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Coinfection of *Mycobacterium malmoense* and *Mycobacterium chimaera* in a kidney transplant recipient: a case report and review of the literature

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Abstract :

Nontuberculous mycobacteria (NTM) are ubiquitous organisms found in soil and water. Solid organ recipients are at increased risk of NTM infections due to impaired immunity. Although the NTM infections rate is low, it increases morbidity and the risk of mortality. Diagnosis is often delayed because of the lack of specific clinical symptoms and requires a high index of suspicion. Management may be challenging: long-term treatment with risks of side effects and interactions with immunosuppressive regimen; reduction of immunosuppression and risk of allograft rejection. Prognosis is widely variable. We report the first case of *Mycobacterium malmoense* chest infection with concomitant *Mycobacterium chimaera* urinary tract infection in a kidney transplant recipient. The evolution was marked by poor tolerance of the treatment with severe adverse events and disabled functional status.

7. Main text:

INTRODUCTION

Nontuberculous mycobacteria (NTM) are ubiquitous environmental pathogens that are responsible of a diverse group of infectious disease. Due to depressed cell-mediated immunity, solid organ transplantation (SOT) recipients have an increased risk of these opportunistic infections [1-5]. We report the first case of a kidney transplant recipient (KTR) with *Mycobacterium malmoense* chest infection and concomitant *Mycobacterium chimaera* urinary tract infection. We review the current literature about the epidemiology, risks factors, diagnosis, treatment and prognosis of NTM infections in SOT. We also briefly review the current literature about *M. malmoense* and *M. chimaera* infections.

CASE REPORT

The patient is a 64-year-old male with history of end-stage renal disease caused by antineutrophil cytoplasmic antibody-associated vasculitis (AAV) treated by prednisolone and azathioprine. His past medical history included anthracosilicosis diagnosed thirty years ago (he had worked in a sandblasting manufactory) and multiple episodes of pneumonia of unknown origin. After four years on hemodialysis, he was transplanted with a deceased-donor kidney. One week after transplantation, he presented fever and chills. The work-up including urinary analysis, blood cultures, thorax radiography, and graft ultrasound showed no origin of infection. The fever had resolved after empirical antibiotic therapy (ceftriaxone). He was discharged two weeks after the transplantation. His immunosuppression regimen includes tacrolimus, mycophenolate mofetil and low-dose methylprednisolone.

Two weeks later, the patient reported daily and prolonged fever with severe asthenia. A comprehensive assessment did not point to the origin of the inflammatory syndrome. Mycophenolate mofetil was switched to azathioprine in the hypothesis of relapsing AAV but without any benefit. The positron emission tomography (PET) with computed tomography (CT) revealed hypermetabolic activity in calcified pulmonary masses and in mediastinal lymph nodes (Figure 1). Increase in lymph nodes size compared to past exams was in favor of active infectious or inflammatory disease other than false-positive images due to anthracosilicosis. Mycobacterial examination of a mediastinal lymph node needle aspiration performed by endobronchial ultrasound showed acid-fast bacilli (AFB). Specimen was inoculated in the Mycobacteria Growth Incubator Tube (MGIT, Becton Dickinson, USA) for culture. Once positive it was first identified by the Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (Bruker Daltonik GmbH, Bremen, Germany) as *M. malmoense*. Confirmation of the identification was performed using the rpoB and the 16S rRNA gene sequencing. Meanwhile, still searching for an insidious infection,

mycobacterial examination of a urine sample showed also acid-fast bacilli. The sample was also inoculated in MGIT tube for culture. MALDI-TOF identified the urine sample as *M. chimaera_intracellulare*_group unable to differentiate both species. We thus performed the rpoB and the 16S rRNA gene sequencing for the differentiation between *M. intracellulare* and *M. chimaera* which further identified *M. chimaera*.

Empirical treatment for NTM infections was initiated and included clarithromycin (1000mg/day), ethambutol (1200 mg/day) and rifampicin (600 mg/day). Azathioprine was stopped and his immunosuppression regimen included tacrolimus (to reach a trough-level of 4 ng/mL) and methylprednisolone (4 mg/day). The next month, the patient suffered from gastro-intestinal intolerance to the treatment. Fever disappeared after two months. At the third month, he presented severe visual loss due to ethambutol toxicity. Ethambutol was replaced by moxifloxacin (400mg/day). Because of slowly growing characteristic of this two mycobacterial species and because of the fact that the analysis were made by another laboratory (national reference center), antimicrobial susceptibility testing (AST) was obtained in the sixth month of treatment (Table 1). The treatment was changed to clarithromycin (1000mg/day), rifabutin (300 mg/day) and amikacin adapted to the trough value (500mg twice a week). Decrease of the inflammatory syndrome was observed in the seventh month. Extremely severe anorexia, sarcopenia, cachexia and weigh loss persisted after eight months, as well as severe sight loss that did not recover after withdrawal of ethambutol. In the meantime, besides NTM infections, he suffered from cytomegalovirus (CMV) reactivation (at sixth month after transplantation) treated by valganciclovir. He was also diagnosed with squamous cell carcinoma of the scalp treated by surgical resection. Currently, nine months after diagnosis and treatment, the patient suffers from severe altered functional status (Karnofsky performance status of 40).

DISCUSSION

NTM include more than 140 ubiquitous saprophytic organisms found in soil and water. Although they are uncommon causes of disease in the general population, they cause more often opportunistic infections in immunocompromised patients [1-8]. Among SOT recipients, skin and soft tissue disease is the most frequent NTM infection. In KTR, NTM cause more often cutaneous or disseminated infections, followed by osteoarticular disease and then pleuropulmonary infections [1,3-6].

In solid organ transplant (SOT), although NTM infection rates are low compared to other infection, they cause significant morbidity and mortality [2,3,5,9]. The epidemiology is not fully defined and varies widely [1,2].

Rates of NTM infection in kidney transplant recipients (KTR) are lower (0.16 to 0.38%) compared to heart transplant recipients (0.2 to 2.8%) or lung transplant recipients (0.5 to 8%) [2-5,10]. NTM infections tend to outnumber *M. tuberculosis* infections in tuberculosis-nonendemic countries[3,4].

Three main factors contribute to higher risk of NTM infection in SOT. First, the immunosuppressive drug regimen may promote the progression to clinical disease among those with environmental exposures [5,10]. Second, structural lung disease may promote airway colonization and disease, particularly among lung transplant recipient [7,10]. In our case, anthracosilicosis was a clear risk factor. Third, the disruption of mucocutaneous barriers during surgery may serve as portals entry for NTM (potential nosocomial transmission of infection) [5,10].

Although the timing of infection after transplantation can vary from early to very late, NTM infections are in general considered as late post-transplant infectious complication (mean of 48 months after transplantation) [3-5]. In the present case, NTM infections occurred very early after transplantation. Interestingly, our patient reported that an old colleague of him, who worked in the same factory of sandblasting and had also anthracosilicosis, was diagnosed with cervical lymphadenitis caused by NTM. Indeed, six years ago, mycobacterial examination of a cervical lymph node showed AFB. 16S RNA gene sequencing by the same national reference center identified *M. malmoense*. Total resolution was observed after treatment including clarithromycin, rifampicin and isoniazid during 18 months despite poor digestive tolerance. We made the hypothesis that ''indolent'' *M. malmoense* pulmonary infection was present before kidney transplantation and that the disease was exacerbated by immunosuppression quickly after transplantation.

Establishing the diagnosis of NTM infection is often difficult because clinical symptoms are nonspecific [1,2,8]. In addition, NTM can be found as an environmental or laboratory contaminant and a positive culture does not necessarily indicate infection [3,4,7]. In 2007, the American Thoracic Society (ATS) published clinical, radiographic and microbiologic criteria required for diagnosis of NTM pulmonary disease [6]. However, although these criteria provide useful reference point for diagnosis of pulmonary infection by NTM in SOT recipients, they have not been validated for the transplanted population [1-3]. Similarly, there are no specific criteria for diagnosis of extrapulmonary disease by NTM in the general population or for SOT recipients. Finally, NTM are not often included in routine diagnostic testing and slow growing culture delay diagnosis. Hence, a high level of clinical suspicion for NTM disease is essential for rapid and accurate diagnosis [1,5,7].

Clinical samples should be sent for acid fast staining, mycobacterial cultures, and histopathology [1,2,5]. Diagnostic at the species level and antimicrobial susceptibility testing (AST) are complex, requiring molecular probes such as 16S rDNA sequencing and MALDI-TOF mass spectrometry, and high expertise of the laboratory evaluating NTM species [2,4,8].

ATS guidelines are available for the treatment of NTM infections in the general population [6]. In SOT recipients, there have been no randomized trials of NTM infections treatment. Data is limited to case series and is based on data from the non-transplant population [5]. However, special considerations are necessary. First, three-drug therapy is recommended to be used in SOT recipients similarly to non-immunosuppressed patients [2,3,8]. Due to the variability of the susceptibility patterns, antimicrobial treatment should be individually adapted according to the in vitro AST of the isolate. However, the correlation between in vitro AST and clinical outcome has only been demonstrated in a limited number of species [2]. Second, the duration of therapy depends on the isolate, on the site of infection, and, most importantly, on the response to therapy. SOT recipients often require a longer course than immunocompetent hosts and it is recommended to treat for more than 12 months [1-5]. Microbiological cure is difficult to achieve because of its prolonged antimicrobial treatment leading to significant toxicity [3,7]. In the case of the presence of 2 NTM species, the treatment may be more challenging. Indeed, adding more and more antimicrobial therapy increases the risk of drug toxicity and drug interactions. In our case, a three-drug regimen had been kept since the beginning until the end of the treatment. Third, whenever possible, initial therapy should include a reduction in the intensity of immunosuppressive therapy [5,8]. However, no studies have specifically looked at particular immunosuppressive drug association with a risk of NTM. Dealing with immunosuppressive therapy can be difficult. Forth, the interactions between antimycobacterial and calcineurin inhibitors (CNIs) or mammalian target of rapamycin (mTOR) inhibitors should be considered. Monitoring of drug levels and clinical toxicities is essential [1-3,5,7]. Fifth, other contributing factors such as concomitant viral infections (in particular CMV infection) should be treated [3].

The outcome of NTM disease in transplant recipients is highly variable due to multiple factors including type of transplant, site of infection, ability to decrease immunosuppression, and the specific NTM species [3]. Among KTR, 44% to 63% reported cure with initial therapy. In 3 to 6% of the cases, death was attributed to NTM infection [1,3].

One atypical characteristic of this case was the presence of two distinct NTM species. *M. malmoense* infections had been described in general population and SOT recipients. *M. malmoense*, named after Malmö (a city in Sweden), was first described by Schröder and Juhlin in 1977 as a respiratory tract

pathogen [11]. In the overall population of northern Europe, it is the second most commonly isolated NTM after *Mycobacterium avium* Complex (MAC) [6]. Two common threads run through the identification of *M. malmoense* infection: its difficulty to grow and its difficulty to discern from other NTM species. Reports of infections by *M. malmoense* were sparse over the decades [12,13]. In a study of 51 patients from the Netherlands with *M. malmoense* isolation, 40 (80%) of them had pulmonary isolates, and 80% of them met the ATS diagnostic criteria. Interestingly, one of the patients in this study was a KTR and had a contact with another patient immunocompetent suffering from *M. malmoense* infection [14]. In contrast to other NTM, only 20% of infections by *M. malmoense* are extrapulmonary, such as cervical lymphadenitis and tenosynovitis [6,15].

To our knowledge, the present case is the first case describing *M. chimaera* infection in a KTR. *M. chimaera* was first described in 2004 by Tortoli, as new specie included in the MAC [16]. It usually has been misreported as *M. intracellulare* (61% to 86% of previously named *M. intracellulare* were in fact *M. chimaera*) [17-19]. This novel specie has been associated with tuberculosis-like pulmonary infections. However, its pathogenicity is still debated. If some authors estimated that *M. chimaera* may be less virulent than other MAC specie, others estimated it as an important virulent NTM pathogen [16-20]. The small number of the population studied and the difference in geography may explain the large variability between these studies. In our case, pathogenicity of this NTM may be subject for discussion. In fact, finding some NTM does not mean real infection. However, because of the prolonged fever, the immunosuppressive condition and the unusual finding in a kidney graft, we preferred to consider both of the two NTM responsible of infection and to treat both of them. Clinical relevance of *M. chimaera* has been highlighted since 2015 when an outbreak of more than 100 prosthetic valve endocarditis and disseminated disease were notified in Switzerland, Germany, the Netherlands, the UK, the USA and Australia. This infection had been associated with contaminated Sorin 3T heater-cooler units used during cardiac surgery with extracorporeal circulation. None of these infections were described in a SOT recipient [21]. In our patient, no origin of the *M. chimaera* could be found (including donor transmission ruled out by polymerase chain reaction (PCR) analysis on the pre-transplant kidney biopsy).

In conclusion, we describe an unusual case of NTM infection in a KTR co-infected with *M. malmoense* and *M. chimaera*. NTM infections are uncommon but important opportunistic infections in SOT. A high level of suspicion must be maintained to ensure prompt diagnosis. Adequate management may be challenging because of a long-term multiple drug therapy that may cause significant adverse effects and drug interactions necessitating a close monitoring of immunosuppressant medication.

8. References

1. Daley CL. Nontuberculous mycobacterial disease in transplant recipients: early diagnosis and treatment. Curr Opin Organ Transplant 2009; 14:619-24.
2. Dorman S, Subramanian A, and the AST Infectious Diseases Community of Practice. Nontuberculous mycobacteria in solid organ transplant recipients. Am J Transplant 2009; 9 Suppl 4: S63-9.
3. Doucette K, Fishman J. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. Clin Inf Dis 2004; 38: 1428–39.
4. Fan M, Hadjiliadis D. Incidence and management of mycobacterial infection in solid organ transplant recipients. Curr Inf Dis Rep 2009, 11: 216 – 222.
5. Keating M, Daly J, and the AST Infectious Diseases Community of Practice. Nontuberculous mycobacterial infections in solid organ transplantation. Am J of Transplant 2013; 13: 77–82.
6. Griffith D, Aksamit T, Brown-Elliot B, et al. An Official ATS/IDSA Statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007; 175: 367–416.
7. Ho T, Rommelaere M, Coche E, et al. Nontuberculous mycobacterial pulmonary infection in renal transplant recipients. Transpl Infect Dis 2010; 12: 138-142.
8. Wilmes D, Coche E, Rodriguez-Villalobos H, et al. Bacterial pneumonia in kidney transplant recipients. Respiratory Medicine 2018; 137: 89–94.
9. Longworth SA, Blumberg EA, Barton TD, et al. Nontuberculous mycobacterial infections after solid organ transplantation: a survival analysis. Clin Microbiol Infect 2015; 21: 43–47.
10. Longworth SA, Vinnard C, Lee I, et al. Risk factors for nontuberculous mycobacterial infections in solid organ transplant recipients: a case–control study. Transpl Infect Dis 2014; 16: 76–83.
11. Schroder KH, Juhlin I. *Mycobacterium malmoens*e sp. nov. Intern Journ of Syst Bact 1977; 27: 241-246.
12. Wayne L, Sramek H. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin Microb Reviews 1992; 5: 1-25.

13. Hoefsloot W, Boeree MJ, van Ingen J, et al. The rising incidence and clinical relevance of *Mycobacterium malmoense*: a review of the literature. Int J Tuberc Lung Dis 2008; 12:987–993
14. Hoefsloot W, van Ingen J, de Lange W, et al. Clinical relevance of *Mycobacterium malmoense* isolation in the Netherlands. Eur Respir J 2009; 34: 926–931.
15. van Grootveld R, Scherer HU, Peters EEM, et al. Tenosynovitis caused by *Mycobacterium malmoense* in two kidney transplant recipients and review of the literature. Transpl Infect Dis 2018; 20:e12810.
16. Tortoli E, Rindi L, Garcia MJ, et al. Proposal to elevate the genetic variant MAC-A, included in the Mycobacterium avium complex, to species rank as *Mycobacterium chimaera* sp. nov. Intern J of Syst and Evolut Microb 2004; 54: 1277–1285.
17. Schweickert B, Goldenberg O, Richter E, et al. Occurrence and clinical relevance of *Mycobacterium chimaera* sp. nov., Germany. Emerg Infect Dis 2008; 14: 1443 – 1446.
18. Soetaert K, Vluggen C, Andre E, et al. Frequency of *Mycobacterium chimaera* among Belgian patients, 2015. Journ of Med Microb 2016; 65, 1307–1310.
19. Boyle D, Zembower TR, Reddy S, et al. Comparison of clinical features, virulence, and relapse among Mycobacterium avium complex species. Am J Respir Crit Care Med 2015; 191: 1310–1317.
20. Moon S, Kim SY, Jhun BW, et al. Clinical characteristics and treatment outcomes of pulmonary disease caused by *Mycobacterium chimaera*. Diagn Microb and Infect Dis 2016; 86: 382–384.
21. Kohler P, Kuster S, Bloemberg G, et al. Healthcare-associated prosthetic heart valve, aortic vascular graft, and disseminated Mycobacterium chimaera infections subsequent to open heart surgery. Eur Heart J 2015; 36: 2745-53.

Authors contributions:

CM managed the patient, collected data, performed the literature review and wrote the manuscript. AD, NK and LB managed the patient and critically revised the manuscript. AM and HR managed the microbiological assessment and critically revised the manuscript.

Table

	Mycobacterium malmoense	*Mycobacterium chimaera*
Amikacin	S	S
Ciprofloxacin	I	I
Clarithromycin	S	S
Ethambutol	S	I
Isoniazid	R	MIC > 8 µg/mL
Rifabutin	S	S
Rifampicin	R	I

Table 1: Antimicrobial susceptibility testing of the two nontuberculous mycobacteria species.

Abbreviations: S, sensitive; I, intermediate; R, resistant; MIC, minimum inhibitory concentration.

Figure

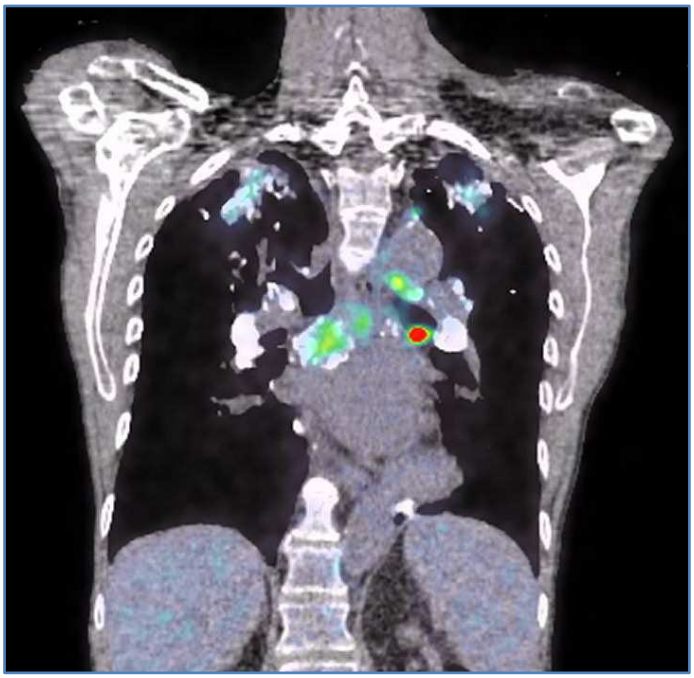

Figure 1: Hypermetabolic activity in calcified pulmonary masses and in mediastinal lymph nodes showed by positron emission tomography (PET) with computed tomography (CT)