**Mycobacterium chimaera**: a report of 2 new cases and literature review

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**Abstract**

*Mycobacterium chimaera* is a non-tuberculous mycobacterium, member of the *Mycobacterium avium* complex (MAC), which has become a global public health concern due to infection following cardiac surgery performed with contaminated heater-cooler units. *M. chimaera* infection is characterized by a long latency, non-specific signs and symptoms and high mortality rates. Thus, the diagnosis is still challenging both for forensic pathologists and for clinicians. Clinical manifestations of *M. chimaera* infection include endocarditis, hepatitis, nephritis, encephalitis and chorioretinitis. A constant histopathologic finding is the presence of non-caseating granulomas, with multinucleated giant cells and histiocytes. Hereby, we present two cases of fatal disseminated *M. chimaera* infection following aortic valve surgery reporting clinical history and post-mortem findings. Further, we provide a brief overview of the literature with a special focus on histopathological characteristics of *M. chimaera* infection. The aim of this article is to provide a complete synopsis of histopathological characteristics useful for forensic pathologists.

**Keywords** *Mycobacterium chimaera* · Granuloma · Cardiovascular surgery · Heater-cooler units · Healthcare-associated infection

**Introduction**

*Mycobacterium chimaera* is a non-tuberculous mycobacterium first identified in 2004 [1], which is part of the *Mycobacterium Avium* complex (MAC). It is an opportunistic pathogen responsible for respiratory infection mainly in immunocompromised subjects and in patients with underlying respiratory diseases such as cystic fibrosis [2].

During last years, *M. chimaera* has become a global public health concern due to infection following cardiac surgery because of contaminated devices, called heater-cooler units (HCU), used to regulate blood temperature during extracorporeal circulation [3]. It seems that *M. chimaera* forms biofilms in heater-cooler unit water tanks of contaminated devices and then spreads through airborne transmission [4].

In 2013, Achermann et al. described the first cases of prosthetic valve endocarditis and bloodstream infection due to *M. chimaera* [5], while an outbreak of *M. chimaera* infections has been reported in 2015 among European patients who underwent open-chest surgery performed using a specific brand of heater-cooler devices (HCD) [6–8]. The first case of *M. chimaera* infection in Italy was described in December 2016, in a woman with a history of cardiac surgery who developed disseminated infection and vertebral osteomyelitis [9].

*M. chimaera* infection is characterized by a long latency between infection and onset of symptoms which varies from 1 to 6 years. Signs, symptoms and laboratory features are often non-specific and include low-grade fever, persistent cough, muscle pain, abdominal pain, pus at the surgical site and vomiting [3]. If not promptly diagnosed and properly treated, *M. chimaera* infections may become life-threatening [10]. Currently, no standardized treatment for *M. chimaera* infection exists [11]. Thus, antibiotic therapy should be guided by the results of a drug susceptibility test performed in a reference center for mycobacterial pathogens [3], and revision surgery has to be evaluated case-by-case.

Here, we present two cases of fatal disseminated *M. chimaera* infection, following valve replacement surgery.
performed with contaminated heater-cooler units, with a special focus on histopathological aspects.

**Case history**

**Case 1** A 74-year-old male underwent bioprosthetic aortic valve replacement for severe regurgitation on December 2015. In February 2018, the patient was submitted to prostate resection. The results of histological analysis on biopsy samples revealed acute and chronic granulomatous inflammation.

In May 2018, the man was admitted to the hospital because of persistent left hemithorax pain and left abdominal pain accompanied by splenomegaly and an episode of dysarthria. Brain MRI with contrast showed bilateral multiple ischemic lesions suggestive of microangiopathic changes. At transoesophageal echocardiogram, no prosthetic vegetation nor abscesses were detected, and blood cultures were negative. On July 2018, a positron-emission tomography and computed tomography (PET-CT) scan showed increased peri-prosthetic metabolic activity. Thus, the man underwent serological analysis and mycobacterial culture that revealed the presence of *M. chimaera* in blood, urine, feces and bone marrow. Antibiotic therapy with clarithromycin, rifabutin and ethambutol was prescribed. Despite targeted drug therapy, the patient died on May 2019, at the age of 77, due to progressive multiple organ failure.

**Case 2** A 66-year-old female underwent bioprosthetic aortic valve replacement associated with aortic root vascular replacement on May 2015.

On March 2017, the woman was admitted to the hospital for persistent fever, somnolence, asthenia, night sweats, hepatomegaly and splenomegaly. Blood cultures were negative while PET-CT scan revealed liver increased metabolic activity. Liver biopsy was performed, and tissue culture showed the presence of *M. chimaera*. After 102 days of hospitalization, she was discharged with the prescription of levofloxacin, rifampicin and clarithromycin.

On August 2017, the woman was hospitalized again due to fever, asthenia and ascites resistant to therapy. Blood cultures demonstrated non-tuberculous mycobacteria, and a new PET-CT scan indicated increased metabolism around the aortic prosthesis. Once again, no signs of endocarditis were pointed out. Antibiotic therapy was modified with the introduction of rifabutin and clofazimine instead of levofloxacin and rifampicin. In January 2019, the hospital where the woman underwent the aortic valve replacement sent alerts regarding the possible risk of *M. chimaera* post-surgical infections.

A brain MRI performed on March 2019 showed bilateral subacute ischemic lesions caused by septic embolization. On the same occasion, splenic infarctions were seen at abdomen CT scan. The patient died on August 2019, at the age of 70.

**Materials and methods**

**Post-mortem examination**

Post-mortem examination was performed 12 days (Case 1) and 2 days (Case 2) after the death and included the revision of clinical records requested to the hospital and the sampling of tissues for histological analysis.

**Histology**

Tissue samples were fixed in formalin, dried, clarified, paraffin embedded and cut with a microtome in order to obtain Sects. 6–8 μm thick. Histological sections were stained with Hematoxylin and Eosin (H&E) or Ziehl–Neelsen. Finally, the slides were observed with an optical microscope. Photomicrographs were taken using a PrimoStar iLED microscope (Zeiss, Germany).

**Microbiology**

Tissues were mechanically homogenized in phosphate-buffered saline (PBS) using a TissueLyser II (Qiagen, Germany). The homogenates were serially diluted and subsequently decontaminated from other environmental microorganism using N-acetyl-L-cysteine sodium hydroxide (NALC- NaOH).

MGIT 960 microbiology system (Becton Dickinson and Co., Sparks, MD) and Middlebrook 7H11 agar for liquid and Lowenstein-Jensen agar for solid were used respectively. Plates were monitored weekly for growth. The presence of mycobacteria on 7H11 media plates as well as in liquid media was confirmed by Ziehl–Neelsen stain. Species identification of the mycobacterium was made with probes from AccuProbe-Hologic, San Diego, CA, USA.

For the identification of the *M. chimaera* species, a genetic analysis was performed with the GenoType NTM-DR VER 1.0 Kit, Hain Lifescience Arnika.

**Review of the literature**

A literature search was first conducted using the Medline Database (PubMed.gov; US National Library of Medicine-National Institute of Health) and free text protocols (i.e. “*Mycobacterium chimaera*”), individually combined through the Boolean operator “AND”. Further studies were identified by reviewing the reference lists of the papers previously found. The search resulted in more than 160 articles, but
our study included only articles that contain references to histologic findings (e.g. granuloma). Data are summarized in Table 1. Approximately 7 articles reported histological examination, and their texts were fully analyzed.

Results

Post-mortem examination

Case 1 The victim is a 77-year-old Caucasian male in quite good overall physical conditions.

At external examination, cadaveric temperature was lower than the environmental one consistent with the stay in a mortuary refrigerator; lividity was reddish, scarce, unbleached on thumb pressure and located at the posterior regions of the body; rigidity appeared completely resolved in the whole body. Mucosal ulcers were observed into the oral cavity, and a linear 27-cm-long scar was seen in the sternal region.

At gross examination, the brain was affected by mild atrophy, oedema and encephalomalacia, particularly in right frontal, parietal and occipital lobes, left temporal and parietal lobes and in the cerebellum. Down the midclavicular line, fractures from the first to the fourth left costa were seen. Bilateral hydrothorax (500 ml in the right pleural space and 600 ml in the left pleural space) and hemoperitoneum (600 ml) were observed, too. The heart was enlarged (750 g) and characterized by adherent pericardium, slight left ventricular hypertrophy and whitish myocardial areas. The aortic bio-prosthesis was correctly located and without signs of endocarditis. Left anterior descending artery showed athero-sclerosis. Lungs appeared expanded, weighting respectively 560 g the right lung and 540 g the left one. Splenomegaly (2240 g) and multiple whitish infarction areas were detected in the spleen. Other findings consisted in hepatomegaly (1400 g), thinner renal cortex and peripancreatic fat necrosis were also observed. The abdominal aorta was atherosclerotic and ectatic.

Histology

Case 1 Brain samples showed cortical-subcortical malacic areas associated with increased glial component, infiltrates of granulocytes and hemosiderin deposition both in the hemispheres and in the brain stem. Perivascular and pericellular optically empty spaces and petechial hemorrhages were also observed. The histopathological examination of cardiac tissue revealed a slight increase in the content of perivascular fibrous tissue, areas of replacement fibrosis and severe and widespread granulomatous lesions consisting of histiocytes, multinucleated giant cells and plasma cells (Fig. 1). Lungs showed anthracosis and airspace enlargement with fragmented alveolar walls alternating with collapsed parenchymal areas. Well-formed granulomas were predominantly detected in the right lung (Fig. 2). Portal inflammation with lymphocytic infiltration, lobular necroinflammatory activity and fibrosis were observed in liver. Arterionephrosclerosis with medial thickening of medium-sized arteries, glomerulosclerosis and tubulointerstitial fibrosis were also noted.

Case 2 Brain samples showed pericellular optically empty spaces and petechial hemorrhages as well as widespread granulomas consisting of lymphocytes, histiocytes and rare multinucleated giant cells surrounded by a lymphocytic and macrophagic infiltrate (Fig. 3). Granulomatous lesions were identified also in heart myocardial samples (Fig. 4), associated with areas of replacement fibrosis and increased perivascular fibrous tissue. The aortic paravalvular tissue examination revealed multinucleated giant cells and fibrosis. Lungs showed pleural thickening and airspace enlargement with fragmented alveolar walls alternating with collapsed parenchymal areas. Liver samples revealed a microscopic pattern of chronic hepatitis consisting in enlargement of portal tracts, fibrosis, lymphocytic infiltrates and portal-portal fibrous bridging. Arterionephrosclerosis with medial thickening of medium-sized arteries and glomerulosclerosis were also seen.
| Authors                  | Age (y) | Sex | Surgery or other                     | Latency (month) | Other tissue involvement | Heart                                      | Kidney       | Liver | Brain | Lungs | Histopathological findings                                                                 | Death |
|-------------------------|---------|-----|--------------------------------------|-----------------|--------------------------|-------------------------------------------|--------------|-------|-------|-------|------------------------------------------------------------------------------------------|-------|
| Trautman C. et al       | 63      | F   | AVR                                  | 72              | Anemia                   | Prosthetic valve vegetations, aortic root abscess | Renal impairment | nd    | nd    | nd    | Bone marrow granuloma, granulomatous interstitial nephritis                               | No    |
| Watanabe R. et al       | 61      | M   | Seronegative rheumatoid arthritis    | /               | Tenosynovitis            | nd                                        | nd           | nd    | nd    | nd    | Inflammatory cell infiltration and multinucleated giant cells in synovial tissue          | No    |
| Böni C. et al           | 51      | M   | Open-heart surgery                   | 16              | Progressive choroiditis  | Endocarditis and/or aortic graft infection | nd           | nd    | nd    | nd    | Yes                                                                                       |       |
|                        | 64      | M   |                                        | 39              | Choroidal lesions        | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
|                        | 49      | M   |                                        | 41              | Progressive choroiditis  | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
|                        | 61      | M   |                                        | 21              | Progressive choroiditis  | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | Yes   |
|                        | 63      | M   |                                        | 22              | Progressive choroiditis  | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | Yes   |
|                        | 64      | M   |                                        | 21              | Choroidal lesions        | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
|                        | 66      | M   |                                        | 36              | Progressive choroiditis  | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
|                        | 50      | M   |                                        | 26              | Choroidal lesions        | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
|                        | 58      | M   |                                        | 25              | Choroidal lesions        | Endocarditis                               | nd           | nd    | nd    | nd    | nd                                                                                       | No    |
| Sandrine A. et al       | 51      | M   | Composite graft replacement           | 16              | Fever, uveitis, vitritis and choroidal lesions, splenomegaly, pancytopenia | Endocarditis with cardiac insufficiency    | Renal impairment | Hepatitis | nd    | Pneumonitis | No                                                                                       |       |
|                        | 65      | M   | Mitral valve reconstruction           | 39              | Uveitis, vitritis and choroidal lesions, splenomegaly, pancytopenia | Endocarditis with cardiac insufficiency    | Renal impairment | Hepatitis | nd    | nd    | No                                                                                       |       |
|                        | 49      | M   | AVR                                  | 41              | Arthritis, choroidal lesions, splenomegaly, pancytopenia | Endocarditis with cardiac insufficiency    | nd           | Hepatitis | nd    | nd    | No                                                                                       |       |
|                        | 61      | M   | Aortic root and arch replacement     | 21              | Splenomegaly, bicytopenia, vertebral osteomyelitis, choroiditis      | Renal failure                              | Hepatitis     | nd    | nd    | nd    | Yes                                                                                      |       |
|                        | 63      | M   | Aortic root and arch replacement     | 22              | Splenomegaly, bicytopenia, choroiditis and anterior uveitis          | Renal impairment                           | Hepatitis     | nd    | nd    | nd    | Granulomatous inflammation of choroid, kidneys and brain                                  | Yes   |
| Authors          | Age (y) | Sex | Surgery or other | Latency (month) | Other tissue involvement | Heart                     | Kidney                     | Liver                     | Brain                     | Lungs                     | Histopathological findings                                      | Death |
|------------------|---------|-----|------------------|-----------------|------------------------|--------------------------|---------------------------|--------------------------|---------------------------|--------------------------|------------------------------------------------------------------|-------|
| Overton K. et al | 83      | F   | AVR              | 13              | Pancytopenia           | Fludeoxyglucose (FDG) avidity around the prosthetic aortic valve | Renal impairment           | Liver function test derangement | nd                        | nd                       | nd                      | Yes                                                              |       |
|                  | 40      | M   | AVR              | 23              |                        | Severe peri-prosthetic aortic valve regurgitation              | Renal impairment           | Liver function test derangement | nd                        | nd                       | Pneumonia               | Reactive changes in bone marrow, renal supplicative granuloma    | No    |
|                  | 79      | M   | AVR + CABG       | 21              | Thrombocytopenia       | Large vegetation on the prosthetic valve, aortic root abscess  | Renal impairment           | Liver function test derangement | nd                        | nd                       | nd                      | No                                                              |       |
|                  | 63      | M   | AVR              | 21              | Pancytopenia           | nd                       | nd                        | Liver function test derangement  | nd                        | nd                       | nd                      | Bone marrow with multiple non-caseating granulomas               | No    |
| Lau D. et al     | 60      | F   | AVR + MVR        | 15              | Pancytopenia, lymphadenopathy, choroidal nodules | nd                       | nd                        | nd                        | nd                       | nd                       | Partially necrotizing granulomatous inflammation in liver, kidneys, heart, brain, lungs, spleen, pancreas and thyroid | Yes   |
| Tan N. et al     | 73      | M   | AVR              | 12              | Choroidal lesions      | nd                       | nd                        | nd                        | nd                       | nd                       | nd                    | Bone marrow with non-caseating granulomas                        | No    |
|                  | 74      | M   | AVR + aortic root repair | 26              | Bilateral chorioretinitis | Fludeoxyglucose (FDG) avidity between the ascending aortic graft and the anterior mediastinum | nd                       | nd                        | nd                        | nd                       | nd                      | No                                                              |       |
|                  | 57      | M   | AVR              | 16              | Splenomegaly, pancytopenia, bilateral chorioretinal lesions | Endocarditis             | nd                        | nd                        | nd                       | Pulmonary infiltrates                                           | Yes   |
| Cai Y. et al     | 63      | F   | AVR              | 60              | Anemia                 | Aortic root abscess, previous mitral valve endocarditis        | nd                        | nd                        | nd                       | nd                       | Bone marrow granulomas, amyloidosis, interstitial nephritis with one granuloma | No    |
| Authors          | Age (y) | Sex | Surgery or other | Latency (month) | Other tissue involvement | Heart           | Kidney                     | Liver   | Brain | Lungs | Histopathological findings                                                                 | Death |
|------------------|---------|-----|------------------|-----------------|-------------------------|-----------------|---------------------------|---------|-------|-------|------------------------------------------------------------------------------------------------|-------|
| Shafizadeh N. et al | 56      | M   | AVR + aortic root repair | 14              | Pancytopenia            | nd              | Acute kidney injury       | nd      | nd    | nd    | Bone marrow granulomas, sinusoidal granulomas with architectural changes of venous outflow obstruction | Yes   |
| 69               | M       | AVR + MVR | 22              | Pancytopenia, bone marrow ill-defined granulomas | Vegetation on both prosthetic valves | nd              | Hepatitis C, liver function test derangement, hepatomegaly | nd      | nd    | nd    | Bone marrow granulomas, macrovesicular steatous, sinusoidal granulomas with architectural changes of venous outflow obstruction | Yes   |
| 76               | M       | AVR | 14              | Thrombocytopenia | nd              | nd              | Liver function test derangement | nd      | nd    | nd    | Sinusoidal granulomas with architectural changes of venous outflow obstruction | Yes   |
| 70               | M       | AVR + aortic root replacement | 21              | nd              | Vegetation on aortic valve | nd              | nd                        | nd      | nd    | nd    | Granulomatous inflammation of bone marrow, kidneys and liver, sinusoidal granulomas with architectural changes of venous outflow obstruction | Yes   |
| 81               | F       | AVR | 20              | nd              | nd              | nd              | Liver function test derangement | nd      | nd    | nd    | Sinusoidal granulomas with architectural changes of venous outflow obstruction | No    |
| 58               | F       | AVR + aortic root replacement | 29              | Leukopenia, anemia | nd              | nd              | Liver function test derangement | nd      | nd    | nd    | Sinusoidal granulomas with architectural changes of venous outflow obstruction | Yes   |
| 62               | M       | AVR | 26              | nd              | nd              | nd              | Liver function test derangement | nd      | nd    | nd    | Sinusoidal granulomas with architectural changes of venous outflow obstruction | No    |
### Table 1 (continued)

| Authors          | Age (y) | Sex | Surgery or other                     | Latency (month) | Other tissue involvement                  | Heart                  | Kidney       | Liver      | Brain      | Lungs | Histopathological findings                                                                 | Death |
|------------------|---------|-----|--------------------------------------|-----------------|------------------------------------------|------------------------|--------------|------------|------------|-------|------------------------------------------------------------------------------------------|-------|
| Sax H. et al     | 58      | M   | MVR                                  | 33              | Splenomegaly, pancytopenia               | Endocarditis           | Renal impairment | Hepatitis | nd         | nd     | Granulomatous nephritis and hepatitis                                                    | Yes   |
|                  | 51      | M   | Composite graft for aortic dissection | 17              | Splenomegaly, pancytopenia, ocular emboli | nd                     | nd           | nd         | nd         | nd     | Granulomatous myocarditis, nephritis and pneumonitis                                       | Yes   |
|                  | 64      | M   | Mitral valve reconstruction           | 42              | Splenomegaly, pancytopenia, ocular emboli, wrist arthritis | Endocarditis           | Renal impairment | Hepatitis | nd         | nd     | Granulomatous endocarditis, osteomyelitis                                                  | No    |
|                  | 49      | M   | AVR                                  | 40              | Splenomegaly, pancytopenia, ocular emboli, pacemaker pocket infection | Endocarditis           | nd           | Hepatitis | nd         | nd     | Granulomatous hepatitis, myositis                                                          | No    |
|                  | 61      | M   | Aortic root and arch replacement     | 19              | Splenomegaly, ocular emboli              | nd                     | nd           | nd         | nd         | nd     | Granulomatous vertebral and sternal osteomyelitis                                         | No    |
|                  | 63      | M   | Aortic root and arch replacement     | 20              | Splenomegaly, multifocal choroiditis      | nd                     | Renal failure  | Hepatitis | nd         | nd     | Granulomatous interstitial nephritis                                                       | No    |
| Authors                  | Age (y) | Sex | Surgery or other                      | Latency (month) | Other tissue involvement                                                                 | Heart                | Kidney | Liver | Brain | Lungs | Histopathological findings                                                                 | Death |
|--------------------------|---------|-----|---------------------------------------|-----------------|------------------------------------------------------------------------------------------|----------------------|--------|-------|-------|-------|------------------------------------------------------------------------------------------|-------|
| Kohler P. et al          | 58 M    |     | Mitral valve reconstruction           | 24              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly                                   | Cardiac insufficiency| nd     | nd    | nd    | nd    | Necrotizing endocarditis                                                                   | Yes   |
|                          | 51 M    |     | Composite aortic graft replacement    | 14              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly                                   | nd                   | nd     | nd    | nd    | nd    | Granulomatous myocarditis, nephritis and hepatitis, granulomatous lesions in brain       | Yes   |
|                          | 64 M    |     | Mitral valve reconstruction           | 26              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly                                   | nd                   | nd     | nd    | nd    | nd    | Granulomatous endocarditis and osteomyelitis                                              | No    |
|                          | 49 M    |     | AVR                                   | 40              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly                                   | Cardiac insufficiency| nd     | nd    | nd    | nd    | Granulomatous pectoral myositis and hepatitis                                              | No    |
|                          | 61 M    |     | Aortic root and arch replacement      | 17              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly                                   | nd                   |         | Nephritis | nd | nd    | Granulomatous endocarditis, osteomyelitis and granulomatous lesions in brain              | Yes   |
|                          | 63 M    |     | Aortic root and arch replacement      | 21              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly, osteomyelitis                     | nd                   | nd     | nd    | nd    | nd    | Granulomatous periportal tissue and granulomatous nephritis                               | Yes   |
|                          | 76 M    |     | AVR                                   | 22              | Anemia, lymphocytopenia, thrombocytopenia, splenomegaly, myositis                         | Cardiac insufficiency| nd     | nd    | nd    | nd    |                                                                         | No    |
|                          | 36 F    |     | Mitral valve reconstruction           | 5               | Anemia, lymphocytopenia, thrombocytopenia, myositis                                      | Cardiac insufficiency| nd     | nd    | nd    | nd    | Granulomatous endocarditis                                                                | Yes   |
|                          | 74 M    |     | AVR + CABG                            | 10              | Anemia, lymphocytopenia, thrombocytopenia                                                | Clinical signs of endocarditis | nd     | nd    | nd    | nd    | Granulomatous osteomyelitis and hepatitis, bone marrow with non-necrotizing granulomas    | No    |
|                          | 1 M     |     | Aortic arch reconstruction             | 13              | Anemia, lymphocytopenia, thrombocytopenia                                                | Cardiac insufficiency| nd     | nd    | nd    | nd    |                                                                         | No    |
### Table 1 (continued)

| Authors                  | Age (y) | Sex | Surgery or other | Latency (month) | Other tissue involvement | Heart                      | Kidney                    | Liver                    | Brain | Lungs                  | Histopathological findings | Death |
|--------------------------|---------|-----|------------------|-----------------|-------------------------|----------------------------|---------------------------|--------------------------|-------|------------------------|--------------------------------|-------|
| Asadi T. et al           | 62      | M   | Aortic root, ascending aorta and aortic arch replacement | 16              | Mild anemia, choroid lesions, vertebral osteomyelitis, walled abscess in the left psoas muscle | nd                         | nd                        | Liver function test derangement | nd    | nd                     | Non-necrotizing granulomatous hepatitis | No    |
| 65                       | M       | AVR + aortic, hemashield graft placement | 36              | Pancytopenia, bone marrow non-caseating granulomas | Aortic root abscess        | Renal failure              | Liver function test derangement | nd    | nd                     | Bone marrow non-caseating granulomas | No    |
| Achermann Y. et al       | 58      | M   | AVR + MVR        | 12              | nd                      | Severe mitral and aortic insufficiency | nd                        | nd                        | nd                        | nd    | nd                     | Respiratory distress Granulomatous inflammation of kidneys and liver, acute necrotizing mycobacterial endocarditis | Yes   |
| 51                       | M       | Composite aortic graft replacement | 16              | Splenomegaly, pancytopenia | Prosthetic valve endocarditis | Progressive renal insufficiency | Liver function test derangement | nd    | nd                     | Acute and chronic granulomatous inflammation of kidneys, liver and spleen | Yes   |
| Rosero C. I. et al       | 66      | M   | Cough, low-grade fever and weight loss, lung mass treated with partial left lung lobectomy | nd              | nd                      | nd                        | nd                        | nd                        | nd    | nd                     | Necrotizing granuloma with acid fast bacilli in left lung | No    |
| Sebastian Haller S. et al| 80      | M   | AVR              | 10              | nd                      | Endocarditis               | nd                        | nd                        | nd    | nd                     | nd No | | No | No |
| 75                       | M       | CABG | 60              | Spondylodiscitis    | nd                      | nd                        | nd                        | nd                        | nd    | nd                     | nd No | | No | No |
| 65                       | M       | AVR | 36              | nd                | Valvular aortic endocarditis, paravalvular leak and abscess | nd                        | nd                        | nd                        | nd    | nd                     | Yes  | | No | No |
| 67                       | M       | AVR + CABG | 48 | nd | Paravalvular abscess | nd | nd | nd | nd | nd | No |
| 53                       | M       | AVR | 36              | nd | Endocarditis | nd | nd | Cerebral abscesses | nd | | No |
| Sacco K. A. et al        | 63      | F   | AVR              | 12              | Leukopenia, thrombocytopenia | Prosthetic valve endocarditis and root abscess | Renal granulomas | nd | nd | Bone marrow with non-specific granuloma | No |
| Joseph Butterworth J. et al | 72 | M   | AVR              | 28              | Pancytopenia, splenomegaly | nd | nd | nd | nd | nd | Bone marrow with non-necrotic microgranulomas | No |

AVR aortic valve replacement, MVR mitral valve replacement, CABG coronary artery bypass grafting, nd non detected
Case 1. *M. chimaera* was detected post-mortem in patient’s bone marrow, lymph nodes, spleen, brain and liver samples.

Case 2. *M. chimaera* was identified post-mortem in patient’s lymph nodes, spleen, brain and peri-prosthetic tissue.

**Discussion**

Since 2013, *Mycobacterium chimaera* infections due to specific brands of contaminated heater-cooler units used in cardiac surgery have been concerning public health worldwide. Many authors have shown that heater-cooler units used to regulate patient’s body temperature during cardiac surgery procedures have been colonized by *Mycobacterium chimaera* [8, 12, 13]. For example, LivaNova Stockert 3T models might have been originally contaminated in German production site [4] even though a contamination during their use cannot be excluded. Since 2014, SORIN Group Deutschland GmbH and Maquet Getinge Group have issued several security alerts finalized to inform about the procedures that have to be adopted in case of specific contaminated units, providing their serial numbers. In particular, the alerts stressed the importance of devices’ cleaning and disinfection, water quality checking and the usefulness of directing the devices’ drain away from the patient. Moreover, the manufacturer recommended to promptly removed from the operating rooms the heater-cooler units suspected to be contaminated [14].

The review of the literature showed that *Mycobacterium chimaera* infections involved patients aged from 12 months to 83 years with a median age of 60.4 years. Regarding the type of surgical intervention, infection followed aortic valve replacement (AVR) alone (*n* = 19) or in combination with aortic root replacement or repair (*n* = 6), mitral valve replacement (*n* = 9), and mitral valve repair (*n* = 6). The most common locations for *M. chimaera* were the hematopoietic, lymphatic, respiratory and central nervous systems.
replacement (MVR) \( n = 3 \) or coronary artery bypass grafting (CABG) \( n = 3 \). Infection followed also aortic root and arch replacement \( n = 7 \), mitral valve reconstruction \( n = 5 \), composite graft replacement \( n = 4 \), CABG \( n = 1 \), MVR \( n = 1 \), aortic arch reconstruction or repair \( n = 2 \), lung lobectomy \( n = 1 \), history of seronegative rheumatoid arthritis treated with methotrexate, tacrolimus and prednisolone \( n = 1 \), nd \( n = 1 \).

The most common presenting symptoms include fever, night sweats and weight loss \([10]\). In addition, lymphopenia, thrombocytopenia, anemia, elevated levels of creatinine, transaminases and C-reactive protein are often encountered \([19]\).

The diagnosis could be difficult because signs and symptoms are non-specific, slight and appear generally from 6 weeks to more than 5 years after surgery. It is interesting to note that some patients were misdiagnosed with sarcoidosis after the discovery of granulomatous involvement and initiated on steroid therapy \([20]\).

According to Sax et al. \([17]\), the latency period is long, with a median of 26 months.

Moreover, extracardiac symptoms may precede the cardiac ones, and a cardiac involvement can be detected only at post-mortem examination.

\textit{M. chimaera} infections mortality rate may reach 60\% \([15]\), probably due to multiple factors including the risk of reoperative surgery, the long latency of the infection, the intrinsic antibiotic resistance of these slow-growing mycobacteria, the prolonged antibiotic therapy and the infected sites that may be challenging for antimicrobial penetration \([19]\).

Patients could experiment prosthetic valve endocarditis, vascular graft infections and/or bacteremia with manifestations that can vary from splenomegaly to arthritis, osteomyelitis, bone marrow involvement with subsequent cytopenia, chorioretinitis, lung involvement, hepatitis, nephritis and myocarditis \([3]\).

Especially, the analyzed studies revealed that patients presented signs of involvement of single or multiple organs including endocarditis \( n = 20 \), cholestatic hepatitis \( n = 20 \), granulomatous nephritis \( n = 12 \), cytopenia \( n = 10 \), osteomyelitis or other bone lesions \( n = 9 \), encephalitis \( n = 7 \), chorioretinitis or vasculitis \( n = 6 \), aortic valve tissue inflammation \( n = 6 \), pneumonitis \( n = 3 \), spleen inflammation \( n = 2 \), myositis \( n = 2 \), uveitis and vitritis \( n = 1 \) and inflammatory cell infiltration of synovial tissue \( n = 1 \).

As routine blood cultures have a low mycobacterial growth sensitivity, suggested methods for diagnosis are mycobacterial blood cultures, performed multiple times on separate days to maximize their sensitivity, together with molecular diagnostics tools such as polymerase chain reaction (PCR) \([3]\). The use of molecular probes with 16S rDNA sequencing and rpoB sequencing is essential to identify \textit{M. chimaera} among other members of the MAC \([17, 18]\).

However, \textit{Mycobacterium} species can require 14–21 days of incubation on culture media before their detection. Thus, a thorough histopathological examination of biotic samples may show a pattern of injury indicative of granulomatous disease, and then it can anticipate the diagnosis.

In fact, the main histologic feature of \textit{M. chimaera} infection is represented by non-caseating granuloma and foamy/swollen macrophages with or without acid-fast bacilli \([19]\).

A granuloma is the result of chronic inflammation and consists of a microscopic aggregation of macrophages transformed into epithelioid cells, surrounded by a collar of lymphocytes and plasma cells. The fusion of epithelioid cells forms the so-called Langhans giant cells with the typical arrangement of nuclei in a horseshoe-shaped pattern near the outer edge of the cell or in cluster at the two poles of the cell \([21]\).

In the examined articles, granulomas involved kidney \( n = 2 \), liver \( n = 3 \), spleen \( n = 1 \), brain \( n = 1 \), heart, lung and choroidal tissue.

In our cases, granulomatous lesions were observed respectively in myocardium and lungs (case 1) and in brain and myocardium (case 2), indicating a disseminated infection.

Therefore, maximum effort should be made to obtain biopsy for histologic analysis: the detection of non-caseating granulomas, foamy macrophages or multinucleated giant cells in cardiac tissue and in other tissues should prompt the clinicians to search for a history of open-heart surgery and to set up the most appropriate diagnostic and therapeutic interventions.

In our case 2, a liver biopsy was performed 2 years before the death as a previous PET-CT scan has revealed liver increased metabolic activity. This allowed the diagnosis of \textit{M. chimaera} infection. Nevertheless, the prognosis has been poor anyway probably due to the dissemination of the pathology that had already occurred.

In some cases, the presence of granulomatous inflammation in multiple organs has led to an initial misdiagnosis of sarcoidosis \([20, 22]\) with consequent administration of immunosuppressive therapies which may also have contributed to poor outcomes. However, the presence of extrapulmonary localizations and bone-marrow involvement is frequent in sarcoidosis and should be properly considered \([23]\).

Hence, it is necessary to stress the importance of a correct differential diagnosis, since the misinterpretation of these cases as sarcoidosis or other immuno-mediated diseases may produce a worse outcome for these patients.

It is also recommended to perform a retinal examination in suspected cases, even without visual symptoms, due to the possibility of detecting rapidly choroidal granulomas that
would be suggestive of a disseminated *M. chimaera* infection [24].

The level of awareness of healthcare professionals is currently improved thanks to specific alerts spread by national or regional government agencies. High clinical suspicion for non-tuberculous mycobacteria infection is strongly recommend in case of cardiac prosthetic valve endocarditis, prothetic vascular graft infection, sternotomy wound infection, mediastinitis and signs of disseminated infection including embolic and immunologic manifestation, in patients who have undergone cardiac surgery requiring heater-cooler units in the 6 years prior the onset of symptoms. Recognition of this pattern of injury can lead to a correct diagnosis so that a suitable antibiotic therapy can be initiated as early as possible in order to reduce morbidity and mortality.

Forensic pathologists need to pay attention to the clinical history of the victim with a thorough examination of clinical records in order to assess the presence of previous cardiopulmonary surgery performed with heater-cooler devices and to ascertain signs and symptoms suggestive of infection. If *M. chimaera* isolation has not been realized when the subject was still alive, post-mortem microbiological investigations have to be carried out. Then, histological analysis of various tissue samples is essential. The detection of granulomatous lesions either localized in only one tissue or spread in different organs could be highly suggestive of mycobacterial infection and, in the same time, could give precious information about the dissemination of the disease.

In conclusion, the management of *M. chimaera* infection is still challenging. Morbidity and mortality are high due to the difficulties related both to diagnosis and to therapy. Forensic pathologists, even if in the absence of a previous diagnosis of *M. chimaera* infection, could easily reach the correct diagnosis based on correlation between clinical history, post-mortem examination and laboratory investigations in which histological analysis plays a fundamental role in order to detect the typical granulomatous lesions.

In Italy, probably there will be an increase in *M. chimaera* infections’ prevalence in the coming years. In fact, until a few years ago, little or nothing was known of this infection related to contaminated heater-cooler units, and the long incubation time of this kind of disease suggests a possible short-term spike in *M. chimaera* infection diagnosis.

Thus, it is essential to increase the level of awareness both among clinicians and among pathologists in order to have skills and tools to face this serious surgical-related infection.

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**Declarations**

**Ethics approval** According to our institution policy on this subject, it is not requested any ethics approval.

**Consent to participate** Not applicable.

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