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Healthcare-associated prosthetic heart valve, aortic vascular graft, and disseminated *Mycobacterium chimaera* infections subsequent to open heart surgery

Philipp Kohler[1], Stefan P. Kuster[1], Guido Bloemberg[2], Bettina Schulthess[2,3], Michelle Frank[4], Felix C. Tanner[4], Matthias Rössle[5], Christian Böni[6], Volkmar Falk[7,8], Markus J. Wilhelm[7], Rami Sommerstein[1], Yvonne Achermann[1], Jaap ten Oever[9], Sylvia B. Debast[10], Maurice J.H.M. Wolfhagen[10], George J. Brandon Bravo Bruinsma[11], Margreet C. Vos[12], Ad Bogers[13], Annerose Serr[14], Friedhelm Beyersdorf[15], Hugo Sax[1], Erik C. Böttger[2,3], Rainer Weber[1], Jakko van Ingen[16]†, Dirk Wagner[17]†, and Barbara Hasse[1]†*

[1]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, University of Zurich, Raemistrasse 100, Zurich 8091, Switzerland; [2]Institute of Medical Microbiology, University of Zurich, Gloriastrasse 30/32, Zurich 8006, Switzerland; [3]National Reference Center for Mycobacteria, University of Zurich, Gloriastrasse 30/32, Zurich 8006, Switzerland; [4]Department of Cardiology, Cardiovascular Center, University Hospital Zurich, University of Zurich, Raemistrasse 100, Zurich 8091, Switzerland; [5]Institute of Surgical Pathology, University Hospital Zurich, University of Zurich, Schmelzbergstrasse 12, Zurich 8091, Switzerland; [6]Department of Ophthalmology, University Hospital Zurich, University of Zurich, Raemistrasse 100, Zurich 8091, Switzerland; [7]Clinic for Cardiovascular Surgery, University Hospital Zurich, University of Zurich, Raemistrasse 100, Zurich 8091, Switzerland; [8]Deutsches Herzzentrum Berlin, Augustenburger Platz 1, Berlin 13353, Germany; [9]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; [10]Laboratory of Medical Microbiology and Infectious Diseases, Isala Clinics, Zwolle, The Netherlands; [11]Department of Cardiothoracic Surgery, Isala Clinics, Zwolle, The Netherlands; [12]Medical Microbiology and infectious Diseases, Erasmus MC, Rotterdam, The Netherlands; [13]Cardiothoracic Surgery, Erasmus MC, Rotterdam, The Netherlands; [14]Centre for Microbiology and Hygiene, University Hospital of Freiburg, Freiburg i.Br, Germany; [15]Department of Cardiovascular Surgery, Heart Center Freiburg University, Freiburg i.Br, Germany; [16]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands; and [17]Department of Medicine, Center for Infectious Diseases and Travel Medicine and Center for Chronic Immunodeficiency, University Medical Center, Freiburg i.Br, Germany

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Aims We identified 10 patients with disseminated *Mycobacterium chimaera* infections subsequent to open-heart surgery at three European Hospitals. Infections originated from the heater–cooler unit of the heart–lung machine. Here we describe clinical aspects and treatment course of this novel clinical entity.

Methods and results Interdisciplinary care and follow-up of all patients was documented by the study team. Patients' characteristics, clinical manifestations, microbiological findings, and therapeutic measures including surgical reinterventions were reviewed and treatment outcomes are described. The 10 patients comprise a 1-year-old child and nine adults with a median age of 61 years (range 36–76 years). The median duration from cardiac surgery to diagnosis was 21 (range 5–40) months. All patients had prosthetic material-associated infections with either prosthetic valve endocarditis, aortic graft infection, myocarditis, or infection of the prosthetic material following banding of the pulmonary artery. Extracardiac manifestations preceded cardiovascular disease in some cases. Despite targeted antimicrobial therapy, *M. chimaera* infection required cardiosurgical reinterventions in eight patients. Six out of 10 patients experienced breakthrough infections, of which four were fatal. Three patients are in a post-treatment monitoring period.

Conclusion Healthcare-associated infections due to *M. chimaera* occurred in patients subsequent to cardiac surgery with extracorporeal circulation and implantation of prosthetic material. Infections became clinically apparent after a time lag of months to years. *Mycobacterium chimaera* infections are easily missed by routine bacterial diagnostics and outcome is

* Corresponding author. Tel: + 41 44 255 25 41, Fax: + 41 44 255 32 91, Email: barbara.hasse@usz.ch; b.hasse@gmx.ch
† Authors contributed equally

poor despite long-term antimycobacterial therapy, probably because biofilm formation hinders eradication of pathogens.

Keywords *Mycobacterium chimaera* • Cardiac surgery • Prosthetic valve endocarditis • Aortic graft infection • Myocarditis • Health-care associated infection

Introduction

Non-tuberculous mycobacteria (NTM) can cause pulmonary disease, particularly in patients with pre-disposing structural lung disease, skin, soft tissue and bone infections, endocarditis, and disseminated infections in immunocompromised hosts. Signs and symptoms are variable and often non-specific. Also, a growing number of case reports of cardiosurgical site infections due to NTM have been reported in recent years.[1–5] Thus far, only rapid-growing NTM have been found to be associated with prosthetic valve endocarditis (PVE).[1–4,6] Recently, we published two cases of PVE and bloodstream infection due to *Mycobacterium chimaera*,[7] a slow-growing NTM and member of the *M. avium* complex (MAC)[8] that has previously been cultured from tapwater in patients' households.[9]

Cardiosurgical outbreaks of NTM infections have been associated with contaminated water used for the cardioplegia solution,[2] contamination during the manufacturing process[4] or use of a contaminated patch for septum defect repair,[3] but source identification often failed.[7,10] In the course of an outbreak at the Zurich Heart Center, *M. chimaera* was cultured from air sampling in the operating theatre and from water tanks of from heater–cooler units (HCUs) serving the heart–lung machine. Identical randomly amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) results indicated that patients were infected by intraoperative contamination of the surgical site due to airborne transmission of microorganisms sprayed from the ventilation outlet of HCUs into the operating theatre.[11,12] As of February 2015 a total of six cases were identified in Zurich (Switzerland). Another four patients were detected in parallel in Freiburg, Zwolle and Rotterdam, where the notification of national public health authorities led to thorough investigation of the respective HCUs pointing to a similar transmission route. On 30 April 2015, an alert was published by the European Centre for Disease Prevention and Control, warning healthcare providers in care of patients who have undergone open-heart surgery to be vigilant for cases of endocarditis or other cardiovascular infections of unknown origin and consider testing mycobacteria.[13,14] Here we aim to give a comprehensive description of the clinical manifestations and outcome of this novel disease entity. In addition, we provide exposure criteria and a case definition to facilitate the detection of potential cases on a global level.

Methods

Exposure criteria

A former open-heart surgery and implantation of a cardiovascular implant were our exposure criteria.

Case definition

Our clinical criteria were: PVE, prosthetic vascular graft infection (PVGI), or disseminated infection including embolic and immunologic manifestations.

Confirmed cases

Confirmed cases were defined as cases meeting the clinical and exposure criteria and *M. chimaera* proven by culture or polymerase chain reaction (PCR) identification from an invasive sample from the cardiac surgery site.

Probable cases

Probable cases were defined as cases meeting the clinical and exposure criteria and detection of *M. chimaera* or *M. avium* complex in blood and/or extracardiac tissue cultures.

Case finding

Mycobacterial cultures are not part of the routine microbiological workup in the case of cardiovascular infections. The first patient was detected by a thorough histopathological analysis of cardiac tissue, which triggered a PCR for non-tuberculous mycobacteria yielding the diagnosis.[7] The remaining patients were detected based on direct 16S rRNA gene-sequencing results of cardiac tissue or bone or on positive mycobacterial blood cultures.

Microbiology of *Mycobacterium chimaera*

Standard methods were used to culture mycobacteria, using the MGIT 960 system (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and Middlebrook 7H11 agar plates incubated at 37°C for 7 weeks or until positive. In Zurich, 16S rRNA gene sequencing was performed as described before.[15] Antimicrobial susceptibility testing was performed in the MGIT 960 system equipped with the TB Exist module for rifampin, rifabutin, amikacin, ofloxacin, moxifloxacin, clarithromycin, and ethambutol.[16] The German strain was identified by sequencing of the 16S rRNA gene and the 16S-26S rRNA Gene Internal Transcribed Spacer, the Dutch strains were identified by the Inno-LiPA Mycobacteria v2 line probe assay, which features a specific probe for *M. chimaera*. The MICs of the German and Dutch strains were determined by broth microdilution in cation-adjusted Mueller Hinton Broth, as recommended by CLSI (document M24-A2, 2011).[17]

Clinical investigations

We obtained patient informed consent to publish their clinical data. Comorbidities were quantified using the Charlson comorbidity index.[18] The information on index surgery included American Society of Anaesthesiologists (ASA) score,[19] type of operation, timing of operation, and the extracorporeal circulation time. All patients were assessed according to the modified Duke criteria.[20] We collected treatment information and, if available, results of therapeutic drug monitoring. In all patients, transthoracic (TTE) and transoesophageal echocardiography (TEE) was performed. Histopathological features of infected tissue before or

after initiation of antimicrobial treatment were collected. In Zurich, patients were screened for ophthalmologic manifestations of the disease, including fundoscopy and multimodal imaging.

We assumed treatment failure if the patient died due to uncontrolled infection or if a patient showed a positive culture for *M. chimaera* despite antimicrobial therapy for at least 3 months.

Results

Population at risk and prevalence

In Zurich, Switzerland, cases were associated with procedures between 13 August 2008 and 30 May 2012. During this period a total of 3706 cardiosurgical procedures with extracorporeal circulation were conducted. We identified six disseminated *M. chimaera* cases, corresponding to a cross-sectional prevalence of 0.16%. Other patients with *M. chimaera* cardiac infection were not detected despite extensive case finding strategies.[11]

After the detection of the first case at the Freiburg University Hospital, Germany, a national alert was issued. In the Netherlands, the second case was identified after publication of the first case in a national newsletter. Review of charts of patients with positive *M. chimaera* cultures yielded one paediatric case in Rotterdam. A case finding protocol has now been implemented in Germany and the Netherlands nationwide.

Patient characteristics

Overall, nine confirmed cases including eight adults and one child, and one probable case are described. For the adult patients the median age and median BMI were 61 years (range, 36–76) and 24.9 kg/m^2 (23.4–35.7), respectively. Details on the index cardiac surgery are shown in *Table 1*. The median extracorporeal circulation time was 191 min (range, 123–294). Two patients had diabetes mellitus, one patient received azathioprine and salazopyrine for Crohn's disease, and one patient had lymphocytopenia of unknown origin. After the index surgery, two patients received corticosteroid treatment for presumptive sarcoidosis and one patient received repetitive intra-articular methotrexate for suspected rheumatologic disease. All patients were HIV negative.

The child with a congenital cardiac anomaly was in neonatal age when he received a correction of the aortic anomalies and banding of the pulmonary artery.

Manifestations of disease

The most common initial complaints in adults were fever, shortness of breath, fatigue, and weight loss. Physical findings were nonspecific with the exception of splenomegaly. All patients had anaemia, pronounced lymphocytopenia, and thrombocytopenia. C-reactive protein, lactate dehydrogenase, transaminases, and creatinine levels were elevated in all subjects. In the infant, clinical suspicion arose due to fever episodes and failure to thrive. A summary of the presenting clinical signs and laboratory analyses are shown in Supplementary material online, *Table S1*, which occurred after a median incubation time of 18 (range, 11–40) months. Details on the microbiological and histopathological findings are summarized in Supplementary material online, *Table S2*.

Confirmed cases

Cardiac manifestations

Five of the nine patients with confirmed diagnosis presented with PVE, two with PVGI and one with myocarditis. The child presented with infection of the prosthetic band and a mycotic aneurysm of the pulmonary artery. All diagnoses were made upon cardiosurgical reintervention with cultures or PCR from cardiac tissue being positive for *M. chimaera*. No other microorganisms were detected in the blood, and there was no serological evidence of a culture-negative endocarditis of other cause (i.e. *Bartonella* spp., *Brucella* spp., *Coxiella burnetii, Tropheryma whipplei*). Diagnosis was delayed with a median duration between index surgery and culture confirmed diagnosis of almost 2 years (21 months; range, 5–40). Affected patients presented with prevailing cardiac complications like severe valve insufficiency and subsequent reduction in ejection fraction, paravalvular abscess, or pseudoaneurysm formation (*Figure 1*). The TEE showed paravalvular regurgitation or leakage, anteroseptal pseudoaneurysm as well as a paravalvular abscess with extension into the interatrial septum (*Figure 1A*). Additionally, vegetations or multiple short, thin and sparse filaments on the ventricular side were detected (*Figure 1B*).

Extracardiac manifestations

In six of nine patients with confirmed diagnosis extracardiac manifestations preceded cardiac disease. Among the first disease manifestations were bone infections (osteoarthritis, spondylodiscitis, or sternal wound infection together with a large retrosternal abscess formation), cholestatic hepatitis, nephritis, or blood stream infection. Mycobacterial blood cultures were positive *a priori* in four patients. At the time of diagnosis, most patients had splenomegaly. In the course of the disease, patients developed bi- or even pancytopenia, panuveitis, or multifocal chorioretinitis (*Figure 1H*), pneumonitis (*Figure 1E*) or cerebral vasculitis. One patient developed a surgical site infection with *M. chimaera* at the removal site of the saphenal vein.

Probable case

The probable case (*Table 1*, Patient 9) presented with fever of unknown origin subsequent to open heart surgery. He had been treated for presumptive sarcoidosis due to granulomatous hepatitis, but persistent fever prompted new diagnostic procedures including a PET/CT scan. Diagnosis of *M. chimaera* infection was ascertained after biopsy of the right sternoclavicular joint, bone marrow, liver, and blood cultures. However, TEE did not reveal any signs of endocarditis.

Antimicrobial therapy

The detailed time course of events and treatment information is depicted in *Figure 2*. Targeted antimicrobial therapy consisted of clarithromycin or azithromycin, rifabutin or rifampicin, ethambutol, plus/minus amikacin, or moxifloxacin. The number of available analyses, mean drug doses, serum maximum observed concentration levels, and the percentage of analyses revealing subtherapeutic drug concentrations are recorded in Supplementary material online, *Table S3*. In more than half of the cases the recommended macrolide drug levels were not reached. The same holds true for rifabutin, ethambutol, moxifloxacin, and amikacin. Antimicrobial drugs, tested drug concentrations, and phenotypic drug susceptibilities of patient

Table 1 Baseline characteristics of patients with invasive infection due to *M. chimaera* at time of the index cardiac surgery

	Patient no.									
	1[a]	2[a]	3	4	5	6	7	8	9	10
Characteristics										
Sex	Male	Male	Male	Male	Male	Male	Male	Female	Male	Male
Age, years	58	51	64	49	61	63	76	36	74	1
BMI, kg/m^2	30.1	24.2	23.4	29.4	30.6	24.9	24.4	35.7	24.3	na
Active or former smoker	Yes	Yes	No	Yes	No	Yes	No	No	No	No
Alcohol use[b]	No	Yes	No	Yes	No	No	No	No	No	No
Comorbidities	COPD Diabetes mellitus Sarcoidosis[c]	Herpes zoster	Mild renal insufficiency, Rheumatoid arthritis[c]	Crohn's disease COPD	None	Severe renal insufficiency	Hypertension CHD	Cardiomyopathy	Diabetes mellitus CHD Hypertension Hyperlipidemia Mild renal insufficiency	None
Charlson Comorbidity Index	5	1	2	2	1	1	1	1	6	0
Immune status										
HIV serology[d]	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
IFNγ autoantibodies[d]	nd	nd	Negative	Negative	nd	Negative	nd	nd	nd	nd
Lymphocytes, G/L	2.09	1.44	1.61	1.66	0.35	1.37	0.75	1.0	0.46	0.66
Immunosuppression	Steroids[c]	None	Methotrexate[c]	Azathioprine Sulfasalazine	None	None	None	None	Steroids	None
Cardiac surgery[e]										
Reason for surgery	Mitral insufficiency CHD	Aortic dissection	Mitral insufficiency	Aortic valve stenosis	Aneurysma spurium of descending aorta[f]	Aortic valve dissection	Aortic valve stenosis CHD	Mitral valve insufficiency Dilated cardiomyopathy	Aortic valve stenosis CHD	Congential cardiac anomaly
Type of surgery	Mitral valve reconstruction	Composite graft replacement	Mitral valve reconstruction	Aortic valve replacement	Aortic root and arch replacement	Aortic root and arch replacement	Aortic valve replacement	Mitral valve robofaciltated reconstruction	Aortic valve replacement combined with CABG	Aortic arch reconstruction Coarctectomie Ductal closure Allograft patch enlargement of the arch Banding of the pulmonary artery
Date of index cardiac surgery	13.08.2008	29.01.2010	12.06.2009	31.10.2009	30.05.2012	26.03.2012	22.08.2011	23.04.2013	16.01.2013	07.04.2011
ASA score	3	5	3	3	4	5	3	4	4	na
Emergency	No	No	No	Yes	Yes	Yes	No	No	No	No
ECC time	191	150	210	166	235	272	123	294	158	na

CHD, coronary heart disease; CABP, coronary artery bypass graft; COPD, chronic obstructive pulmonary disease; BMI, body mass index; HIV, human immunodeficiency virus; IFNγ, interferon gamma; ASA, American Society of Anesthesiology; ECC, extracorporeal circulation time; na, not available

[a]Patients 1–6.[7,11]

[b]Alcohol use: severe (female subjects, 140 g/day; male subjects, 160 g/day) or moderate (female subjects, 20–40 g/day; male subjects, 40–60 g/day).

[c]Diagnosis or treatment initiation after index surgery.

[d]Investigation done after manifestation of the disease.

[e]Implants differed in types of material and manufacturers.

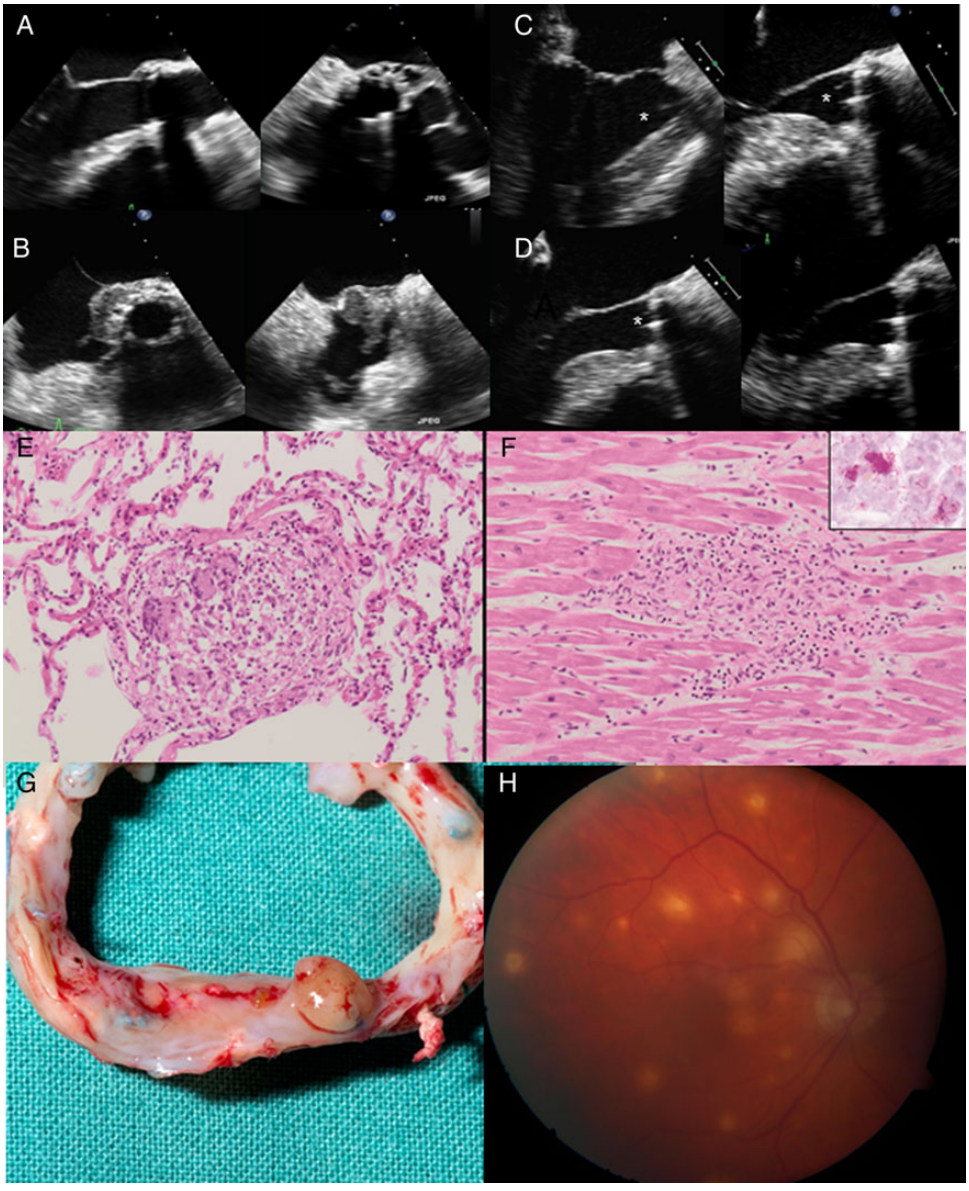

Figure 1 Clue echocardiographic findings of patient four and five with *M. chimaera* prosthethic valve endocarditis after open-heart surgery (*A*–*D*). Histopathological findings of pulmonary (*E*) and cardiac tissue (*F*) of Patient 2. Biofilm formation on mitral ring of Patient 3 after 12 months of antimycobacterial therapy (*G*), and fundoscopy of Patient 5 while being on treatment for almost 6 months (*H*). (*A*) Patient 4 at presentation. Mid-oesophageal biplane view of the aortic mechanical prosthesis (ATS 24 mm) with a paravalvular abscess as well as an anteroseptal pseudoaneurysm. (*B*) Patient 5 at presentation. Midesophageal long-axis view showing an echo-dense structure around the aortic valve prosthesis suggesting a local inflammation as well as multiple sparse and thin filaments with a maximal length of 10 mm. (*C*) Patient 4 after replacement of the mechanical prosthesis with a Freestyle bioprosthesis 25 mm in 2013 and after more than 6-month of antibiotic therapy. A mid-oesophageal biplane view focusing on aortic root and interatrial septum is shown. A moderately echodense and slightly inhomogenous mass surrounds the aortic valve, extends into the interatrial septum, and protrudes into the right atrium consistent with a persistent infection. (*D*) Patient 5- after 6-month of therapy. Mid-oesophageal view showing multiple, persistent, sparse, and thin filaments correlating with therapy-recalcitrant endocarditis.

isolates are provided in *Table 2*. Drug susceptibility testing of breakthrough isolates was unchanged.

Outcome

At least eight patients experienced therapy failure according to our definition. Five patients died, four of them due to uncontrolled *M. chimaera* infection despite being under targeted combination therapy for 15, 31, 270, and 375 days, respectively. Tissue cultures from Patient 3 (bone and annuloplasty ring (*Figure 1G*)), Patient 4 (sternoclavicular mass, epicardial pacemaker wire), Patient 8 (annuloplasty ring) and blood cultures from Patients 6 and 9 became positive for *M. chimaera* despite prolonged antimicrobial therapy

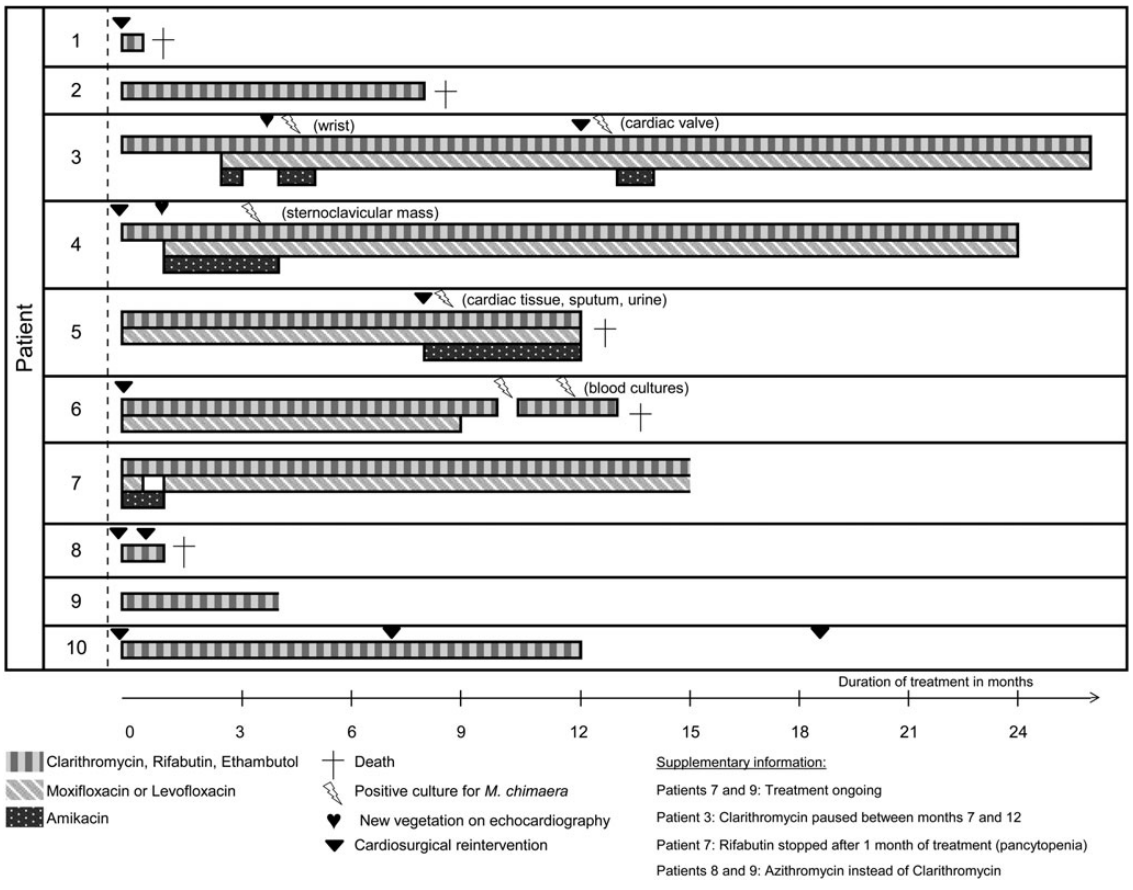

Figure 2 Treatment and clinical course of *M. chimaera* cardiovascular infections. Note: Start of antimycobacterial therapy represents day 1.

(median 8 months, range 0.5–12). Persistent signs of infection (Patients 4 and 5, *Figure 1C* and *D*) and progressive chorioretinal lesions (Patient 5, *Figure 1H*) represented an indication for immediate cardiosurgical reintervention. Of note, all these patients were previously considered inoperable due to presumptively high perioperative mortality, but the risk to benefit assessment changed in the light of uncontrolled *M. chimaera* infection. Currently, three patients are in a post-treatment monitoring period.

Discussion

As of February 2015, 10 heart surgery patients from four hospitals in three different European countries have been diagnosed with disseminated *M. chimaera* infection. Airborne contamination of the operation region and/or prosthetic material with *M. chimaera* during cardiac surgery is the most likely source of infection.[11] This new clinical entity may manifest itself after an incubation time of several months or even years after surgery. The patients present with non-specific clinical signs and symptoms and a variety of local or disseminated infection sites, which may hamper the diagnosis. Furthermore, diagnosis of mycobacterial infection is delayed as culture for mycobacteria is not part of the routine diagnostic work-up. Despite surgical reintervention and long-term antimicrobial therapy, the outcome is mostly poor.

Based on the disease prevalence in the four affected centres, we estimate a minimum of one to two *M. chimaera* infections per 1000 patients undergoing open-heart surgery. In total, 8 out of 16 tested Swiss hospitals, one out of one tested German hospital, eight out of eight tested Dutch hospitals performing cardio-surgical procedures have detected *M. chimaera* in the water system of their HCUs, and in some hospitals also in air cultures of the operating theatre, suggesting a high significance of our findings. In addition, a recent investigation from England reported that *M. chimaera* was found in the water within HCUs (air investigation ongoing).[21] Of note, until now cases were only detected in hospitals where the HCUs are placed inside the operating theatre, hence some public health authorities now recommend to put HCUs outside the operating theatre.[22,23] However, these epidemiological findings need to be extended. Ongoing whole-genome sequencing efforts indicate a match between patient isolates and air samples from the proximity of the heat cooler units.

In our patients, mycobacterial infection occurred in the absence of severe immunodeficiency. Apart from infection of the cardiac prosthetic material, disease manifestations were similar to what has been described for other disseminated NTM disease. This involves constitutional symptoms such as fever, night sweats and

Table 2 Phenotypic drug susceptibility testing of 15 *M. chimaera* isolates of the 10 study patients

Patients	1	2		3		4		5		6		7	8	9	10
Sample date	30.06.11	27.07.11	10.05.12	20.03.13	07.02.14	06.03.13	13.06.13	06.01.14	10.09.14	14.01.14	26.11.14	12.06.13	23.04.13	16.01.13	30.01.13
Material	Mitral ring	Bone marrow	Urine	Bone Wrist	Mitral ring	Cardiac tissue	Pocket tissue	Vertebral bone	Urine	Cardiac tissue	Blood culture	Aortic valve	Mitral valve	Bone	Cardiac tissue
MIC (mg/L)															
Clarithromycin	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	≤4	2	1	2	0.5
Moxifloxacin	2.5	2.5	2.5	2.5	2.5	0.5	0.5	2.5	2.5	2.5	2.5	4	4	4	2
Linezolid	ND	ND	ND	4	16	ND	16	4	4	16	16	16	8	16	16
Amikacin	20	20	20	4	4	4	4	4	4	4	20	8	8	8	8
Rifampicin	>1<20	>1<20	>1<20	4	4	ND	4	4	4	4	4	2	2	2	1
Rifabutin	>0.1<2	>0.1<2	>0.1<2	0.4	0.4	2	0.4	0.4	0.4	2	2	0.5	0.5	≤0.25	0.5
Ethambutol	≤5	≤5	≤5	≤5	≤5	ND	12.5	≤5	≤5	12.5	12.5	8	8	4	8

Data are minimum inhibitory concentrations, in mg/L.
ND, not done, minimum inhibitory concentrations, MICs.
MGIT method applied in Patients 1–6, the broth dilution method has been applied in Patients 7–10.

weight loss as well as non-specific laboratory findings such as anaemia and high lactate dehydrogenase serum levels. In most patients, manifestation of bacteriemic embolization preceded symptoms of cardiac infection. We therefore recommend a high clinical suspicion for NTM infection in patients with cardiac prosthetic material and a history of cardiac surgery presenting with signs of disseminated disease, e.g. osteomyelitis or other bone lesions, cholestatic hepatitis, or granulomatous nephritis without identification of a causative pathogen. The same accounts for patients with multifocal chorioretinitis or vasculitis of unknown origin. As routine blood cultures have a low sensitivity for mycobacterial growth, suggested methods for mycobacterial blood cultures include the BacTec myco Lytic/F bottles (BD Bioscience) and the use of Isolator tubes (Isolator 10, Oxoid; Isostat® System, Wampole™). These cultures should be performed multiple times on separate days to maximize their sensitivity. In addition, maximum effort should be taken to obtain biopsy or other samples of affected organs and tissues (e.g. bone, liver, and bone marrow), specifically including cardiac valve samples for culture in specific mycobacteria media together with molecular diagnostics such as mycobacterial genus PCR.[15]

As in all foreign body infections, a removal of the prosthetic material and a surgical debridement have to be thoroughly discussed with the cardiovascular surgeon in the case of invasive infection with *M. chimaera*.[24] Excisional surgery without antimicrobial therapy is not advisable, but the most appropriate timing of surgery is unknown. If the diagnosis is made prior to surgical intervention, it is prudent to wait for antimicrobial therapy in an attempt to sterilize/decrease the bacterial load at the site where prosthetic valves have to be reinserted.

Mycobacterium chimaera strains were uniformly susceptible to clarithromycin (MIC <8 mg/L).[25] We used the combination of clarithromycin, rifabutin, and ethambutol as the basis of treatment regimens.[17] As for severe pulmonary *M. chimaera* disease, it appears prudent to add amikacin during the first 3 months of treatment,[17] akin to staphylococcal and streptococcal endocarditis.[26] No statement can be made regarding the duration of treatment since there are no data regarding cardiac implants infected with NTM. According to ATS/IDSA guidelines, a minimum of 12 months of therapy after immune restoration is indicated for non-HIV patients with disseminated MAC disease.[17] Despite our attempts to optimize therapy with therapeutic drug monitoring, breakthrough infection occurred in most of patients. Low drug concentrations of macrolides, rifabutin, and moxifloxacin due to drug–drug interactions[27] were recorded. The relevance of these findings remains unknown. Macrolides have a strong tissue penetration and hence, serum concentration is much lower than the concentration at the surgical site. A beneficial role of therapeutic drug monitoring has not yet been proved in NTM diseases.[27,28] Most anti-mycobacterial agents are associated with a high rate of side effects and increased macrolide or rifabutin doses were not tolerated due to QT-interval prolongation and liver or bone marrow toxicity. The *in vitro* activity against an organism may not necessarily translate to the *in vivo* situation, especially in the context of potential biofilm formation, where the interpretation of traditional *in vitro* susceptibility testing is problematic.[29]

Our findings have important implications. First, infections with *M. chimaera* and other NTM have to be considered in the differential

Table 3 Recommendations for future case detection

Exposure criteria
A patient having undergone surgery requiring cardiopulmonary bypass prior to symptoms of infection
Clinical criteria
Prosthetic valve endocarditis
Prosthetic vascular graft infection
Sternotomy wound infection
Mediastinits
Fever of unknown origin
Disseminated infection including embolic and immunologic manifestations (e.g. splenomegaly, arthritis, osteomyelitis, bone marrow involvement with cytopenia, chorioretinitis, cerebral vasculitis, pneumonitis, myocarditis, hepatitis, nephritis)
Microbiology
Positive heparin blood cultures for *M. chimaera*
Detection of *M. chimaera* by culture or PCR in cardiac tissue in the proximity of the prosthetic material
Histopathology
Detection of non-caseating granuloma and foamy/swollen macrophages with/without acid fast bacilli in cardiac tissue in the proximity of the prosthetic material
Additional criteria
Negative conventional blood cultures
Serologic exclusion of Coxiella, Bartonella, Brucella, Tropheryma whippeli, Legionella, Mycoplasma, Chlamydia

Confirmed cases
Meet clinical and exposure criteria
AND
M. chimaera is detected by culture and polymerase chain reaction (PCR) identification from invasive sample (blood, pus, biopsy or prosthetic material).
Probable cases
Meet clinical and exposure criteria
AND
M. chimaera is detected by polymerase chain reaction (PCR) identification from invasive sample (blood, pus, biopsy or prosthetic material)
operating theatre
M. avium complex (MAC) is detected by culture and polymerase chain reaction (PCR) identification from invasive sample (blood, pus, biopsy or prosthetic material).
operating theatre
Detection of non-caseating granuloma and foamy/swollen macrophages with acid fast bacilli in cardiac tissue in the proximity of the prosthetic material or in specimen from sternotomy wound
EU protocol for case detection, laboratory diagnosis and environmental testing of *Mycobacterium chimaera* infections potentially associated with heater-cooler units (available to the member states through the EPIS AMR-HAI platform).[31]

diagnosis of patients with previous cardiac surgery and extracorporeal circulation, even in the absence of severe immunosuppression. A few more case patients are already identified in Europe[21,30] and more cases are likely to be found in the future when clinicians are alerted and, as a consequence of active case finding (recommendation for case detection, *Table 3*). Second, HCUs as potential source of *M. chimaera* and other waterborne microorganisms in the operating theatre have to be identified and avoided by either placing HCUs outside of the operating theatre with independent air flow control, by making the water reservoir and piping air-tight or by reliable disinfection of the water circuits and reservoirs. Recommendations for the prevention of these waterborne, aerogenic infections in cardiac surgery are strongly warranted. Third, these infections are recalcitrant to classic antimycobacterial therapy, because of intrinsic antibiotic resilience, notoriously challenging infection sites such as bone tissue, and biofilm formation on the cardiovascular implant. More studies are needed with regard to the clinical phenotype of disseminated *M. chimaera* infection, its epidemiology, virulence mechanisms, and susceptibility to antibiotics.

Supplementary material

Supplementary Material is available at *European Heart Journal* online.

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Authors' contributions

P.K., B.H., J.t.O., S.D., M.W., M.V., D.W., and J.v.I. reviewed and gathered patient data. G.B., B.S., E.C.B., A.S., and J.v.I. performed microbiological analyses and M.R. performed histopathology. M.F. and F.T. reviewed echocardiographic studies. C.B. reviewed ophthalmic examinations. V.F., M.W., B.B., A.B. contributed surgical data. H.S., S.K., P.K., R.S., Y.A., D.W., and J.v.I. conducted outbreak investigations. P.K. and B.H. wrote the first draft, and P.K., S.K., H.S., D.W., J.v.I., R.W., E.C.B., and B.H. wrote the final version of the manuscript. All investigators contributed to data collection and interpretation of the data, reviewed drafts of the manuscript, and approved the final manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

References

1. Robicsek F, Daugherty HK, Cook JW, Selle JG, Masters TN, O'Bar PR, Fernandez CR, Mauney CU, Calhoun DM. *Mycobacterium fortuitum* epidemics after open-heart surgery. *J Thoracic Cardiovasc Surg* 1978;**75**:91–96.
2. Kuritsky JN, Bullen MG, Broome CV, Silcox VA, Good RC, Wallace RJ Jr. Sternal wound infections and endocarditis due to organisms of the *Mycobacterium fortuitum* complex. *Ann Int Med* 1983;**98**:938–939.
3. Strabelli TM, Siciliano RF, Castelli JB, Demarchi LM, Leao SC, Viana-Niero C, Miyashiro K, Sampaio RO, Grinberg M, Uip DE. *Mycobacterium chelonae* valve endocarditis resulting from contaminated biological prostheses. *J Infect* 2010;**60**:467–473.
4. Vukovic D, Parezanovic V, Savic B, Dakic I, Laban-Nestorovic S, Ilic S, Cirkovic I, Stepanovic S. *Mycobacterium fortuitum* endocarditis associated with cardiac surgery, Serbia. *Emerg Infect Dis* 2013;**19**:517–519.
5. Jonsson G, Rydberg J, Sturegard E, Christensson B. A case of *Mycobacterium goodii* prosthetic valve endocarditis in a non-immunocompromised patient: use of 16S rDNA analysis for rapid diagnosis. *BMC Infect Dis* 2012;**12**:301.

6. Bush LM, Paturi A, Chaparro-Rojas F, Perez MT. Mycobacterial prosthetic valve endocarditis. *Curr Infect Dis Rep* 2010;**12**:257–265.
7. Achermann Y, Rossle M, Hoffmann M, Deggim V, Kuster S, Zimmermann DR, Bloemberg G, Hombach M, Hasse B. Prosthetic valve endocarditis and bloodstream infection due to *Mycobacterium chimaera*. *J Clin Microbiol* 2013;**51**: 1769–1773.
8. Tortoli E, Rindi L, Garcia MJ, Chiaradonna P, Dei R, Garzelli C, Kroppenstedt RM, Lari N, Mattei R, Mariottini A, Mazzarelli G, Murcia MI, Nanetti A, Piccoli P, Scarparo C. Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int J Syst Evol Microbiol* 2004;**54**(Pt 4):1277–1285.
9. Wallace RJ Jr, Iakhiaeva E, Williams MD, Brown-Elliott BA, Vasireddy S, Vasireddy R, Lande L, Peterson DD, Sawicki J, Kwait R, Tichenor WS, Turenne C, Falkinham JO 3rd. Absence of *Mycobacterium intracellulare* and presence of *Mycobacterium chimaera* in household water and biofilm samples of patients in the United States with *Mycobacterium avium* complex respiratory disease. *J Clin Microbiol* 2013;**51**: 1747–1752.
10. Nagpal A, Wentink JE, Berbari EF, Aronhalt KC, Wright AJ, Krageschmidt DA, Wengenack NL, Thompson RL, Tosh PK. A cluster of *Mycobacterium wolinskyi* surgical site infections at an Academic Medical Center. *Infect Control Hosp Epidemiol* 2014;**35**:1169–1175.
11. Sax H, Bloemberg G, Hasse B, Sommerstein R, Kohler P, Achermann Y, Rossle M, Falk V, Kuster SP, Bottger EC, Weber R. Prolonged outbreak of *Mycobacterium chimaera* infection after open chest heart surgery. *Clin Infect Dis* 2015;**61**:67–75.
12. Massnahmen für höhere Patientensicherheit in der Herzchirurgie. Bundesamt für Gesundheit. **https://www.news.admin.ch/message/index.html?lang=de&msg-id=53774 (14 July 2014)**.
13. Investigation of Mycobacterium chimaera infection associated with cardiopulmonary bypass. Public Health England. **https://www.gov.uk/government/publications/health-protection-report-volume-9-2015/hpr-volume-9-issue-15-news-30-april (19 May 2015)**.
14. Risk assessment on Mycobacterium chimaera infections associated with heater-cooler units. ECDC. **http://ecdc.europa.eu/en/publications/Publications/mycobacterium-chimaera-infection-associated-with-heater-cooler-units-rapid-risk-assessment-30-April-2015.pdf (19 May 2015)**.
15. Peter-Getzlaff S, Luthy J, Voit A, Bloemberg GV, Bottger EC. Detection and identification of *Mycobacterium* spp. in clinical specimens by combining the Roche Cobas Amplicor *Mycobacterium tuberculosis* assay with *Mycobacterium* genus detection and nucleic acid sequencing. *J Clin Microbiol* 2010;**48**:3943–3948.
16. Hombach M, Somoskovi A, Homke R, Ritter C, Bottger EC. Drug susceptibility distributions in slowly growing non-tuberculous mycobacteria using MGIT 960 TB eXiST. *Int J Med Microbiol* 2013;**303**:270–276.
17. Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, Holland SM, Horsburgh R, Huitt G, Iademarco MF, Iseman M, Olivier K, Ruoss S, von Reyn CF, Wallace RJ Jr, Winthrop K. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
18. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987;**40**:373–383.
19. M. S. Grading of patients for surgical procedures. *Anesthesiology* 1941;**2**:281–284.
20. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, Bashore T, Corey GR. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 2000;**30**:633–638.
21. Investigation of Mycobacterium chimaera infection associated with cardiopulmonary bypass: an update. Public Health England. **https://www.gov.uk/government/publications/health-protection-report-volume-9-2015/hpr-news-volume-9-issue-18-21-may (21 May 2015)**.
22. Hartcentra nemen maatregelen om hartoperaties veiliger te maken. Inspectie voor de Gezondheitsvorg. **http://www.igz.nl/actueel/nieuws/hartcentra_nemen_maatregelen_om_hartoperaties_veiliger_te_maken.aspx (25 June 2015)**.
23. Problematik kardiovaskulärer Infektionen durch Mykobakterium chimaera im Zusammenhang mit Heater-Cooler-Systemen bei Herzoperationen. **http://www.bfarm.de/SharedDocs/Risikoinformationen/Medizinprodukte/DE/Hypothermiegeraete.html?nn=3495464. Bundesinstitut für Arzneimittel und Medizinprodukte (10 July 2015)**.
24. Darouiche RO. Treatment of infections associated with surgical implants. *N Engl J Med* 2004;**350**:1422–1429.
25. Clinical and Laboratory Standards Institute (CLSI). 2003. *Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; approved standard*. CLSI document M24-A.
26. Baddour LM, Wilson WR, Bayer AS, Fowler VG Jr, Bolger AF, Levison ME, Ferrieri P, Gerber MA, Tani LY, Gewitz MH, Tong DC, Steckelberg JM, Baltimore RS, Shulman ST, Burns JC, Falace DA, Newburger JW, Pallasch TJ, Takahashi M, Taubert KA, Committee on Rheumatic Fever E, Kawasaki D, Council on Cardiovascular Disease in the Y, Councils on Clinical Cardiology S, Cardiovascular S, Anesthesia, American Heart A, Infectious Diseases Society of A. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. *Circulation* 2005;**111**:e394–e434.
27. van Ingen J, Egelund EF, Levin A, Totten SE, Boeree MJ, Mouton JW, Aarnoutse RE, Heifets LB, Peloquin CA, Daley CL. The pharmacokinetics and pharmacodynamics of pulmonary *Mycobacterium avium* complex disease treatment. *Am J Respir Crit Care Med* 2012;**186**:559–565.
28. Koh WJ, Jeong BH, Jeon K, Lee SY, Shin SJ. Therapeutic drug monitoring in the treatment of Mycobacterium avium complex lung disease. *Am J Respir Crit Care Med* 2012;**186**:797–802.
29. Frei E, Hodgkiss-Harlow K, Rossi PJ, Edmiston CE Jr, Bandyk DF. Microbial pathogenesis of bacterial biofilms: a causative factor of vascular surgical site infection. *Vasc Endovasc Surg* 2011;**45**:688–696.
30. Epidemiological update: invasive infections with *Mycobacterium chimaera* potentially associated with heater-cooler units used during cardiac surgery. ECDC. **http://ecdc.europa.eu/en/press/news/_layouts/forms/News_DispForm.aspx?ID=1223&List=8db7286c-fe2d-476c-9133-18ff4cb1b568&Source=http%3A%2F%2Fecdc.europa.eu%2Fen%2Fpress%2Fnews%2FPages%2FNews.aspx (22 May 2015)**.
31. Protocol for case detection, laboratory diagnosis and environmental testing of *Mycobacterium chimaera* infections potentially associated with heater-cooler units. ECDC. **http://ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/about_programme.aspxEU**.