American Journal of Transplantation 2013; 13: 802–807
Wiley Periodicals Inc.

© Copyright 2013 The American Society of Transplantation and the American Society of Transplant Surgeons

doi: 10.1111/ajt.12063

Case Report

HLA-DRB1 Typing by Micro-Bead Array Assay Identifies the Origin of Early Lymphoproliferative Disorder in a Heart Transplant Recipient

M. Fedrigo[a], F. Poli[b], G. Esposito[c], G. Feltrin[a], G. Toscano[a], C. d'Agostino[a], B. Schiavon[a], G. Gerosa[a], A. Amadori[c,d], M. Valente[a], G. Thiene[a] and A. Angelini[a,*]

[a] *Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Italy*
[b] *Department of Regenerative Medicine Transplant Immunology, IRCCS Ca' Granda Policlinic Hospital, Milan, Italy*
[c] *Istituto Oncologico Veneto, IOV-IRCCS, Padua, Italy*
[d] *Department of Oncology and Surgical Sciences, University of Padua, Italy*
**Corresponding author: Prof. Annalisa Angelini, annalisa.angelini@unipd.it*

We report the case of a 68-year-old woman who underwent heart transplantation for hypertrophic cardiomyopathy. Two months after the transplant she developed mild fever and dyspnea with a marked drop in left ventricle ejection fraction of 31%. Coronary angiography was negative for cardiac allograft vasculopathy. Endomyocardial biopsy revealed ischemic damage with no evidence of acute cellular rejection, antibody-mediated rejection or viral myocarditis. A neoplastic process was suspected even though full-body computerized tomography was negative for malignancy. The patient died 4 months after transplantation. The autopsy showed acute antero-septal myocardial infarction due to a nodular epicardial EBV-related posttransplant lymphoproliferative disorder (PTLD) infiltrating the left anterior descending coronary artery with occlusive neoplastic thrombosis. We highlight two major aspects of this case: (1) the unusual occurrence of early PTLD involving the cardiac allograft and causing a fatal outcome, (2) the application of an immunological technique for HLA-DRB1 typing to posttransplant paraffin-embedded autopsy material to identify the recipient origin of this early malignancy, thus excluding a possible donor-transmitted neoplasm.

Key words: Heart transplant, HLA-typing, PTLD

Abbreviations: EBV, Epstain-Barr virus; PTLD, Post-Transplant Lymphoproliferative Disease; HLA-DBR1, Human Leucocyte Antigen-DRB1 alleles; IVIG, Intravenus Immunoglobulin; CVVH, Continuous veno-venous hemofiltration; ECG, Electrocardiogram; EF, Ejection Fraction; EMB, Endomyocardial Biopsy; CT, Computerized Tomography; HTx, Heart Transplant; PCR-SSP, Polymerase Chain Reaction-Sequence Specific Primers; PCR-SSO, Polymerase Chain Reaction-sequence-specific oligonucleotides; STR, Sequence Tandem Repeated; MRI, Magnetic Resonance Imaging.

Received 10 August 2012, revised 01 November 2012 and accepted for publication 19 November 2012

Introduction

Posttransplant lymphoproliferative disorder (PTLD) is a lymphoid proliferation that spans the spectrum of appearances from Epstein–Barr virus (EBV) infection to high-grade malignant lymphoma. It is a major cause of morbidity and mortality following solid organ transplantation, with an overall incidence of approximately 2–6% in cardiac transplant recipients (1,2) and is more frequent in children (2,3). It usually occurs late after transplantation when it involves mainly lymph nodes. However extranodal disease has been reported in early-onset PTLD (within 6 months of transplantation) (4). PTLD involvement of the allograft is rare (5,6). Its onset early after transplantation raises the question of its origin. We report the case of a patient who died 4 months after transplantation with localization of PTLD to the cardiac allograft and to which we applied HLA-DRB1 typing by micro-bead array assay to identify its origin.

Case Report

A 68-year-old woman underwent heart transplantation (HTX) for hypertrophic cardiomyopathy. Two months later she presented with bilateral hydrothorax and a pericardial effusion with marked deterioration of left ventricular (LV) function. The patient had received induction therapy (7). The electrocardiogram (ECG) showed tachycardia and right bundle branch block. Blood tests confirmed congestive heart failure with an increase in hepatic enzymes and gamma-GT. Serologic antibodies and viral genomic DNA tests for HSV1 and 2, adenovirus, echovirus, coxackievirus, CMV and influenza A were negative. Peripheral blood EBV-DNA copies were <300 copies/mL. Echocardiography showed a decrease in global function of LV with an ejection fraction (EF) of 31% and functional mitral and

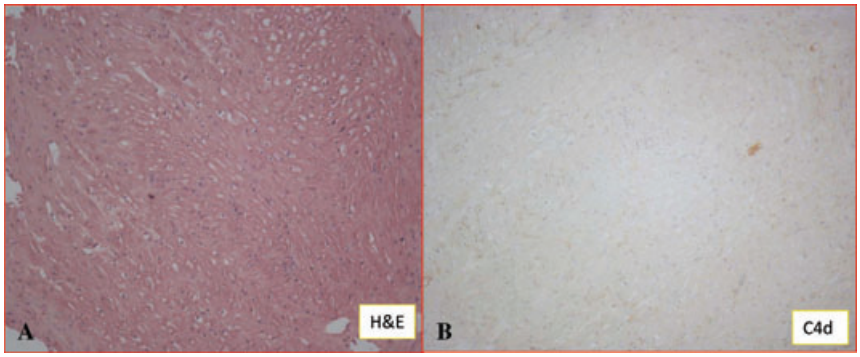

Figure 1: Last EMB: (A) Sign of ischemic damage with diffuse intracytoplasmatic vacuolization without interstitial cellular infiltration (10× magnification). (B) C4d staining showed no capillary complement deposition (10× magnification).

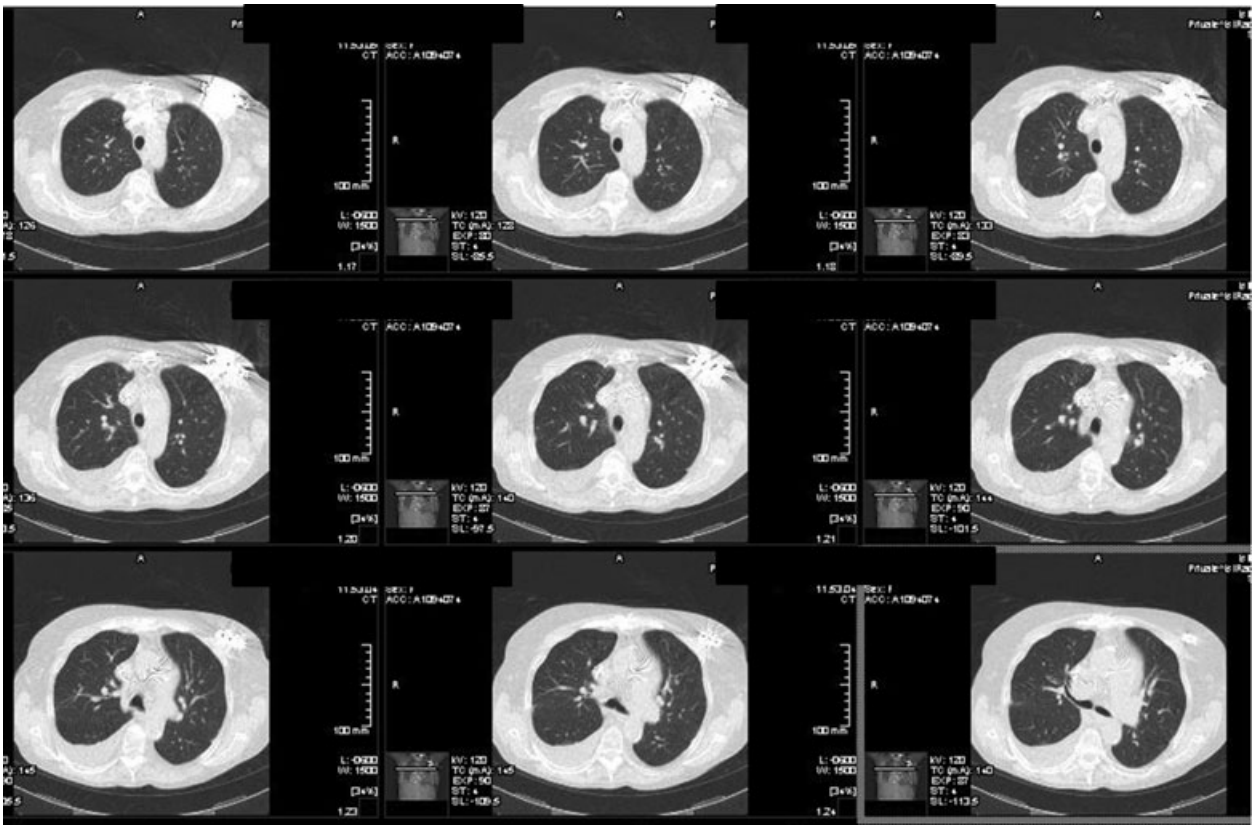

Figure 2: Chest computerized tomography (CT): negative for malignancy.

tricuspidal regurgitation. Chest X-ray showed bilateral pleural effusions and a few slightly enlarged mediastinal lymph nodes (maximum diameter 15 mm). Endomyocardial biopsy (EMB) showed ischemic damage but no signs of acute cellular or humoral rejection or viral myocarditis (Figure 1). Coronary angiography was negative for cardiac allograft vasculopathy. A neoplastic process was suspected, however a total body computerized tomography (CT) scan was negative (Figure 2). Despite the negative biopsy the patient was treated with triple therapy, IVIG and a bolus of corticosteroids. Over the next month, she became hemodynamically unstable, dyspnoeic and oliguric and was transferred to the intensive care unit, mechanically ventilated and hemofiltered using continuous veno-venous hemofiltration (CVVH). ECG showed tachycardia and reduced voltage but no signs of acute myocardial infarction (Figure 3). Troponin I was slightly increased at 0.35 ng/mL (normal <= 0.15 ng/mL) and 4600 copies/mL of EBV-DNA were detected in peripheral blood. Four days later she developed progressive and intractable bradycardia resulting in cerebral anoxia with death 4 months after HTx. The autopsy showed an acute antero-septal myocardial infarction, with a nodular epicardial mass 3 cm in diameter, encircling and infiltrating the left anterior descending coronary artery with neoplastic thrombotic occlusion (Figure 4 and supplementary video).

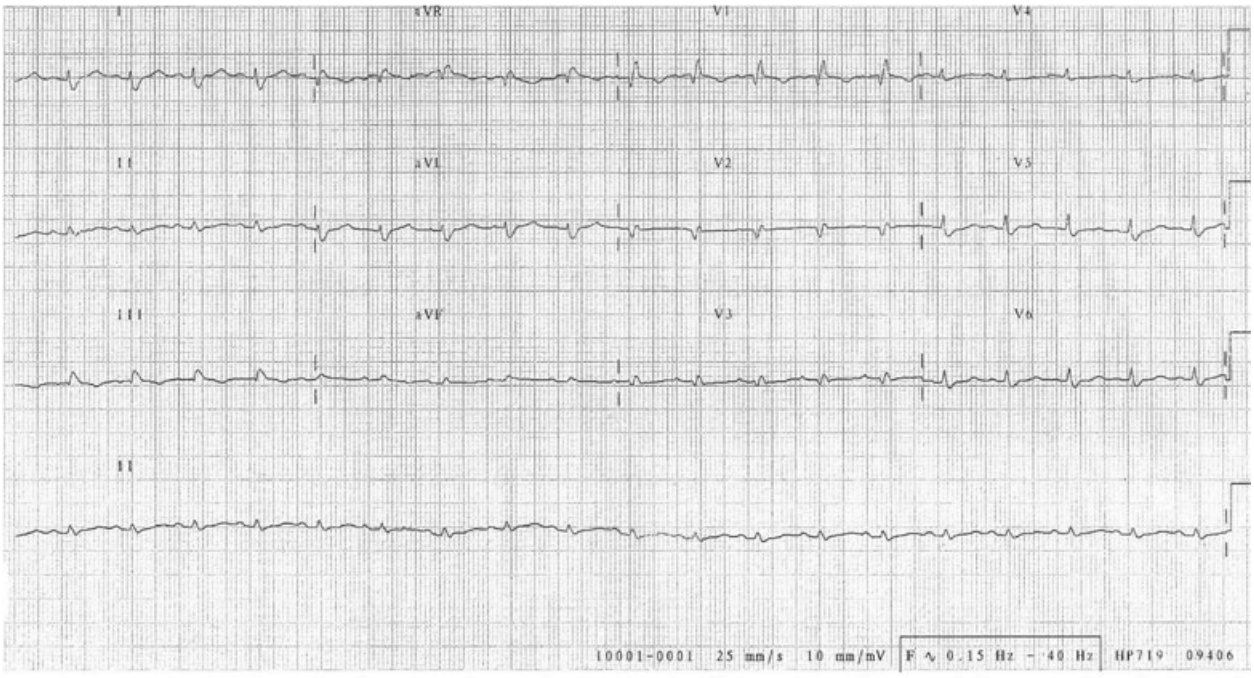

Figure 3: Electrocardiogram: the last ECG with no signs of infarction.

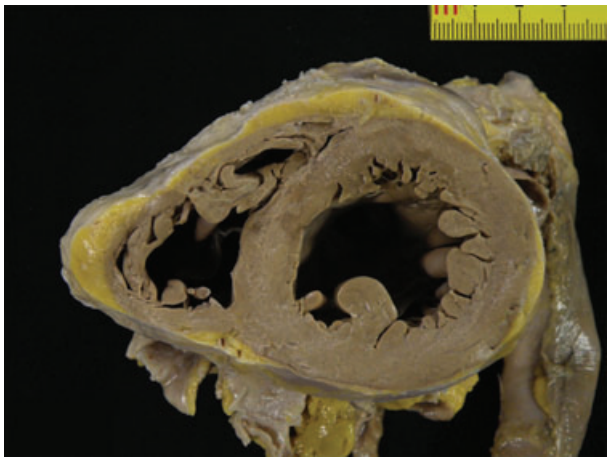

Figure 4: Heart. Autopsy findings of antero-septal acute myocardial infarction due to infiltration by neoplasm of proximal left anterior descending coronary artery.

No tumor was seen in the remainder of the autopsy. Histopathological examination and immunophenotyping of the tumor gave a diagnosis of EBV-related PTLD with a high-grade malignant lymphoma phenotype (Figure 5A–I).

As symptoms developed early after HTX, and as the tumor was confined to the donor allograft, we favored origin in the graft but could not exclude origin in the recipient. To resolve this issue we used laser microdissection to isolate single neoplastic cells from histological sections of the neoplasm using Laser Microdissection System Leica LMD 6000 (Leica Microsystems, Wetzlar, Germany) (Figure 1E). Single neoplastic cells were selected by a pulsed UV laser beam cutting the plastic film along the line drawn on the borders of each cell selected. A total of 500 neoplastic cells were collected. In our institution donor and recipient HLA-DRB1 typing analyses are usually performed before transplantation using PCR-SSP and SSO typing respectively on blood samples (8–10). In our case donor and recipient HLA-DRB1 were identical at low resolution, both showing HLA-DRB1*07, *11. In an attempt to identify whether DRB1 allelic typing was informative, high resolution DRB1 typing of donor and recipient was carried out retrospectively on stored blood using a microarray bead-based technique (Lambda Array Beads Multi-Analyte System LABMAS, Canoga Park, CA, USA; Catalog RSSOH2B1#006-2) 6 (11). The two samples differed at allelic level, the assigned alleles being DRB1*07:01, 11:04 for the donor and DRB1*07:01, 11:01 for the recipient. HLA-DRB1*11 typing results were confirmed by PCR-SSP (Olerup SSP AB, SItsjobaden, Sweden, lot#36K) confirming that DRB1*11: 11:04 was indicative of donor origin. HLA-DRB1 allelic typing, carried out on the DNA obtained from the neoplastic cells isolated by laser microdissection, was DRB1*11:01, thus confirming origin in the recipient and excluding origin from the donor.

Our final diagnosis was early EBV-related PTLD, with extranodal localization to the donor graft but of recipient

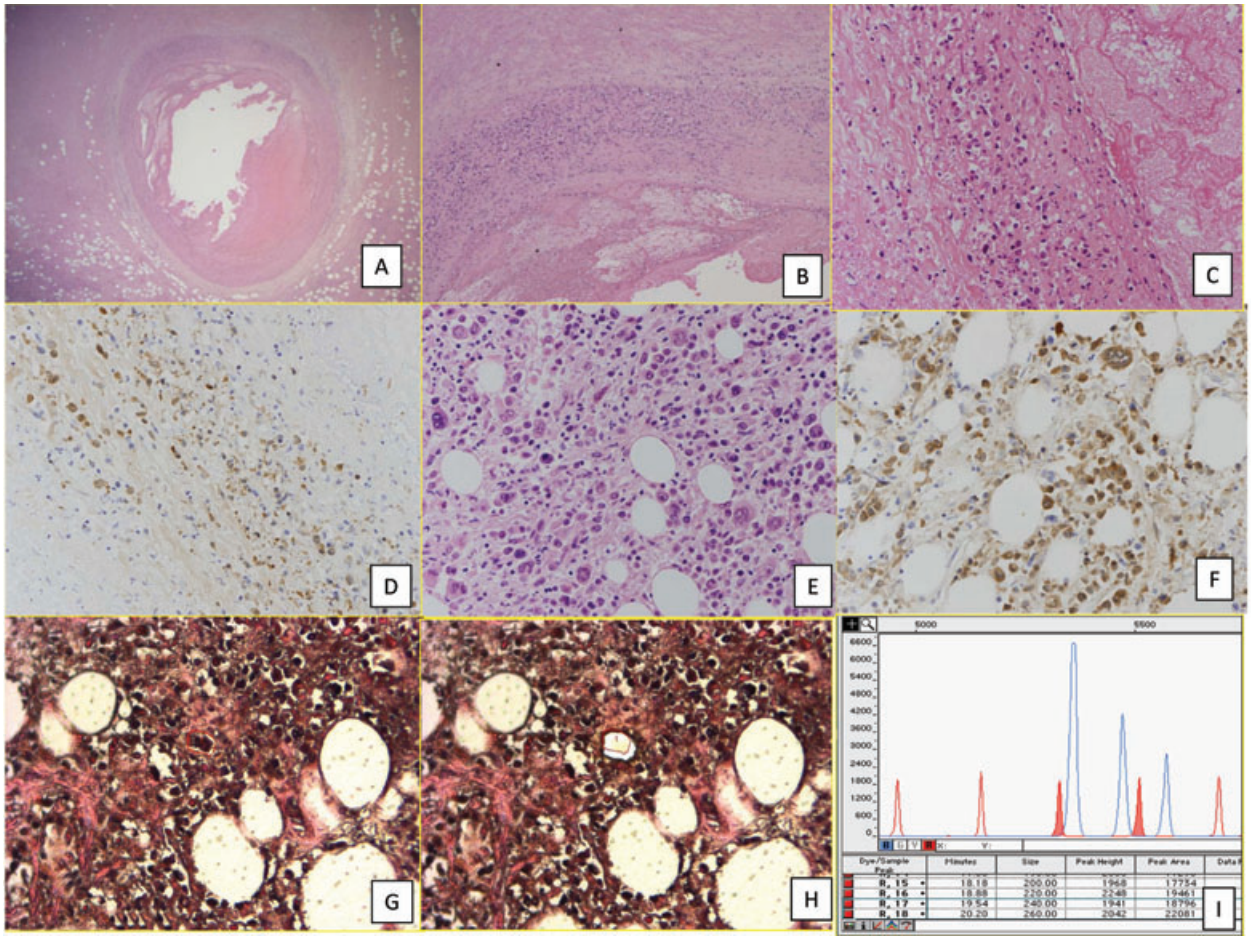

Figure 5: Epicardial mass showing polymorphic, anaplastic tumor cells infiltrating coronary artery from adventitia to intima with occlusive neoplastic thrombosis (A–C at progressively higher magnification). Immunophenotypic characterization of neoplastic cells showing a B cell subtype (CD20 positive) (D). *In situ* hybridization for EBV was strongly positive (E (H&E) and F). Laser micro-dissected tumor cells (G and H). Molecular cloning using nested PCR showed monoclonality for B cells and polyclonality for T cells (I).

origin, causing death though occlusion of the left anterior descending coronary artery (Figure 5).

Discussion

As far as we know, there is only one other case reported in the literature with a fatal outcome due to PTLD confined to the cardiac allograft and manifesting as an acute myocardial infarct. This was due to extensive thrombosis of the distal left anterior descending coronary artery (14). However that case was a late manifestation of PTLD, 14 months after transplantation, and was characterized by diffuse lymphocytic arteritis. In contrast our case presented as early-onset disease with focal involvement of the proximal left anterior descending coronary artery with an otherwise normal epicardial and intramyocardial coronary artery tree.

Two important aspects of this case are highlighted: (1) the early onset of EBV-related PTLD (15) confined to the cardiac allograft and triggering the fatal outcome; and (2) novel use of a genotyping method for HLA-DRB1 on paraffin-embedded tissue to identify its origin (16,17) despite suboptimal DNA quality in tumor tissue obtained from the autopsy. This was of crucial importance as the allograft was from a multiorgan donor. Had the neoplasm been of donor origin, it would have led to five other recipients undergoing neoplastic screening and a clinical risk assessment procedure.

STR analysis is the technique most frequently used to detect the origin of blood and tissue tumor cells and was our first choice. However, in our case, this technique failed to give a positive result due to the poor quality of the autopsy tissue samples available to us. Novel application of HLA-DRB1 typing to laser-dissected tumor cells resolved

the dilemma of donor or recipient origin. DNA typing of HLA class II alleles of the DRB1/3/4 and DQB1 loci using sequence-specific oligonucleotide probes and polymerase chain reaction-amplified DNA are used in the large-scale donor typing in Transplant Units (10). We recommended adapting this genotyping technique to clinical dilemmas such as ours after transplantation.

An *in vivo* diagnosis was not reached in our patient because the total body CT scan failed to detect the epicardial mass. With hindsight its localization and dimensions might have been identified though cardiac MRI with its high diagnostic accuracy for tissue characterization (18–20). Had a correct *in vivo* diagnosis been made a different therapeutic strategy could have been tried. Knowledge of involvement of the allograft early by PTLD would have prompted more detailed investigation, as would the presence of ongoing ischemic damage on surveillance EMBs.

In early PTLD, identification of donor or recipient origin presents a challenge in other solid-organ transplants. PTLD of donor origin in the transplanted lung is reported to be more common than previously thought, presenting in two out of six patients with early onset PTLD and identified using HLA-typing on optimally preserved fresh tissue from allograft biopsies containing tumor tissue (21).

In conclusion early onset PTLD requires tissue characterization to identify donor or recipient origin of the neoplastic cells. We have also shown that HLA genotyping can be done successfully on autopsy material and on formalin-fixed paraffin-embedded tissue using micro-dissection techniques.

Acknowledgments

The authors wish to thank Drs. Elisabetta Baliello and Marco Pizzigolotto for their assistance with micro-dissection and DNA extraction from paraffin-embedded tumor samples. This paper has been supported by CPDA108809 University of Padua Reseach Grant, Padua, Italy.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation.*

References

1. Swinnen LJ, Mullen GM, Carr TJ, Costanzo MR, Fisher RI. Aggressive treatment for postcardiac transplant lymphoproliferation. Blood 1995; 86: 3333–3340.
2. Crespo-Leiro MG, Alonso-Pulpon L, Vazquez de Prada JA, et al. Malignancy after heart transplantation: Incidence, prognosis and risk factors. Am J Transplant 2008; 8: 1031–1039.
3. Webber SA, Naftel DC, Fricker FJ, et al. Lymphoproliferative disorders after paediatric heart transplantation: A multi-institutional study. Lancet 2006; 367: 233–239.
4. Opelz G, Dohler B. Lymphomas after solid organ transplantation: A collaborative transplant study report. Am J Transplant 2004; 4: 222–230.
5. Ghobrial IM, Habermann TM, Maurer MJ, et al. Prognostic analysis for survival in adult solid organ transplant recipients with post-transplantation lymphoproliferative disorders. J Clin Oncol 2005; 23: 7574–7582.
6. Ghobrial IM, Habermann TM, Macon WR, et al. Differences between early and late posttransplant lymphoproliferative disorders in solid organ transplant patients: Are they two different diseases? Transplantation 2005; 79: 244–247.
7. Crespo-Leiro MG, Alonso-Pulpon L, Arizon JM, et al. Influence of induction therapy, immunosuppressive regimen and anti-viral prophylaxis on development of lymphomas after heart transplantation: Data from the Spanish Post-Heart Transplant Tumour Registry. J Heart Lung Transplant 2007; 26: 1105–1109.
8. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 1992; 39: 225–235.
9. Zetterquist H, Bengtsson M, Backstrom G, et al. Report from the HLA class II typing by PCR-SSP Multicentre Study. Eur J Immunogenet 1997; 24: 191–199.
10. Ng J, Hurley CK, Baxter-Lowe LA, et al. Large-scale oligonucleotide typing for HLA-DRB1/3/4 and HLA-DQB1 is highly accurate, specific, and reliable. Tissue Antigens 1993; 42: 473–479.
11. Dunbar SA. Applications of Luminex xMAP technology for rapid, high-throughput multiplexed nucleic acid detection. Clin Chim Acta 2006; 363: 71–82.
12. Colinas RJ, Bellisario R, Pass KA. Multiplexed genotyping of beta-globin variants from PCR-amplified newborn blood spot DNA by hybridization with allele-specific oligodeoxynucleotides coupled to an array of fluorescent microspheres. Clin Chem 2000; 46: 996–998.
13. Pindolia K, Janakiraman N, Kasten-Sportes C, et al. Enhanced assessment of allogeneic bone marrow transplant engraftment using automated fluorescent-based typing. Bone Marrow Transplant 1999; 24: 1235–1241.
14. Murray DL, Pereira NL, Miller DV. An unusual presentation of post-transplant lymphoproliferative disorder mimicking vasculitis in heart transplantation. J Heart Lung Transplant 2008; 27: 1257–1261.
15. Bakker NA, van Imhoff GW, Verschuuren EA, et al. Early onset post-transplant lymphoproliferative disease is associated with allograft localization. Clin Transplant 2005; 19: 327–334.
16. Penn I. Transmission of cancer from organ donors. Ann Transplant 1997; 2: 7–12.
17. Penn I. Occurrence of cancers in immunosuppressed organ transplant recipients. Clin Transplant 1998; 12: 147–158.
18. Baumwol J, Delgado DH, Cusimano RJ, et al. Atrial masses post cardiac transplantation: Diagnostic and treatment dilemmas. Am J Transplant 2012; 12: 2237–2241.
19. Bruce CJ. Cardiac tumours: Diagnosis and management. Heart 2011; 97: 151–160.
20. Hoey ET, Mankad K, Puppala S, Gopalan D, Sivananthan MU. MRI and CT appearances of cardiac tumours in adults. Clin Radiol 2009; 64: 1214–1230.
21. Verschuuren EA, Popa ER, Van Der Bij W, Harmsen MC, The TH, Hepkema B. Donor or recipient origin of post transplant

lymphoproliferative disease after lung transplantation. J Heart Lung Transplant 2001; 20: 199.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Supplemental video: In this video, we show the epicardial mass encircling the proximal segment of left anterior descending coronary artery. The transverse cut shows the white 3 cm in diameter nodular tumor infiltrating epicardial fat, with acute thrombotic occlusion of the coronary artery at the bottom of the cut.