. 4

NTM AND HIV



The knowledge of NTM in infectious disease comes from HIV, in which it was mostly a disseminated disease from M. Avium Complex. It has a medium/high incidence: 10-20% in patients with AIDS patients without treatment, mostly because of CD4+ numbers decrease (low WBC count), but it decreases significantly in patients under HAART.
NTM in AIDS patients are correlated with high mortality, therefore prophylaxis with clarithromycin (1000mg/die) and azithromycin (12000 mg/week) is highly suggested.
An important element is that according to data from the EU, there is still 20% patients presenting with AIDS, 30% late presentation with less than 350 CD4+/mm3, meaning that there is still an high possibility for newly HIV positive patients to develop NTM (as shown in fig.5).

[5] He probably meant an IMPAIRED ciliary clearance as for example in Immotile Cilia Syndrome.

CLINICAL CASE

Fig. 6

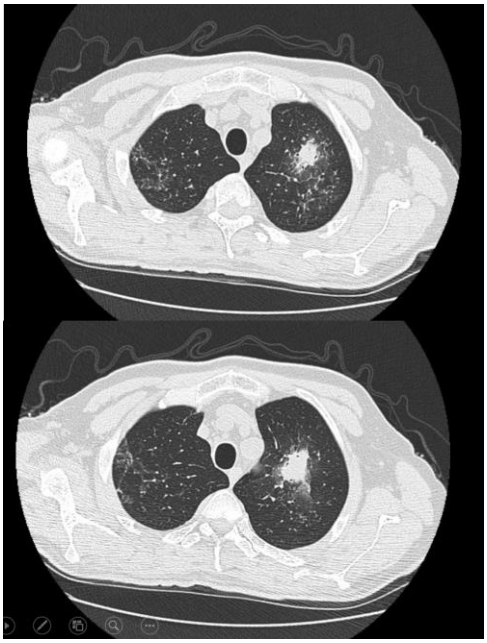


Fig. 6 is a CT scan of a patient with AIDS hospitalized last year. He presented late, with less than 100 CD4 lymphocytes count. Although it wasn't possible to find any sign of infection, in the CT scan, a nodule is visible with a round glass appearance[6] around the nodule. A BAL (bronchoalveolar lavage) was performed, in which fungi and neutrophils were found, culture for candida albicans was positive[7]. Candida pneumonia leads to a severe situation observed in neutropenic and immuno-depressed patients. Moreover, tests for PJ (pneumocystis) was negative and CMV DNA were found in low copies. After four weeks it was confirmed that the culture for Mycobacterium avium was positive.

Therefore, this was a MAC pneumonia in an AIDS patient without a disseminated disease, just localized in the lungs.

the figure below shows a graph that results from a study conducted in Catalonia, where they monitored the incidence of several NTM over time. One can see that M.kansasii was the most frequent in HIV+ patients until 2004. The introduction HAART lead to a better state of HIV+ patients, there is a steep increase in 2 NTM: MAC (red) and abscessus (blue). There is also a difference between the diagnosis of MAC (MAC isolation) and and the pulmonary/disseminated disease, suggesting that in most of the cases there is just a colonization. Treating HIV+ patients and improving their condition changed also the ecology [8] of NTM in the region.

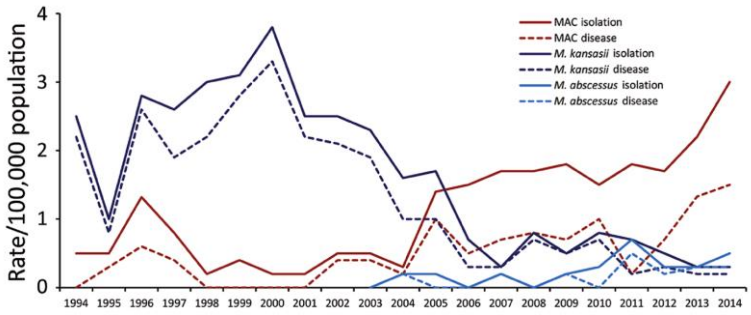


Fig. 7

In fig.8 you can see anti-INFγ antibodies, which are one of the pathways involved in favouring NTM replication observed in Sjogren syndrome and systemic lupus erythematosus. Some patients with immunological disorders have antibodies against INF-γ that may favor NTM, therefore they have high risk of developing NTM pulmonary disease.

[6] This is exactly what he said, although it is not so clear what does it mean.

[7] Remember that candida albicans is usually on skin and several mucoses, finding it in the bronchi means that it was brought it there from the oral cavity during the BAL procedure.

[8]

There have been several studies that tried to find out which genetic variants are associated with high risk of NTM[9].
It is relevant to remember that CFTR (the protein involved in cystic fibrosis) is also a risk factor itself, so when NTM pulmonary disease is diagnosed, doctors discuss with pulmonologist about the risk of an adult onset of cystic fibrosis.

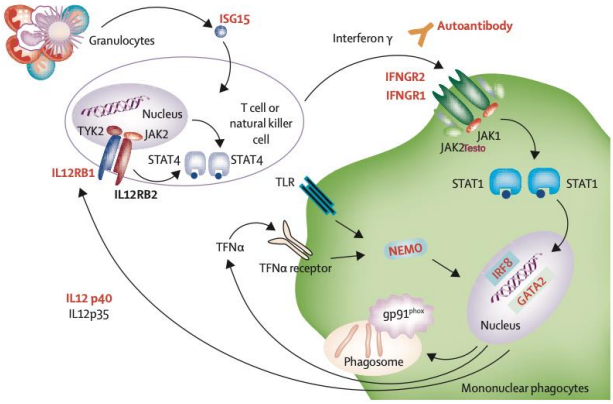


Fig. 8

CO-INFECTIONS

Several co-infections were reported, such as the one with Aspergillus or CMV. Moreover, 10 to 30% patients with Mycobacterium Tuberculosis has a co-infection with NTM and sometimes it can be misdiagnosed. The two infections have a longer time of incubation and are harder to treat.

NTM RISK IN IMMUNO-DEPRESSED HOST

Immuno-depressed patients/hosts present:

- a higher risk of developing NTM pulmonary disease;
- a less acute clinical presentation and the treatment is more difficult because of drug interactions with immunosuppressant drugs, or because of side effects.

Talking about a patient with rheumatoid arthritis, he has a risk of having NTM is around 164.8 /100K, if he is on treatment for rheumatoid arthritis it's risk almost double. Usually patients get diagnosed years later.

IMMUNO-DEPRESSED HOSTS ARE THOSE THAT PRESENT:

- Solid organ transplant;
- Hematological malignancies;
- Autoimmune disorders;
- Treatment for many of the aforementioned issues. Drugs that prevent graft versus host disease or avoid complication of bone marrow transplant can lead to immunocompromising. For example, in Rheumatoid arthritis[10] patients, the risk seems to be around 164,8 over 100000 patients, but in those treated with drugs the risk is almost double (240/100000). So, both the condition and the treatment increase the predisposition of develop NTM pulmonary disease.

SJOGREN'S DISEASE

In Sjogren's disease there is a prevalence of 1% of NTM. It is not as common as in rheumatoid arthritis, but it appears usually during the first year after the diagnosis and is associated with aggressive immunosuppression correlated to methotrexate and cyclophosphamide. However, we don't know if it is a drug side-effect or proxy of a more aggressive disease.

[9] professors said that this figure is just for those of us who are interested in genetics.
[10] autoimmune disorder

CLINICAL PRESENTATION ASSOCIATED WITH NTM

	Most common	Less common	
Chronic broncopulmonary disease	*M.avium complex (M.avium,M.intracellulare), kansasii, abscessus*	*M.xenopi, malmoense, szulgai, smegmatis, simiae, scrofulaceum, immunogenum*	Inhalation from water and soil
Lymphadenitis	*M.avium complex*	*M.haemophilum, scrofulaceum, abscessus, fortuitum*	Ingestion, inhalation, direct contact [laterocervical!]
Disseminated	*HIV+: M.avium, kansasii HIV-: M. abscessus/chelonae*	*M.genavense, xenopi marinum, kansasii*	Inhaled or ingested (years before) [immunedepressed]
Skin and soft tissues	*M.fortuitum, chelonae, abscessus, marinum, ulcerans*	*M.kansasii, haemophilum, smegmatis, genavense goodii, immunogenum*	Trauma, surgical interventions (plastic), water and pools
Bone and joint	*M.marinum, avium complex, fortuitum, chelonae, abscessus,*	*M.haemophilum, scrofulaceum, smegmatis*	Trauma, surgical interventions
CVC-associated	*M.fortuitum, chelonae, abscessus*	*M. mucogenicum, immunogenum, septicum*	Surgical intervention

Fig. 9

In the left column of fig.9, there are the six most common presentations of NTM are shown and the species of Mycobacterium that cause them are divided in most common and less common. The six most common diseases are explained below.

Chronic bronchopulmonary disease is the most common disease in HIV negative[11] individuals, the most frequent NTM that lead to it are: M. avium complex, M. xenopi (Central Europe), M. malmoense (North Europe), M. kansasii (US); M. abscessus; M. immunogenum (contaminated bronchoscopies)[12].

- Incidence:

Incidence seems to be low but can be higher in some population than in other. For example, in USA and japan it is 1,3 cases each year per 100k inhabitants, while in Svizzera is 0,9 cases each year per 100k inhabitants.[13]

- Risk factors:

There are multiple risk factors: smoking, alcohol, previous TB, HIV, bronchiectasis, cancers, silicosis, cystic fibrosis, alveolar proteinosis. Also medications and treatment for other diseases may be a risk factor

- Pathogenesis:

First event is the inhalation of Mycobacterium, after months or years there is the development of an alveolar infection (takes less time if there is an altered pulmonary clearance), then there is the development of granulomas. *(from the slides)*

You might see granulomas or incomplete granulomas and they might, but they are non-producing caseum (differently from MT)

- Clinical presentation:

Clinical presentation includes specific sign and symptoms with a subacute/chronic clinical course: chronic productive cough (80%), asthenia, weight loss (50%), night sweating (20%), fever (10% - not so common),

[11] HIV negative.
[12] there is a geographical difference for the other species.
[13] from slide 22.

hemoptysis, malaise and dyspnea. Recognizing the presentation may not be easy because of how heterogeneity is present. You can see different types of presentations, like some very painful nodules, which may be present both in arms and legs. Sometimes there may be cavities produced.

An example may be M. Marinum. This type of lesion is typical of some tropical diseases, it takes months to develop and it is not painful. Usually it gets to a very severe stage due to the lack of diagnosis and treatment in that geographic area.

Lastly this is the case of mycobacterium chimaera, and specifically there was an epidemic in the field after cardiac surgery due to contaminated surgical tools.

DIAGNOSIS:

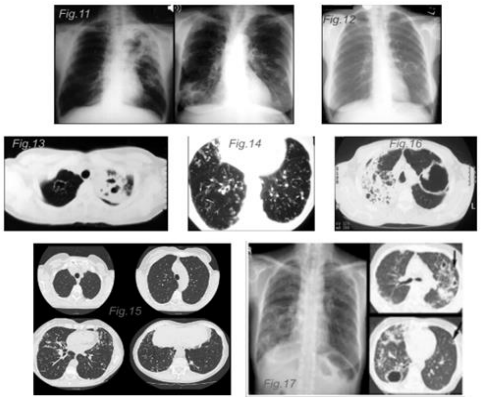
Diagnosis is based on two elements: radiological imaging and microbiological criteria. So, diagnosis is made thanks to chest x-ray, CT (HRCT), sputum staining and culture (at least two positive culture for the same NTM), BAL. PCR is in development (*from slides)*.
In chest X-ray presents fibro-nodular or cavitary lesions, usually located in the upper lobes. These two, cavitation and nodules, are the most common elements observable in an X-rays. Cavitation are also more common in this situation than in TB, but their appearance is different: thinner cavity wall and a larger dimension[14]. There may be also a bilateral pleural thickening in 50% of the cases. Pleural effusion is almost never observed.
Diagnosis is usually made with the CT scan in which you can see bronchiectasis. The presence of nodular lesion, bronchiectasis and the clinical presentation point towards the diagnosis of NTM.
Diagnostic Criteria of the American Thoracic Society
The American Thoracic Society divides the diagnostic criteria in clinical and microbiological.
There are also some physical characteristics typical in patients, which include pectus excavatum, and low BMI.

MICROBIOLOGICAL CRITERIA IS THE MOST IMPORTANT:

1. Positive culture results from at least two separate expectorated sputum samples. So, if you isolate MAC and then chimera or if you have isolated MAC (from here onwards this is the acronym used for Mycobacterium Avium Complex) and gordone, the result is not valid. This is because the same culture from 2 different sputum sample is needed. If the results from a single one is non-diagnostic, consider repeat sputum AFB smears and cultures.
2. Positive culture result from at least 1 bronchial wash or lavage.
3. Transbronchial or other lung biopsy with mycobacterial histopathologic features (granulomatous inflammation or AFB) and positive culture for NTM or biopsy showing mycobacterial histopathologic features (granulomatous inflammation or AFB) and one or more sputum or bronchial washings that are culture positive for NTM.
4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination.
5. Patients who are suspected of having NTM lung disease but do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded.[15]

[14] with respect to the TB ones.
[15] From the slides

6. Making the diagnosis of NTM lung disease does not, *per se,* necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients.



CLINICAL FINDINGS IN THE RADIOLOGICAL IMAGING

In the first two chest x-rays, consolidation is observed. *(fig, 11)*
In the second chest x-rays, cavitation can be appreciated. *[fig.12]*
Some CT scans show a massive involvement very similar to TB. *[fig.13]*
Bronchiectasis, some nodules and minimal disease (mostly in the medial lobe of the right lung). *[fig.14 and fig.15]*
There might be both nodules and cavitation.
In the last CT scan, there is a large cavitation with a thin wall: this is a situation that make suspect NTM or aspergilloma. These two conditions can even coexist and the treatment is very complicated. *[fig.17]*

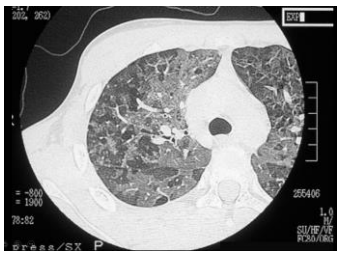


Fig. 18

CLINICAL VARIANTS OF CHRONIC BRONCOPULMONARY DISEASES:

1. Cystic Fibrosis
2. Lady Windermere Syndrome, which is a chronic pulmonary NTM disease, usually is observed in female patient over 50 years old with no previous concomitant pulmonary disease. Lady Windermere syndrome is characterized by chronic cough with no constitutional symptoms and mild lung involvement, few nodules and bronchiectasis. It is a monolateral disease and cavitation are found in only 25% of the cases. There is a very strong association between this syndrome and depression. The disease is named after the character of the Oscar Wilde novel.
3. Hypersensitivity pneumonia: inhalation of an aerosol of heated water containing MAC. In the CT scan there is mostly an interstitial involvement of the two lungs.

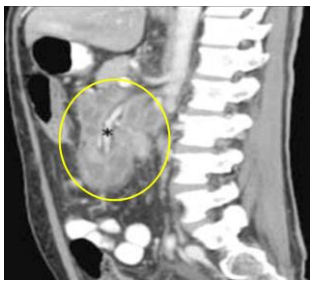


Fig. 19

II. NMT LYMPHADENITIS

NMT Lymphadenitis will be dealt with starting with its presentation in HIV negative patients, and then HIV positive patients.

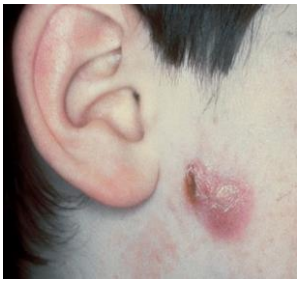


Fig. 20

2.1 NTM Lymphadenitis in HIV-negative patients: NTM Lymphadenitis is common in children. The most frequent one is MAC. Usually there is an involvement of the anterior laterocervical lymph nodes, it is monolateral, non-painful and without fever in most cases. There could be an involvement of mediastinal lymph nodes. In some children there is a fast clinical course and risk of fistulation.
Mantoux test is intermediate: between 5 and 10mm, sometimes more than 10mm. Some research used

intradermal reaction to MAC antigens, but it is not part of the clinical practice, yet.

Fig. 21

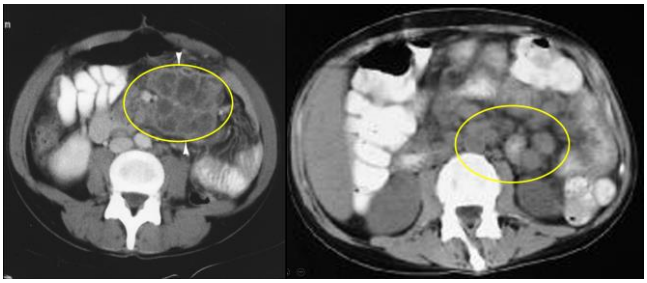
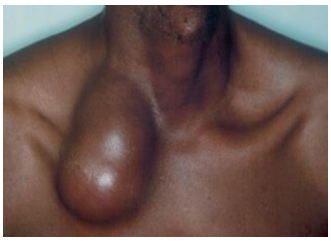


Fig. 22



Diagnosis is usually made with an excision biopsy or fine needle biopsy of the lymph node from which the culture is then performed, although it is often negative. Pathology: granulomas without caseous center (sometimes with) ± AFB (Ziehl-Neelsen, PCR on tissue slices).
Differential Diagnosis: TB lymphadenitis, cat-scratch disease (Bartonella), lymphomas, Toxoplasmosis, EBV, Metastatic tumors.
Treatment: consists of lymph node excision, which is usually enough, but in some scenarios a combination of antibiotics is needed.
Example: a cervical submandibular adenitis with fistulation. It presents red skin and infection. There can be abdominal lymph node involvement; the symptoms present in this case are abdominal pain and maybe fever.

2.2 NTM Lymphadenitis in HIV positive patients:

NTM Lymphadenitis is observed in patient with AIDS or severe immune-depression, it might present itself as IRIS[16]. It is mostly a painful lymphadenitis with fever due to its systemic nature. Usually the involvement of deep lymph nodes causes abdominal pain.
Diagnosis: with this presentation abdominal CT scan is useful to get an idea of what's going on. Often biopsy is necessary to confirm the diagnosis and exclude alternative disorders.
There can be involvement of abdominal, lateral and cervical lymph nodes.
Fig 22 shows an HIV positive patient with an over clavicular mass. In the CT scan shown in fig.21 it is possible to see a colliqued collection, and a communication with the lung. Usually it is either a pleural nodule or a lung nodule that opens up outside and the biopsy will be positive for a non-caseous granuloma.

III. DISSEMINATED DISEASE IN HIV-POSITIVE PATIENTS

In HIV+ patients with advanced disease - AIDS patients - there is also a disseminated disease most commonly caused by MAC. This is a very late presentation, when CD4+ count is below 50/mm3, in particular the median count is of 13 CD4/mm3 (for reference, HIV negative patients have between 800 to 1400 CD4+ count). Patients present with:

- Very high fever, weight loss, night sweat, abdominal pain, diarrhea and diffuse lymphadenitis.
- Often there is liver and spleen enlargement, while lung involvement is not that common (10% of the cases with alveolar infiltrates, nodules, cavitation). Potentially involvement of: suprarenal glands, stomach, GI, bone marrow (often found in Mycobacterium blood culture or bone marrow aspirate).
- Laboratories abnormalities include pancytopenia (anemia is the most relevant) and elevated alkaline phosphatase.

Disseminated disease is present in other immuno-depressed patients such as:

- Transplant patients;
- Solid and bone marrow transplant recipients;

[16] IRIS: Immune Reconstitution Inflammatory Syndrome

- Chronic steroid treatment;
- LLC (chronic lymphocytic leukemia);
- Congenital immune-depression in children (highest if deficits of IFN-γ and IL-12 receptor and IL-12 deficits).

Diagnosis: usually based on positive hemoculture for mycobacteria (BACTEC, with a liquid culture ground, with sensitivity > 90%), biopsy of lymph node, in certain cases of pancytopenia culture from bone marrow aspirate, liver/spleen biopsy («Myco-f-lytic» means) -> 8-14 days, specific PCR.
Differential diagnosis includes TB, CMV, Salmonella infections, lymphomas, wasting syndrome, malignancies and rare fungal infections such as cryptococcosis, histoplasmosis.

IV. SKIN AND SOFT TISSUES INFECTIONS

Skin and soft tissues infections are mostly related to RGM (rapid growing mycobacterium): *M. fortuitum, chelonae, abscessus; M. marinum,* which appear either after trauma or over dermatological lesions*;* and *M. ulcerans; MAC* which are less common.
RGM:

- *M. fortuitum: chronic skin ulcers after traumas (sharp metals, motorbike accidents) mostly to feet and legs, pimples after «skin care», post-surgical (plastic surgery).*
- *M. chelonae: immunodepressed hosts (rheumatoid arthritis, SLE, chronic steroid use) or «over» skin lesions.*
- *M.abscessus: immunocompetent and immunosuppressed hosts, skin lesions, spondylitis.*[17]

Fig. 23 shows: example of lesions. They are not painful and often tend to become chronic ulcers with time, and that's the reason why biopsy is performed to have a diagnosis.

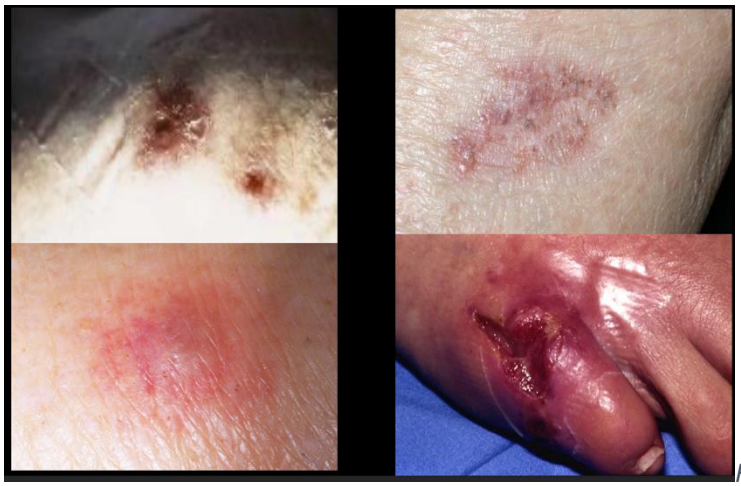


Fig. 23

Two other less rapid growing mycobacterium can give skin and soft tissues infections, shown in *fig.24*:

- *M. marinum*: mostly seen in patients handling fish tanks (contaminated water/fishes). This mycobacterium presents an incubation period of 2-3 weeks. It's infection presents with papular lesions on hands and arms (ulcers).
  The diagnosis is done through **history** and **biopsy** (histology + culture).
- *M. haemophilum: immunosuppressed skin papula lesions and ulcers on arms and legs.*[18]

[17] From the slides
[18] From the slides

- *M. ulcerans:* called also Buruli's ulcer. It is a tropical disease with a very long incubation period (8-12 weeks). Nodular lesions over time can transform into destroying ulceras (mostly lower limbs), causing debilitating lesions. These lesions are not painful. In slide 55 you can find a map of the diffusion of this agent. The areas that are mainly affected are: tropical countries, Africa, Central America and Australia.

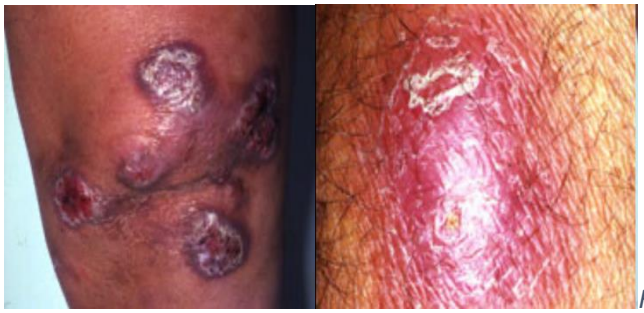


Fig. 24

In fig.25 there are other example of lesions. Nodules and ulcers are visible, specially the devastating ulcers. Ulcers can appear in arms, ears, thorax or everywhere.
These lesions are very hard to be treated, surgical approach is sometimes needed.

Fig. 25

V. BONE AND JOINT INFECTIONS

Bone and joint infections are chronic inflammation with granulomas, usually coming from direct inoculation from traumas, surgical procedures, injections. Many species are associated with these infections:

- *MAC, M. marinum: hand (tenosinovitis) infection;*
- *M. chelonae, M. haemophilum: immuno-depressed, potential disseminated disease;*
- *M. haemophilum: bone and joints, usually with skin fistulizations and bacteremia;*
- *M. fortuitum, M. abscessus: sternal osteomyelitis after cardiac surgery.*[19]

Diagnosis requires **Biopsy** and **culture**.

VI. CVC OR PERITONEAL CATHETERS INFECTIONS

[19] From the slides (the professor did not mention them)

CVC or peritoneal catheters infections are usually caused by RGM (*M. fortuitum, chelonae, abscessus*).
Clinical presentation includes:

- fever;
- secretions from the insertion site;
- bacteremia;
- secondary infiltrates in other organs like lung or liver, where can specifically lead to granulomatous hepatitis.

Therapy: Therapeutic strategies consist in CVC/catheter removal and antibiotics directed specifically against the species isolated (6-12 weeks).

MYCOBACTERIUM CHIMAERA (MYCOBACTERIUM AVIUM COMPLEX)

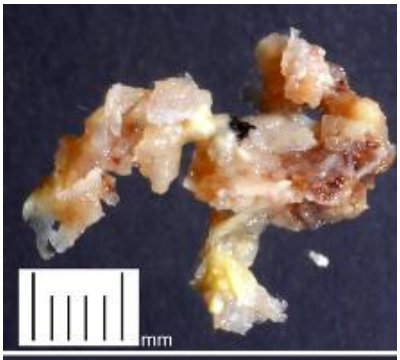
M. chimera is included in *MAC*, but it can be genetically differentiated from *Mycobacterium Avium* and *Mycobacterium Intracellulare*. Recently, it's been shown that disseminated *M.chimera* infection can be associated with heater-cooler units after cardiac surgery such as aortic valve surgery without endocarditis.

Fig. 26 presents a review where all the cases related to heater-cooler units are reported. It is interesting to note the very long latency, meaning that from surgery to the appearance of symptoms elapsed months or even years (up to 6 or 7 years). While from symptoms to diagnosis elapsed even 1.6 years. This means that it's not easy to recognise *M. chimera* as causative agent of infections in patients that have undergone cardiac surgery months or years before.

	Latency		
Outbreak Location/N/Citation	Surgery to Symptoms	Symptoms to Diagnosis	Mortality (%)
Europe/10/[7]	Median, 18 months	Median, 21 (5–40 months)	5/10 (50)
United Kingdom/30/[28]	Median, 14.5 months (range, 1.5–60 months)	Median, 7 weeks	18/30 (60)
Germany/5/[17]	Range, 5–60 months	NR	1/5 (20)
Pennsylvania/8/[26]	NR	Median, 1.2 years (1–27 months)	5/8 (63)
United States/24/[25]	NR	Mean, 1.6 years (range, 0.1–6.3 years)	11/24 (46)
New York/2/[31]	NR	Mean, 14.5 months (range, 12–17 months)	0
Montreal, Canada/2/[21]	Range, 13–16 months	Additional 2–3 months from presentation	0
Florida/1/[24]	72 months	NR	0
Minnesota/3/[22]	Range, 16–26 months	NR	2/3 (67)
Italy/1/[27]	14 months	12 months	0

Fig. 26

Fig. 27



Gross examination of the periaortic debris shows yellow-to-tan tissue with some fibrous tissue strands (white tissue).

Clinical manifestation years after surgery includes:

- Fever (80%), malaise (80%), weight loss (60%), cough (37%), and dyspnea (33%);
- Splenomegaly, hepatomegaly and chorioretinitis;
- Cytopenias which is similar to disseminated disease in immunocompromised patients;
- Elevated inflammatory markers, transaminase levels, and creatinine;
- Histopathology is consistent with granulomatous lesions, including dissemination to several organs leading to hepatitis, nephritis, pneumonitis, chorioretinitis, myocarditis, osteomyelitis, and myositis.

In Fig.27 of a periaortic debris, there is a thick biofilm. It is difficult to diagnose and treat unless tissue is removed.

Fig. 28

MAC (n=170)	RE	REH
No.	37	38
Deaths (All causes)	32%	39%
Deaths (Myco)	0%	8%
Failure/relapse	41%	16%
Alive and cured at 5 yrs	27%	34%

Laboratory assessment:
The gold standard for diagnosis is a *M.chimera*-positive cultures obtained from an *invasive sample* (blood, pus, tissue biopsy, or implanted prosthetic material).

Clinical assessment:
The diagnosis should moreover include:

- Prosthetic valve endocarditis;
- Prosthetic vascular graft infection;
- Sternotomy wound infection;
- Mediastinitis;
- Bloodstream infection;
- Disseminated infection, including embolic and immunologic manifestations (splenomegaly, arthritis, osteomyelitis, bone marrow involvement with cytopenia, chorioretinitis, lung involvement, hepatitis, nephritis, myocarditis) .

Therefore, it is very hard to diagnose it.

Exposure assessment: If the **past medical history** include surgery requiring cardiopulmonary bypass, an infection from *M. chimera* should be taken into account, either disseminated or affecting a single organ.

TREATMENT OF NTM INFECTIONS

The following are some of the principles of treatment. The treatment for NTM is very long: guidelines suggest 12 months from negative cultures, and it might take between from 18 to 24 months. Moreover, all the drugs present significant side effects and poor efficacy rates. The following should be taken into consideration when prescribing medications:

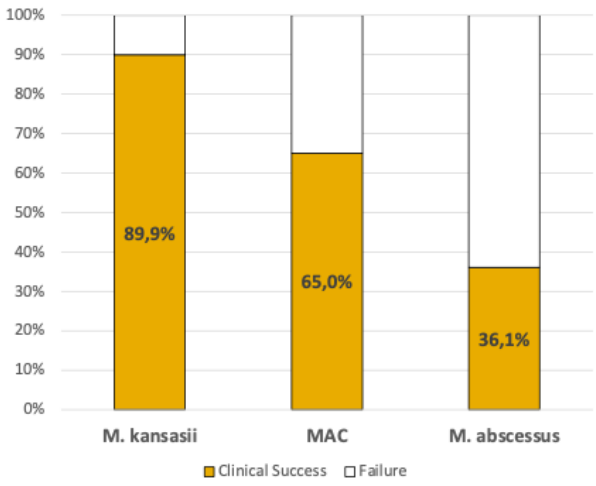
- ATS criteria for infection versus colonization;
- We should always weigh benefits and potential harms in terms of radiological and clinical microbiological outcome;
- Patients should be involved in the treatment decision and should be informed that the treatment could take 2 years and that there could be side effects.
- The type of patients must be considered, their lifestyles,their age, their comorbidities. In fact, a 40 years old patient with cystic fibrosis will require a different treatment with respect to a 90 years old woman that just reports asthenia.

There are very few RCT[20] guide treatment, therefore, dosage or amount of drugs to be taken and the length of the treatment changes according to the species, severity of the disease and concomitant disorders.
Usually the treatment is a combination of **macrolides** (azithromycin, clarithromycin) plus ethambutol and rifabutin/rifampicin. Thus, is a standard 3 drug regimen, and every species has a different set of drugs. For instance, *M. abscessus* had a completely different regimen that involves 4 weeks of Intravenous therapy at

[20] Randomized clinical trials

the beginning and then continuous therapy with oral drugs.

Fig. 29



The guidelines of the American Thoracic Society and the British Thoracic Society represent the best source of knowledge for the clinical management of these infections.[21]

As shown in figure 20 the outcomes are poor, even using the 3 drugs regimen for *MAC*- correlated pulmonary disease, 40% of the patients doesn't survive (32% for all causes while only in 8% deaths is related to mycobacterium),16% risk of relapse and the percentage of 5 years survival is only 27%.

Recap: very long treatment, side effects present and poor outcomes.

FAILURE

Failure should be distinguished in three fields:

- **Clinical failure:** patients do not improve, although the microbiological picture improves;
- **Microbiological failure** with non-sputum conversion: all the selection resistance[22]
- **Radiological failure:** lesions do not improve or even worsen under treatment.

Outcomes are set with the patient, if after a year we do not have improvement with this treatment, the outcomes may be weighted again with the patient and he should be involved in the decision regarding his future treatment.

The rate of failure and/or relapse:

- MAC-PD presents a failure rate around 13-16% with 3-drug regimen, which is not very high. *Up to 48% in some case series*, the rate of reinfection is very high, around 75%. *Higher incidence if previously treated, macrolide resistance, non-sputum conversion after 6 months. Better outcomes by adding «new» drugs (fluoroquinolones, clofazimine, linezolid)*.
- *M. Abscessus*-PD presents a failure rate of 42% with standard treatment (4 weeks i.v. plus maintenance). Further distinguishing between *M. abs. abscessus* and *M. abs. massiliense*, the former present a failure rate of 75%, whereas the latter of 22%. Therefore *M. abs. abscessus* is the most difficult to treat. *Relapse rates are: 24-42% con 1/2 iv drugs 58% in Myc abs abscessus vs. 5% in Myc abs massiliense. 52% in the US*[23]

INHALED LIPOSOMIAL AMIKACIN

There are several trials for new drugs, including those discussed for resistance TB: bedaquiline, clofazimine and lanesolate, tenozalide . The only very specific drug for NTM in development (not already approved by the FDA) and evaluation under EMA is inhaled liposomal amikacin. Amikacin is used for the treatment of NTM, but Intravenous amikacin has the disadvantage that it must be given IV in day hospital. Moreover, it is associated with renal insufficiency that may be irreversible and also with ear toxicity (ototoxicity), leading to several symptoms and correlated with a genetic predisposition. Using inhaled liposomal amikacin renal

[21] These guidelines present complete informations on every single species. They are not required to be read for the exam but keep in mind that whenever there's a doubt on a treatment for a particularly rare species, these are the literature that doctors refer to.

[22] Audio at slide 68 (microbiological failure at the time 00:09 min) - Not so clear.

[23] Everything in *Italic* is integrated from the slides

insufficiency is totally avoided, while IR toxicity is only partially avoided. The liposomal formulation increases the amount of drugs that get inside alveolar macrophages (which are the target sites) and decreases the amount of drugs that is systemic available, thus affecting the tolerability of the patient.

TREATMENT OF MACROLIDE-R MAC-PD.

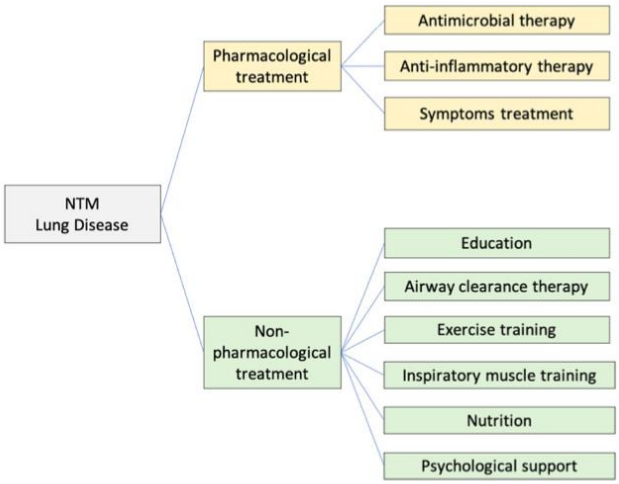
We should always ask to the laboratory if our NTM is resistant or susceptible to macrolides. Macrolides are the cornerstone. Moreover, there is a genetic association, meaning that there is a genetic probe that will indicate if the NTM is resistant. It is a slow and complex phenotypic test, therefore only few laboratories are able to perform this phenotypic assessment of NTM susceptibility to drugs.
In case of MAC-pulmonary disease, that is macrolide resistant, in which the efficacy is around 20%, a combination of four drugs is used: rifampicin, ethambutol, ionized FQ/clofazimine and inhaled amikacin.

In case of *M. abscessus* PD (macrolide resistant) there are few weeks of intravenous treatment and a very long phase of oral drugs, including new drugs. However, there are side effects: linezolid is a very powerful anti-Gram+ drug and is associated with peripheral neuropathy, anemia and thrombocytopenia, so is hard to use it for 2 years. Almost all these drugs have side effects that make it difficult to have a long-term treatment based on them.

MULTIDISCIPLINARY MANAGEMENT

Fig. 30

Usually the pharmalogical treatment is combined with a non pharmacological one as shown in figure 30.
Physical exercise is very important, as well as airway clearance therapy, followed by a pneumologist, in order to open the airways.
Depression is very common, so a phycological support is recommended.



Calcagno A, et al. Infection 2024