

Fatal Atypical Mycobacterial Infection in a Cardiac Transplant Recipient

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A 37-year-old female underwent heart transplantation for giant cell myocarditis. The patient died within three-and-a-half months of cardiac transplantation. Postmortem specimens from the heart and lung showed multiple necrotizing granulomas with numerous acid-fast bacilli. Polymerase chain reaction done on both the postmortem samples confirmed the presence of atypical mycobacterial infection. This fatal case of atypical mycobacteriosis in a cardiac transplant patient is reported for its rarity. (**Indian Heart J 2001; 53: 100–103**)

Key Words: Transplantation, Infection, Myocarditis

Giant cell myocarditis (GCM) is a rare and frequently fatal disorder of unknown origin that is defined histopathologically as diffuse myocardial necrosis with multinucleated giant cells in the absence of sarcoid-like granulomas.^{1,2} Cardiac transplantation is the treatment of choice though the disease is known to recur in the donor heart.² Occurrence of atypical mycobacterial infection in a cardiac transplant recipient is rare. We present a fatal case of atypical mycobacterial infection in a cardiac transplant recipient who underwent heart transplantation for GCM.

Case Report

This 37-year-old female was clinically diagnosed as dilated cardiomyopathy. Pre-transplant endomyocardial biopsy revealed non specific features, e.g. mild focal thickening of the endocardium, myocyte hypertrophy with nucleomegaly and sarcoplasmic degenerative changes. There was no myocarditis in the material examined. The patient underwent orthotopic cardiac transplantation for intractable cardiac failure. In the postoperative period, the patient was on immunosuppressive therapy with cyclosporin, azathioprine and prednisolone. Four endomyocardial biopsies were taken on days 10, 20, 30 and 60 following transplantation. The first two biopsies revealed acute rejection of grade IA (ISHLT). The third biopsy showed focal ischemic changes while the fourth one showed interstitial fibrosis and myocyte hypertrophy. The patient

expired on day 100 following transplantation. Postmortem biopsies from the heart and left lung were taken for pathological examination.

Pathology:

The native heart: The heart weighed 235 g. Due to the operative technique, parts of both the atria and outflow tracts of the ventricles were not included in the specimen. On external examination, the pericardial surface showed a few small 0.1–0.2 cm grey-white necrotic areas. On opening the heart, part of the right atrium included in the specimen and the tricuspid valve were normal. The right ventricular cavity had focal endocardial thickening which was especially prominent over the septal wall. The inflow tract of the right ventricle just beneath the tricuspid valve had a

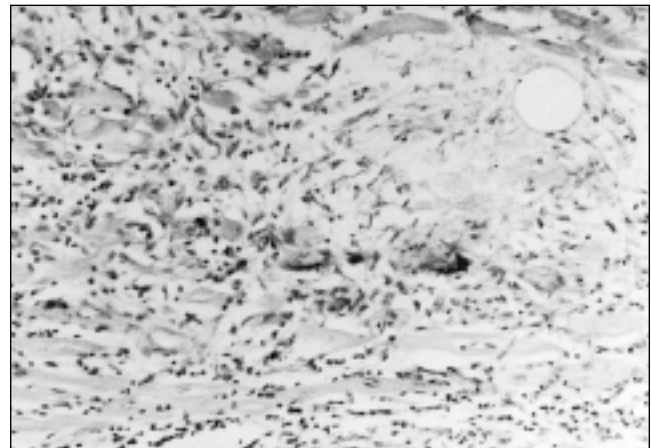


Fig. 1. Photomicrograph from the explanted native heart showing necrosis, lymphohistiocytic infiltrate and giant cells. (H&E× 180)

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thrombus, 0.5 cm in diameter. The left atrium and the mitral valve were normal. The left ventricular cavity was dilated and showed diffuse endocardial thickening. The ventricular myocardium had multiple small grey-white areas 0.1–0.2 cm in diameter. Segments of the epicardial coronary arteries included in the specimen were normal.

On light microscopic examination, multiple sections from all the chambers showed large areas of myonecrosis and lymphohistiocytic infiltrate with numerous giant cells (Fig. 1). There was endocardial thickening, widespread myocyte degeneration and focal replacement fibrosis. No definite epithelioid cell granuloma was noted. Periodic acid Schiff and silver methenamine stains for fungus did not demonstrate any fungal profile. Ziehl–Neelsen and auramine–rhodamine stains for acid-fast bacilli were negative. The coronary arteries were histologically normal. In view of the presence of numerous giant cells with myonecrosis in the absence of distinct sarcoid-like granulomas, the diagnosis of giant cell myocarditis was considered.

Postmortem biopsy specimens: Postmortem biopsy samples included tissues from the donor heart and the left lung of the patient. On light microscopic evaluation, the endocardium was normal. The myocardium revealed non-specific changes like myofibre hypertrophy and focal interstitial edema. The pericardium had multiple discrete necrotizing granulomas showing central necrosis with a few epithelioid and giant cells. Sections from the lung showed similar epithelioid cell granulomas (Fig. 2). Ziehl–Neelsen stain for acid-fast bacilli demonstrated numerous acid-fast *Mycobacterium tuberculosis*-like organisms both in the pericardial and pulmonary granulomas. No fungal profile or inclusion of cytomegalovirus infection was identified in

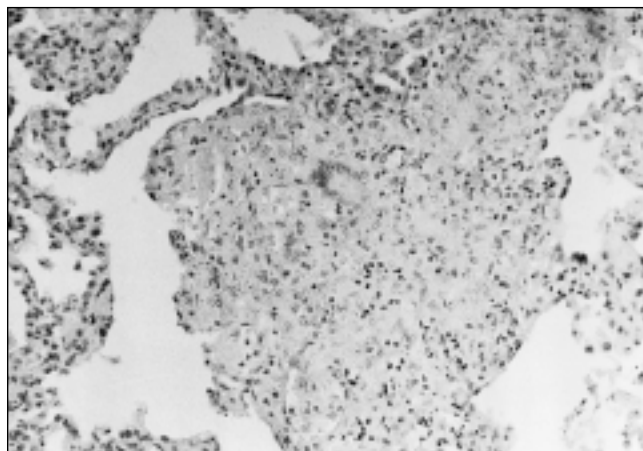


Fig. 2. Photomicrograph from the lung showing epithelioid cell granuloma with Langhan's giant cells. (H&E×180)

the specimen examined. A diagnosis of tuberculosis involving the pericardium and lung was suggested. Subsequently samples from the explanted native heart (on which the diagnosis of GCM was earlier made) and postmortem biopsies from the heart and lung (histologically diagnosed as tubercular) were subjected to polymerase chain reaction (PCR) analysis for the detection of *Mycobacteria* in general and *M. tuberculosis* in particular.

Polymerase chain reaction:

Analysis of the native heart and postmortem samples: Samples for PCR were retrieved in the form of scrapings of paraffin-embedded tissue from paraffin blocks and transferred to 1.5 ml polypropylene tubes (Axygen Inc.). The samples were deparaffinized by extraction with xylene. Residual xylene was removed by extraction with 100% ethanol, tissue pellets were dried using acetone and DNA was extracted using 10% chelex 100.³ Polymerase chain reaction was performed with the supernatant as follows:

- (1) 23 S rDNA PCR assay specific for genus⁴ *Mycobacterium* was done. An amplification product of 174 bps was seen as expected in the postmortem lung (Fig. 3a) and donor

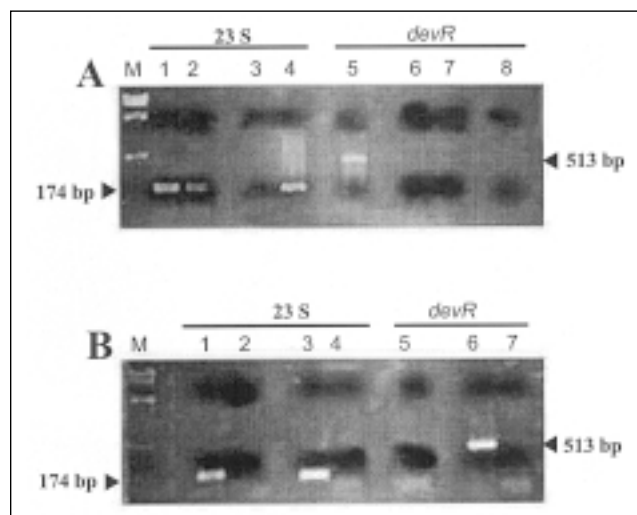


Fig. 3a. Amplification of mycobacterial DNA from paraffin-embedded lung tissue. Amplification reaction products were electrophoresed and visualized by ethidium bromide staining. Lane M, molecular weight marker; lanes 1 and 2, DNA from postmortem lung tissue (undiluted and 1:10 dilution, respectively); lane 3, DNA-negative control; lane 4, *M. tuberculosis* DNA-positive control; lane 5, *M. tuberculosis* DNA-positive control; lanes 6 and 7, DNA-negative control.

Fig. 3b. Amplification of mycobacterial DNA from paraffin-embedded heart tissue. Amplification reaction products were electrophoresed and visualized by ethidium bromide staining. Lane M, molecular weight marker; lane 1, DNA isolated from implanted donor heart tissue (postmortem); lane 2, DNA isolated from explanted native heart; lane 3, *M. tuberculosis* DNA-positive control; lane 4, DNA negative-control; lane 5, DNA isolated from implanted donor heart; lane 6, *M. tuberculosis* DNA-positive control; lane 7, DNA-negative control.

heart (Fig. 3b) samples, indicating the presence of an organism belonging to the genus *Mycobacterium*. PCR failed to amplify 23 S rDNA sequences from the explanted native heart (Fig. 3b) and thereby, the possibility of tuberculosis in the native heart could be ruled out.

- (2) DevR-based PCR assay was not performed in the explanted heart tissue as the 23 S rDNA-based genus-specific PCR assay failed to amplify mycobacterial DNA (Fig. 3b). Following step 1, devR-based PCR was performed in the postmortem samples from the lung and donor heart. This assay generates a 513 bps DNA product specific for *M. tuberculosis*⁵ which was negative in these samples (Figs 3a and 3b). The positivity of 23 S rDNA assay and a negative *M. tuberculosis* complex-specific assay suggested the presence of nontubercular *Mycobacteria* (atypical *Mycobacteria*) in the postmortem samples of the heart and lung. The causative mycobacterial species could not be identified further due to the nonavailability of species-specific PCR for assays of nontubercular *Mycobacteria* in our PCR laboratory.

Discussion

Cardiac transplantation is an accepted therapeutic modality in patients with intractable heart disease. GCM is a rare disorder characterized histologically by the presence of diffuse inflammatory infiltrates with multinucleated giant cells in the absence of sarcoid-like granulomas. Cardiac transplantation is a recommended therapeutic procedure in this condition as the disease is otherwise fatal.^{2,6} Histological examination of the explanted native heart in the present case revealed myocardial necrosis with lymphohistiocytic infiltrate and giant cells. There was no obvious granulomatous reaction or presence of acid-fast bacilli and thus the diagnosis of GCM was made.

In the post-transplant period, the patient underwent episodes of acute rejection and finally succumbed to disseminated atypical mycobacterial infection involving the heart and lungs. While examining the pathological material from transplant recipients, the possibility of infection should always be considered as these patients are immunosuppressed. The two most common infections which can be identified and diagnosed on endomyocardial biopsy are cytomegalovirus infection and toxoplasmosis.⁷ Though tuberculosis has occasionally been described in cardiac transplant recipients,^{8,9} the occurrence of atypical mycobacterial infection in such patients is distinctly rare.¹⁰ The incidence of tuberculosis among patients undergoing antirejection therapy is considerably higher than that in the

general population and heart transplant recipients have been found to carry the highest risk of tuberculosis.⁹ Though infections due to nontubercular *Mycobacteria* in solid organ transplant recipients are infrequent, they may be a major cause of morbidity in such patients. The reported prevalence of disease in a heart transplantation program due to nontubercular *Mycobacteria* is 0.24%. In the disseminated form of the disease, the lungs and subcutaneous tissue are commonly involved. Intestinal involvement has rarely been reported in the literature.¹¹ Our patient is unique as she had a fatal outcome due to atypical mycobacterial infection involving the lungs and heart within 100 days of transplantation. Other organs could not be examined as an autopsy was not conducted on the patient.

A high degree of clinical suspicion of tubercular infection is required in cardiac transplant recipients and the diagnosis needs to be confirmed by histological examination and/or cultures. Postmortem specimens from the heart and lung in the present case revealed multiple small necrotizing granulomas. Ziehl–Neelsen stain demonstrated numerous acid-fast bacilli and a provisional diagnosis of tuberculosis was offered. The final diagnosis of nontubercular mycobacterial infection could only be made following PCR analysis of tissues retrieved from the paraffin block as the genus-specific PCR product was amplified without amplification of *M. tuberculosis* DNA. Polymerase chain reaction has revolutionized the entire spectrum of molecular biology as it can offer rapid definitive diagnosis of an infective organism including its species specification and antibiotic resistance profile. Retrieval of tissue from paraffin-embedded material and subsequent PCR analysis can provide an accurate etiological diagnosis even when the culture report is not available to the pathologist, as in the present case. While examining the postmortem specimens, we had a re-look at the original diagnosis of GCM made on the explanted native heart. The possibility of undiagnosed mycobacterial infection with widespread necrosis and giant cell reaction could be ruled out due to the absence of granulomas, negative Ziehl–Neelsen and auramine–rhodamine staining and non-amplification of genus-specific PCR product on retrospective PCR analysis. Thus, heart transplantation should be considered an unheralded risk factor for mycobacterial infection, particularly in countries such as India where the disease is prevalent.

References

1. Cooper LT, Berry GJ, Rizeq M, Schroeder JS. Giant cell myocarditis. *J Heart Lung Transplant* 1995; 14: 394–401

2. Cooper LT Jr, Berry GJ, Shabetai R. Idiopathic giant cell myocarditis—natural history and treatment. *N Engl J Med* 1997; 336: 1860–1866
3. Lambeverie X de, Zandotti C, Vigneli C, Bollet C, de Micro PA. One-step microbial DNA extraction method using "Chelex-100" suitable for gene amplification. *Res Microbiol* 1992; 143: 785–790
4. Verma A, Rattan A, Tyagi JS. Development of a 23 S rRNA-based PCR assay for detection of *Mycobacteria*. *Indian J Biochem Biophys* 1994; 31: 288–294
5. Singh KK, Navi MD, Radha Krishnan K, Tyagi JS. Utility of PCR assay in the diagnosis of en-plaque tuberculoma of the brain. *J Clin Microbiol* 1997; 37: 467–470
6. Laruelle C, Vanhaecke J, Van de Werf F, Flameng W, Verbaken E, Meyns B, et al. Cardiac transplantation in giant cell myocarditis. A case report. *Acta Cardiol* 1994; 49: 279–286
7. Tazelaar HD. Spectrum and diagnosis of myocardial rejection. *Cardiol Clinics* 1990; 8: 119–139
8. Munoz RN, Pulpon LA, Yebra M, Segovia J, San Martin M, Salas C. Three cases of tuberculosis after heart transplantation in Spain. *Eur J Clin Microbiol Infect Dis* 1998; 17: 801–806
9. Dipetri G, Luzzati R, Forni A, Allegranzi B, Cazzadori A, Bonora S, et al. Fatal primary multidrug resistant tuberculosis in a heart transplant recipient. *Transpl Int* 1998; 11: 305–307
10. Le Mense GP, Van Bakel AB, Crumbley AJ III, Jodson MN. *Mycobacterium scrofulaceum* infection presenting as lung nodules in a heart transplant recipient. *Chest* 1994; 106: 1918–1920
11. Munoz RM, Alonso-Pulpon L, Yebra M, Segovia J, Gallego JC, Daza RM. Intestinal involvement by nontuberculous mycobacteria after heart transplantation. *Clin Infect Dis* 2000; 30: 603–605

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