Title: Diagnostic challenges in Mycobacteria chimaera infection

Authors: Hall H[1], Cosgrove C[2,3], Houston A[2], Macallan DC[2,3], Aul R[1].

Corresponding author:

Dr Helen Hall
Dept of Respiratory Medicine
St George's Hospital
Blackshaw Road
Tooting
London SW17 0QT

h.hall2@nhs.net
Tel 07765 636873

Affiliations:

1. Department of Respiratory Medicine, St George's University Hospitals NHS Foundation Trust, London, UK

2. Infection Care Group, St George's University Hospitals NHS Foundation Trust, London, UK

3. Institute for Infection and Immunity, St George's, University of London, London, UK

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Case History

A 35 year old woman presented to her local hospital with fever, night sweats and malaise. Her initial investigations revealed anaemia (Hb 91g/L), hypercalcaemia (2.75mmol/L) and deranged liver function - bilirubin 38umol/l; ALT 424u/L, AST 402u/L, ALP 1107u/L, GGT 202u/L. Viral (including HIV), TB (Quantiferon) and autoimmune screens were negative but serum angiotensin converting enzyme (ACE) levels were elevated (257 iU/L). A CT scan showed hepatosplenomegaly without lymphadenopathy or pulmonary nodules. Liver biopsy showed non-necrotising granulomas and on the basis of clinical, biochemical, radiological and histological features, a diagnosis of sarcoidosis was made.

Her symptoms and biochemistry improved with Prednisolone, but relapsed on dose reduction. Azathioprine was introduced but discontinued due to pancytopenia. Bone marrow examination was normal. Repeat CT abdomen showed massive splenomegaly. She underwent splenectomy, histology of which showed multiple non-necrotising granulomas with Acid-fast bacilli (AFB) seen on microscopy (samples were not sent for AFB culture). She was started on quadruple therapy for tuberculosis, but this was not tolerated (vomiting and worsening liver function). PCR from the splenectomy sample was negative for *M tuberculosis* and TB treatment was therefore stopped. Repeat liver biopsy again demonstrated granulomas with AFB on microscopy. Cultures grew a mycobacterium, identified as *Mycobacterium intracellulare* (MAI). This was considered a contaminant and she remained on Prednisolone for presumed sarcoidosis.

She presented to our centre a year later, with persistent systemic upset and marked hepatomegaly. Reviewing her history, we noted previous mitral valve repair for acute mitral regurgitation secondary to chordae tendineae rupture in 2014. In view of her surgical history, the possibility of disseminated Mycobacterium chimaera infection was considered. Subsequently, an echocardiogram showed a vegetation on the mitral valve (fig 1), and reassessment of the isolate previously identified as MAI confirmed its true identity as *M chimaera*.

The patient was started on Rifabutin, Clarithromycin, Ethambutol and Amikacin. Amikacin was later substituted with Linezolid (because of hearing loss) which was replaced with Moxifloxacin (due to neuropathy). After 6 months, her clinical symptoms and hepatomegaly have resolved, and liver function improved. The mitral valve vegetation persists and valve replacement is being considered.

Discussion

The presence of visible AFB in liver and spleen in the absence of a positive TB-PCR suggests disseminated non-tuberculous mycobacterial infection. The history of previous cardiac surgery raised the possibility of *Mycobacteria chimaera* infection. Similar cases of *M chimaera* infections acquired from

contaminated heating-cooler units used in cardiopulmonary bypass machines have been described since 2014[1-6]. *M chimaera* is very similar to MAI - only one base discriminates the two species on 16S rRNA sequencing - so misidentification is common[1,2].

Manifestations of *M chimaera* infection typically occur 3 months to 5 years after surgery and include endocarditis, wound or bone infection, splenomegaly, pancytopenia, hepatitis, renal involvement and embolic phenomena[1,3-6]. Treatment requires a prolonged course of combination therapy guided by macrolide sensitivity, typically Rifabutin, Clarithromycin, and Ethambutol with or without a quinolone or Amikacin[4,5]. Patients frequently need repeat surgical intervention. Death may occur despite prolonged antimicrobial therapy and mortality rates are high, up to 50%[3-5].

Differentiating a rare mycobacterial infection such as chimaera from sarcoidosis in the context of a multisystem granulomatous inflammation can be notoriously difficult. Features such as raised ACE levels and hypercalcaemia are non-specific and routine AFB culture techniques may be misleading[1,2,4,6]. Aggressive immunosuppression used for severe sarcoidosis may aggravate *M chimaera* infection[4-6]. Although action has been taken to limit newly acquired infections, it is likely that further *M chimaera* cases will emerge. Awareness of the association between a history of cardiopulmonary bypass and *M chimaera* acquisition is therefore crucial.

Learning Point

Mycobacterium chimaera was first identified as contaminating cardiopulmonary bypass equipment in 2014. Patients with previous cardiac surgery are at risk of having acquired this infection. Presenting symptoms and investigations may mimic other conditions including sarcoidosis. Awareness of the risk for *Chimaera* infection in this cohort of patients is therefore crucial.

Acknowledgments

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Competing Interests

Dr R Aul has accepted lecturing fees from Pfizer. There are no other relevant disclosures.

References

1. Sax H, Bloemberg G, Hasse B, Sommerstein R, Kohler P, Achermann Y, et al. Prolonged outbreak of Mycobacterium chimaera infection after open-chest heart surgery. *Clin Infect Dis*. 2015;61(1): 67-75.

2. Public Health England. Mycobacteria chimaera infections associated with Cardiopulmonary bypass: clinical guidance for secondary care. Version 1. *PHE London.* 2017; 2-11.

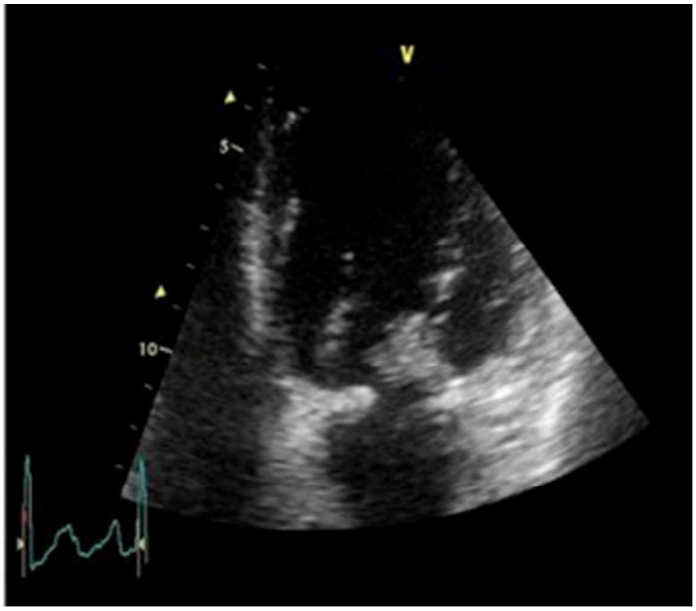
3. Sommerstein R, Hasse B, Marschall J, Sax H, Genoni M, Schlegel M et al. Global health estimate of invasive Mycobacterium chimaera infections associated with heater-cooler devises in cardiac surgery. *Emerg Infect Dis.* 2018;24(3): 576-578.

4. Kohler P, Kuster SP, Bloemberg G, Shulthess B, Frank M, Tanner FC, 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: 2745-2753.

5. Chiesi S, Piacentini D, Duccio Salerno N, Luise D, Peracchi M, Concia E, et al. Disseminated Mycobacterium chimaera infection after open heart surgery in an Italian woman: a case report and a review of the literature. *Infez Med.* 2017;25(3): 267-269.

6. Chand M, Lamagni T, Kranzer K, Hedge J, Moore G, Parks S, et al. Insidious risk of severe Mycobacterium chimaera infection in cardiac surgery patients. *Clin Infect Dis.* 2017;64(3): 335-342.

Figure 1: Echocardiogram showing a 2.2 x 1.5cm vegetation on the mitral valve



List of acronyms:

ACE – angiotensin converting enzyme
AFB – acid fast bacilli
ALP – alkaline phosphatase
ALT – alanine transaminase
AST – aspartate aminotransferase
CT – computed tomography
GGT – gamma-glutamyl transferase
Hb – Haemoglobin
HIV – human immunodeficiency virus
MAI – Mycobacterium avium intracellulare
MTB – Mycobacterium tuberculosis
PCR – polymerase chain reaction
rRNA – ribosomal ribonucleic acid
TB – Tuberculosis