Title:

***Mycobacterium chimaera* infection following cardiac surgery in the United Kingdom: clinical features and outcome of the first 30 cases**

Running title:

Clinical features of *M. chimaera* disease

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Abstract

Objectives

Mycobacterium chimaera infection following cardiac surgery, due to contaminated cardiopulmonary bypass heater-cooler units, has been reported worldwide. However, the spectrum of clinical disease remains poorly understood. To address this, we report the clinical and laboratory features, treatment and outcome of the first 30 UK cases.

Methods

Case note review was performed for cases identified retrospectively through outbreak investigations and prospectively through on-going surveillance. Case definition was *Mycobacterium chimaera* detected in any clinical specimen, history of cardiothoracic surgery with cardiopulmonary bypass, and compatible clinical presentation.

Results

Thirty patients were identified (28 with prosthetic material) exhibiting a spectrum of disease including prosthetic valve endocarditis (14/30), sternal wound infection (2/30), aortic graft infection (4/30), and disseminated (non-cardiac) disease (10/30). Patients presented a median of 14 months post surgery (maximum 5 years) most commonly complaining of fever and weight loss. Investigations frequently revealed lymphopenia, thrombocytopenia, liver cholestasis and non-necrotising granulomatous inflammation. Diagnostic sensitivity for a single mycobacterial blood culture was 68% but increased if multiple samples were sent. 27 patients started macrolide-based combination treatment and 14 had further surgery. To date, 18 patients have died (60%) a median of 30 months (IQR 20-39) after initial surgery. Survival analysis

identified younger age, mitral valve surgery, mechanical valve replacement, higher serum sodium concentration and lower CRP as factors associated with better survival.

Conclusions

Mycobacterium chimaera infection following cardiac surgery is associated with a wide spectrum of disease. The diagnosis should be considered in all patients who develop an unexplained illness following cardiac surgery.

Funding

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Introduction

There is now widespread recognition of the global *Mycobacterium chimaera* outbreak associated with surgery on cardiopulmonary bypass (1-5). Contaminated heater-cooler units (HCU) have been identified as the likely source resulting in aerosolisation of *M. chimaera* into the operating theatre environment and direct contamination of the surgical field and/or prosthetic material (6-8). Phylogenetic analysis of sequenced *M. chimaera* strains from patients and HCUs has demonstrated strong clustering suggesting a point source outbreak likely originating during manufacture (3,4,9).

The spectrum of disease associated with *M. chimaera* remains poorly understood. Case reports have described both prosthetic valve endocarditis (PVE) and disseminated disease (2,10,11), with significant diagnostic delay and poor outcome (2,7). Given the long incubation period and unknown number of exposures it is vital that clinicians are well informed regarding the wide range of pathology associated with *M. chimaera* infection. Here we report the clinical, laboratory, echocardiographic and radiological features of the first 30 cases identified in the UK, along with outcome data and prognostic markers.

Methods

Case identification

Cases were identified during a UK national outbreak investigation co-ordinated by Public Health England (PHE),[7]. PHE has approval from the Confidentiality Advisory Group of the Health Research Authority for the processing of confidential patient information through its devolved responsibility under regulation 3 of section 251 of the NHS Act 2006.

Retrospective cases were identified by cross-referencing national mycobacterial reference service data with National Health Service (NHS) data for surgery with cardiopulmonary bypass or extracorporeal membrane oxygenation using unique patient NHS numbers. Prospective cases were identified through diagnostic laboratory reporting. A case was defined as: detection of *M. chimaera* in any clinical specimen, prior cardiothoracic surgery involving cardiopulmonary bypass, and a compatible clinical presentation (e.g. endocarditis, surgical site or disseminated infection; isolated pulmonary infection was excluded). A maximum incubation period of 4 years was initially used for retrospective case finding but extended to 10 years for prospective surveillance in light of subsequent reports.

Mycobacterial identification

Mycobacterial cultures provisionally identified as *M. intracellulare* by line probe assay (GenoType Mycobacterium CM, HAIN Lifescience) were identified as *M. chimaera* by internal transcribed spacer (ITS) sequencing and additionally in 18 patients by whole-genome sequencing (WGS) (12). WGS was performed using an Illumina (San Diego, California) MiSeq sequencing platform that identified

mycobacterial species using a gene presence or absence algorithm (compared against a catalogue of 169 sequenced mycobacterial strains) (7,13). 16s PCR with DNA sequence analysis was performed in-house following a previously detailed method (14). Clarithromycin sensitivity testing was performed at clinician's request using either standard broth microdilution with AIM Sensititre® plates (Trek Diagnostics Systems) or by direct agar proportion method on Lowenstein-Jensen slope media (15).

Data collection

Case note review was performed by local clinicians detailing clinical, laboratory and radiological findings, treatment, clinical outcome (alive/dead) and date of death. Outcome was re-checked on 30th September 2017 when the database was closed. Echocardiography reports were considered diagnostic of PVE if they met Duke major criteria (16). Radiological reports were independently categorised by two investigators with differences resolved through discussion.

Statistical analysis

Database creation and management was conducted using EpiData Manager and EntryClient (v4.0.1.45, EpiData, Denmark). Clinical data were double-entered by two independent investigators. Chi-squared testing was performed to compare frequency of symptoms between clinical groups; cox proportional hazards modelling was performed to assess risk factors linked to all-cause mortality from date of surgery. STATA (v14.2, StataCorp, USA) was used for all statistical analysis.

Results

Patients

The median age at diagnosis was 65 years (range 8-82); 23 (77%) were male. Comorbidities included ischaemic heart disease (10/30), hypertension (6/30), and diabetes (7/30). Significant immune suppression was present in one patient. All patients had negative HIV serology. Cardiac surgery was performed in 17 different cardiac centres in the UK between 2007 and 2015 as detailed in **Table 1.** A marked increase in cases was observed in patients who had surgery after 2011 (**Figure 1**). Stöckhert 3T HCUs were in use in all centres during the relevant time periods. Earlier investigations isolated *M. chimaera* from 17 out of 35 3T HCUs sampled (7).

Clinical features

The median time between surgery and developing symptoms was 14.5 months (IQR 9.8-19.4) but ranged from 6 weeks to five years; 80% of patients became unwell within 2 years of surgery **(Figure 2).** The median duration of symptoms was 7 weeks (IQR 4-15) but the longest was one year. Unexplained fever was the most common presentation (80%); five patients had signs of chronic sternal wound infection (**Table 2)**. PVE was diagnosed in 14 patients and aortic graft infection in four, 10 had disseminated (non-cardiac) infection affecting a variety of organs (liver, spleen, bone marrow, spine, skin, bone) and 2 had isolated sternal wound infection. Patients with PVE and disseminated disease were significantly more likely to present with fever (*P*=.003), while aortic graft and sternal wound infections more likely to present with chest pain (P<.001).

Laboratory tests

Twenty-three patients (77%) had abnormal full blood counts (FBC): lymphopenia ($<1x10^9$/L) was present in 19 (63%) and thrombocytopenia ($<150x10^9$/L) in 14 (47%) (**Table 2**). Deranged liver function tests (LFTs) were also common and typically displayed a cholestatic pattern with raised alkaline phosphatase (>130 IU/L), in 21/30 patients (70%). Only three patients had both normal FBC and LFTs at presentation (all with sternal wound infections) and only one patient at the time of diagnosis. CD4 counts were measured in four patients: absolute counts were below normal (range 263-390/μL) but the percentage was preserved, consistent with a generalised reduction in all lymphocytes.

Mycobacteriology

The median time between presentation to hospital and microbiological diagnosis was 10 weeks (range 2-100). *M. chimaera* was cultured from a range of fluids/tissues, the most common being blood and urine (**Table 3)**. Prior to initiation of therapy the overall diagnostic sensitivity for a single mycobacterial blood culture was 68% (28/41 samples positive) but decreased to 34% (11/32) once treatment started. Diagnostic yield was increased with multiple samples: all patients with at least two mycobacterial blood cultures isolated *M. chimaera* from at least one sample (**Supplementary Table 1**). Blood cultures were negative in four patients when taken immediately after revision valve surgery. *M. chimaera* was isolated from explanted prosthetic valves/cardiac tissue from all 10 patients who underwent repeat surgery or post mortem examination (**Table 3**). Five of these 10 were taking anti-mycobacterial therapy at the time of sampling (median 6 months, range 1-14 months). *M. chimaera* was cultured from sternal tissue in all five patients who presented with a chronic

sternal wound infection. No macrolide resistance was detected in 15 patients at baseline, two patients who were failing treatment and on one patient who relapsed.

Echocardiography

Prosthetic valve endocarditis (PVE) was diagnosed in 11/29 (38%) patients who underwent echocardiography during diagnostic workup (5/24 had positive transthoracic echocardiography [TTE]; 10/22 had positive transoesophageal echocardiography [TOE]) (Supplementary **Table 2)**. Major echocardiographic findings included vegetations (7/29), aortic root abscess (5/29) and valvular regurgitation (4/29). TTE had a sensitivity of 33% and specificity of 88% compared to TOE in the 17 patients who had both investigations performed. Three patients with normal initial echocardiography were later diagnosed with PVE by either follow-up TOE or post mortem examination.

Radiology

Computerised tomography (CT) scanning commonly revealed splenomegaly (14/26) and pulmonary consolidation/infiltrate (10/26); other important findings included mediastinal/aortic graft infection (4/26) and fluid around the aortic root (5/26) (**Supplementary Table 3**). Ten patients had a positron emission tomography (PET)-CT scan performed. This provided additional evidence of aortic graft infection in 3 patients in whom standard CT scanning had been equivocal.

Histopathology and post mortem examinations

Non-caeseating granulomatous inflammation was observed in 15 out of 19 patients who underwent biopsy. The most commonly biopsied tissues included liver (7/8

patients with granulomas) and bone marrow (7/10 with granulomas) but in some the changes were scanty. Post mortem examination was performed in four patients and revealed varying degrees of granulomatous inflammation and embolic disease affecting the heart, lungs, kidneys, liver, spleen, bone marrow, vertebrae, sternum and brain; PVE was noted in two patients.

Treatment

27 patients started combination therapy (three died prior) comprising a macrolide (clarithromycin n=23; azithromycin n=4), rifamycin (rifampicin n=18; rifabutin n=8) and ethambutol (n=27); additional treatment consisted of intravenous amikacin (n=5) and/or quinolone (n=3) (**Supplementary Table 4**). Eleven patients developed significant drug toxicity within the first 6 months including, deranged liver tests, renal dysfunction, hearing loss, gastrointestinal upset and hypercalcaemia. Eight patients had intensification of treatment with amikacin and/or quinolones. Interferon-gamma was given to three patients but two developed inflammatory reactions. Four received corticosteroids without anti-mycobacterial treatment (presumed sarcoidosis) and had an initial clinical, biochemical and radiological improvement but eventually deteriorated with worsening infection. Fourteen patients underwent further surgery a median of 23 months (IQR 17-37) after the original surgery. Operations performed included aortic valve replacement (2/14), aortic valve replacement with repair of aortic root abscess (6/14), mitral valve repair (1/14), and sternal wound debridement (5/14). 30-day survival following repeat surgery was 92%.

Outcome

As of 30th September 2017, 18 out of 30 patients had died (60%), a median of 30 months (IQR 20-39) after initial surgery and 9 months (IQR 2-14) after treatment was started. Kaplan-Meier estimates of 5-year survival were 0.45 (95%CI 0.24-0.63) (**Figure 3**). 8 patients remain on treatment (median 19 months, IQR 9-22), two of whom are re-treatments after relapsing within a year of treatment cessation. Four patients have completed treatment and remain well (median 14 months off treatment; range 4-40 months).

An exploratory survival analysis identified younger age, white British ethnicity, mitral valve repair, mechanical valve replacement, higher serum sodium concentration and lower CRP to be associated with significantly improved survival **(Supplementary Table 5)**. No particular treatment was significantly associated with improved survival but a non-significant trend towards improved survival was observed for patients who underwent further surgery (HR .51, 95% CI .2-1.3, *P*=.16**)** **(Supplementary Figure 1)**.

Discussion

This study is largest cohort of cardiac surgery associated *M. chimaera* infections reported to date and provides important information regarding the clinical features, laboratory abnormalities, sensitivity of diagnostic tests and prognostic markers. Consistent with earlier reports *M. chimaera* infection was associated with a spectrum of disease including PVE, disseminated disease, aortic graft and sternal wound infections (2,5,10,17). Diagnosis was frequently delayed and response to treatment was poor.

Immune suppression was not a required predisposing factor. Although lymphopenia was a common finding this was not documented prior to cardiac surgery and is likely to be a consequence of infection. Indeed, progressively worsening lymphopenia due to lymphocyte apoptosis has been observed in mouse models of disseminated *Mycobacterium avium* (18). All but two patients in the cohort had prosthetic material inserted during surgery. These two developed isolated sternal wound infections following CABG with no evidence of cardiac or disseminated disease. Given the large numbers of CABG procedures performed each year (16,791 procedures/year, UK 2012) the overall risk for patients undergoing CABG remains extremely low (19). However, a report of *M. chimaera* spondylodiscitis following CABG suggests disseminated infection may occasionally occur in the absence of prosthetic material (8).

Identifying patients with *M. chimaera* is difficult due to the insidious onset, non-specific symptoms and prolonged incubation period. However, splenomegaly, abnormal LFTs, cytopenias and granulomatous inflammation were common findings and could be used to guide microbiological testing (2,5,10). Fundoscopy may also be a helpful diagnostic adjunct given the choroidoretinitis reported here and elsewhere (10,11).

Culture of *M. chimaera* from peripheral blood was the most common method of microbiological diagnosis. Sensitivity was increased by sending multiple samples but reduced by anti-mycobacterial therapy. The combination of mycobacterial culture and 16S rRNA gene sequencing of excised cardiac material was positive in all patients in whom material was available. This data has informed UK guidance which

recommends three mycobacterial blood cultures and early morning urine samples in suspected patients, along with mycobacterial culture and 16s PCR testing of tissue or valve specimens (20).

Consistent with previous studies in bacterial PVE, TOE was more sensitive than TTE in diagnosing *M. chimaera* PVE (21). However, the later development of PVE in patients with an initially normal TOE suggests serial assessment should be considered. Aortic graft infections were frequently associated with an overlying sternal wound infection and CT, MRI or CT-PET scanning were helpful assessing deeper infection/graft involvement. CT-PET has also proved useful elsewhere in the diagnosis of *M. chimaera* spondylodiscitis and PVE (5).

Mortality due to *M. chimaera* was high (60%) and the estimated 5-year survival of 45% is markedly worse than that reported from large cardiac surgery outcome studies (76-95% 5-year survival) (22,23). Significantly better survival was observed among patients who had undergone mitral valve repair compared to aortic valve or root surgery. This could be a result of differing areas of prosthetic material available for biofilm formation. However, studies in bacterial PVE have not reported such differences (24,25).

Optimal treatment for *M. chimaera* is unknown and guidelines for *Mycobacterium avium* complex (MAC) treatment were followed (26,27). No drug treatment was associated with significantly better survival but a non-significant trend was observed among patients who underwent repeat surgery. Repeat valve surgery is frequently required to cure PVE caused by biofilm-forming organisms (28-30), and a survival

benefit has been demonstrated in a meta-analysis of observational studies in bacterial PVE (31). Given the known potential of *M. chimaera* to form biofilms (32), and the clear failure in this cohort to sterilise valves with medical treatment alone, it is possible that a sustained cure in *M. chimaera* PVE may not be possible without removal of infected material (6,7). However, given the technical difficulties of repeat surgery and the inherent risk this may pose to patients, such decisions cannot be taken lightly and more outcome data are required before this question can be addressed. Studies reporting culture-positivity rates of explanted valves from *M. chimaera* patients will be helpful to inform clinical decision-making.

This study has limitations. It is a small retrospective observational study with low statistical power; it is subject to case selection bias and likely underestimates of the total case burden. As such, these results should be interpreted with caution and ideally verified in larger cohorts. However, it is the largest study of cardiac surgery-associated *Mycobacteria chimaera* infection to date and the first to examine diagnostic, treatment and prognostic factors. We also report the identification of *M. chimaera* using WGS, a rapid and cost-effective method of mycobacterial diagnostics that is increasingly used in UK mycobacterial reference laboratories (13).

Large numbers of patients undergo cardiac surgery every year (UK~150,000) (19). Whilst the overall risk to patients remains very low (7), it is likely there are more patients who have been infected. Clinicians must remain vigilant and *M. chimaera* infection should be strongly suspected in patients with a history of cardiac surgery (especially with prosthetic material) who develop unexplained fevers, weight loss, splenomegaly, cytopenias, hepatitis, granulomatous inflammation or chronic sternal

wound infections; particular caution should be taken when making a diagnosis of sarcoidosis. Efforts are now required to combine international data to better inform treatment, particularly the use of additional anti-mycobacterial drugs and the indications for repeat valve surgery.

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Conflicts of Interest

None of the authors has any conflict of interest to declare.

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Contributions

The study was conceived by JES, GS, MC and MZ, and co-ordinated by JES, AS and MC. Cases were identified by outbreak investigations lead by TL, EGS, TF, MC and MZ. Data collection was performed by JES, AH, TC, VC, OMK, TM, OR, AR, SW, PW, DM, DA, ED, ML, SS, AS, MC, MH, JT, and ST. Data entry was performed by JES and AS. Statistical analysis was performed by JES and NQV. SK, MD, and TL provided additional expertise in data interpretation. The manuscript was written by JES with input from all authors.

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Figure 1 – Histogram displaying year of original cardiac surgery for patients who developed *M. chimaera* infection in UK (n=30).

Figure 2 – Violin plot demonstrating the distribution of times between cardiac surgery and the development of symptoms, hospital admission and diagnosis of *M. chimaera* infection. Plots comprise a symmetrical kernel-density plot (red) showing the full distribution of times with a superimposed boxplot (blue) demonstrating the median times with interquartile ranges. Time is quantified in days post original cardiac surgery. Vertical height of each plot represents the kernel probability density of data.

Figure 3 – Kaplan-Meier survival curve illustrating survival after cardiac surgery for patients diagnosed with *Mycobacterium chimaera* infection. Numbers at risk are shown below the plot. Shading indicates 95% confidence intervals. Kaplan-Meier estimate of 5-year survival is 0.45 (95%CI 0.24-0.63). The low numbers of patients with follow-up data ≥5 years mean survival estimations after this point should be interpreted with caution.