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CASE REPORT

Endothelial cell antibody-mediated rejection and successful retransplantation in a heart transplanted patient

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Abstract

Antibody-mediated rejection (AMR) plays a significant role in cardiac allograft dysfunction, and recently a consensus regarding the diagnosis of AMR has been published. To our knowledge, it has not previously been reported that acute graft failure related to AMR, and antiendothelial cell antibodies can successfully be diagnosed to allow the patient to receive the outlined treatment and undergo a subsequent retransplantation.

Keywords: Cardiac transplantation • Retransplantation • Antibody mediated rejection • Antiendothelial cell antibodies • Non-HLA antibodies

INTRODUCTION

Antibody-mediated rejection (AMR) is rare compared with acute cellular rejection (ACR) in heart transplanted (HTx) patients, and the incidences of AMR and ACR are reported to be approximately 10% and 40–60%, respectively, within the first year post-heart transplant [1, 2]. Several cases of hyperacute and acute AMR caused by anti-human leucocyte antigen (HLA) antibodies have been described [3]. Endothelial cells of the donor organs are targets for the host's immune system during allograft rejection, and it has been claimed that antiendothelial cell antibodies (AECA) are clinically important in HTx [4]. We report a successful outcome after HTx and early cardiac retransplantation in a patient who suffered graft loss due to AECA-mediated AMR.

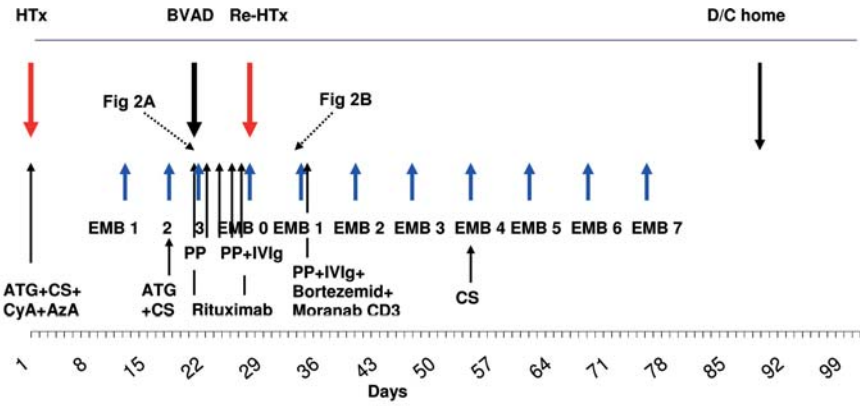
CASE REPORT

A 42-year-old male patient with ankylosing spondylitis and ulcerative colitis in remission was diagnosed with giant cell myocarditis (GCM). According to endomyocardial biopsies (EMBs), the GCM regressed on cyclosporine (CyA), azathioprine and corticosteroids. He was stable for 4 years, but deteriorated thereafter with advanced heart failure.

The pretransplant work up included HLA typing (A, B and DRβ1 loci) of the patient and the donor, screening for anti-HLA antibodies (LABScreen® Mixed, One Lamda Inc., CA, USA) and flow cytometric crossmatch tests. The patient had no HLA antibodies and the crossmatch test was negative with regard to donor T- and B-cells.

The patient underwent HTx and received induction therapy with antihuman thymocyte immunoglobulin (ATG; total dose 3.5 mg/kg over 2 days) and methylprednisolone (MP; total dose 1375 mg over 2 days). CyA (3 mg/kg/day) and azathioprine (150 mg/day) were started on the first postoperative day. CyA through the level (C0) was 200 ng/ml within the first week and 250 ng/ml at the second week. Prednisolon (0.2 mg/kg/day) was started on the third day. Azathioprine and CyA were continued as the colitis was well managed with this regime. The total CD3+ cell count was 0.08×10^9/l 3 days postoperatively. There were three HLA class I and one HLA class II allele mismatches between the donor and the recipient. Major case events are shown in Figure 1.

The first EMB, 2 weeks after HTx, showed mild rejection [International Society of Heart and Lung Transplantation grade 1 cellular rejection (ISHLT 1R)] and negative C4d. The patient was asymptomatic, and all parameters were normal except that cytomegalovirus (CMV), measured with the quantitative method (PCR), was elevated by 3.24 $\log_{10}$ (1700 Geq/ml). Valganciclovir was initiated and CMV PCR levels subsequently decreased. The second EMB (3 weeks postoperatively) showed severe macrophage infiltration (CD68+), diffuse eosinophilic and lymphocytic infiltration and a negative C4d. He had fever, no clinical infection, echocardiography showed mild right ventricular dysfunction and CyA through the level was 350 ng/ml. Rejection therapy with ATG (total dose 5 mg/kg) and MP (500 mg over 3 days) was started, and the immunosuppressive therapy was intensified. The patient stabilized initially, but developed cardiogenic shock 2 days later. Extracorporeal membrane oxygen was initiated and converted the next day to biventricular assist device (BVAD). EMB showed myocyte necrosis, severe antibody and cellular-mediated rejection (ISHLT 3R, AMR), strongly positive for C4d (Fig. 2A). However,



CDC T/B cells	–/–		Na	–/–	Na	Na	–/(+)	Na	Na	Na	Na	Na	Na	Na	
Donor ECXM (IgM / IgG)	–/–		+/–	+/–	+/–	–/–*	–/–	–/–	–/–	Na	Na	Na	Na	Na	
Auto ECXM (IgM / IgG)	–/–		–/–	+/+	Na	–/–	–/–	–/–	–/–	–/–	–/–	Na	Na	–/–	
ISHLT	Na		1R	3R	3R	Na	0R	1R	1R	0R	2R	1R	1R	0R	
CD68	Na		+	+	+	Na	–	+	+	+	+	Na	+	–	
C4d	Na		–	–	+	Na	–	–	–	–	–	Na	–	–	

Figure 1: Timeline of major events between cardiac transplantation and discharge to home. HTx: heart transplantation (red arrows); EMB: endomyocardial biopsy (blue arrows); BVAD: biventricular assist device; PP: plasmapheresis; ATG: antihuman thymocyte immunoglobulin; CS: corticosteroids; CyA: cyclosporine; AzA: azathioprine; IVIg: intravenous immunoglobulin; Na: not available; *: Donor 2.

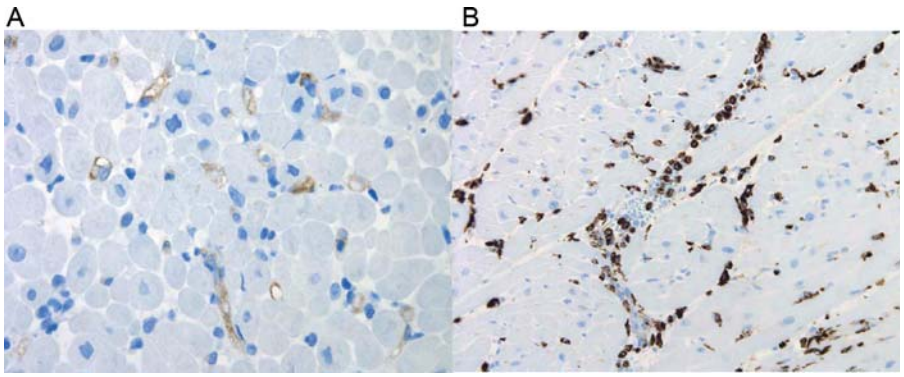


Figure 2: (**A**) A full thickness myocardial biopsy was taken introperatively at the time of conversion from extra corporeal membrane oxygenation to BVAD, which illustrates antibody-mediated rejection with positive immune staining for capillary C4d deposition. (**B**) Endomyocardial biopsy taken 1 week after retransplantation with signs of macrophage activity (CD68+ staining).

the cytotoxic T- and B-lymphocyte crossmatch tests were negative. The AMR protocol was initiated with 5 plasmapheresis (PP, plasma exchange with albumin) over 7 days with intravenous immunoglobulin (IVIg, 1 g/kg/day) given after each PP and one dose of rituximab (700 mg).

An endothelial precursor cells crossmatch test (EPC-XM; XM-ONE®, AbSorber AB, Stockholm, Sweden) was performed. The test measured the amount of AECA in the patient serum, either against donor-specific EPCs or autoreactive EPCs (the patient's own EPCs) [5, 6]. We retrospectively investigated the presence of donor-specific and autoreactive AECAs in the serum from our biobank, which was taken at the same time as the protocol EMBs (Fig. 1).

In the pretransplant serum, both donor-specific and autoreactive AECAs were negative. In the first week post-transplant, the donor-specific AECA was positive during a low-grade ACR (ISHLT 1R), but autoreactive AECA was negative. At the time of acute graft failure, the test was positive for both donor-specific and autoreactive IgM AECA, but not IgG AECA. Hence, with severe graft failure, negative lymphocyte but positive EPC-XM, profound macrophage invasion and C4d capillary deposits in the graft, our assumption was a severe AMR caused by non-HLA AECAs.

The AECAs were measured regularly until negative after 5 days on BVAD. The patient was then accepted for urgent retransplantation, and a donor organ was available within 1 day. The lymphocytic crossmatch test against the second donor was negative with regard to the T-cells (complement dependant cytotoxicity test) and the flow cytometric cross match test. Prior to retransplantation, the patient received an additional round of PP, IVIg (1 mg/kg) and rituximab (700 mg). In conjunction with cardiac transplantation, two doses of ATG (2 mg/kg) and MP (1375 mg over 3 days) were given, followed by tacrolimus (0.075 mg/kg/day, C-0 level 7 and 14 ng/ml between the 4th and 10th day, respectively) and mycophenolate mofetyl (MPA, 2 g/day, area under curve 73 mg/l measured at C-0, C-30

and C-120 min). Prednisolon was kept at 0.2 mg/kg/day. The tacrolimus and MPA concentrations were kept high (15–22 ng/ml and 70–80 mg/l, respectively) during the first weeks.

The next EMB (1 week after retransplantation) showed normal myocardium, minor infiltration of lymphocytes, increased amount of macrophages (CD68+), but C4d was negative (Fig. 2B). However, EPC-XM was negative against the EPCs of the second donor. Echocardiography showed normal cardiac function, and the patient was clinically stable. Considering the high risk of a new acute AMR, extended therapy was given with PP, IVIg (0.5 g/kg/day), muronomab-CD3 (5 mg/day) and MP (750 mg/day) for 3 days and he received one bolus of bortezomib (2.8 mg). The following EMB showed the regression of macrophage infiltration and C4d was negative. One month after retransplantation, the EMB showed ACR (ISHLT 2R), but no AMR. Seven weeks after retransplantation, the EMB showed normal myocardium, without macrophages (ISHLT 0R), no giant cells and negative C4d staining. Thirteen weeks after the first transplantation, the patient was discharged, and he is still doing well 30 months later.

DISCUSSION

We have demonstrated the presence of donor-specific IgM AECA early post-transplant and later during a cardiac allograft rejection and graft failure. The EBM showed a C4d deposition in the endothelial microvascular bed, but in the absence of HLA antibodies. The EPC-XM test allowed the diagnosis and monitoring of the AMR treatment, by measuring the AECAs in the patient serum during the non-HLA allograft AMR. The AMR was treated with rituximab and IVIg after each PP, before and after the retransplantation, and OKT3 and bortezomib added after the first retransplant EMB, to prevent progression of AMR in the second graft. We successfully desensitized the patient, making him eligible for a second transplant, after which no clear signs of AMR have reoccurred.

Interestingly, the donor-specific IgM AECA was positive at least 10 days prior to deterioration, but not the autoreactive AECA. It has been shown that the presence of non-donor-specific or so-called autoreactive antibodies correlates with lower graft survival and poor transplant function [7]. Our patient had autoimmune diseases, and the question was raised if a possible reactivation of an underlying autoimmune disease was triggering a post-transplant alloreactivity. However, autoantibodies that develop after transplantation have been shown, in most cases, to represent a *de novo* autoantibody development rather than a recurrence of preexisting autoimmune disease [8].

Our case indicates that the EPC-XM test should be performed in cases with a negative HLA test and a high suspicion of AMR, with or without acute graft failure, as a positive donor-specific AECA early post-transplant may precede a complete graft failure.

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REFERENCES

[1] Gonzalez-Stawinski GV, Cook DJ, Chui J, Gupta S, Navia JL, Hoercher K *et al*. A comparative analysis between survivors and nonsurvivors with antibody mediated cardiac allograft rejection. J Surg Res 2007;142:233–8.

[2] Fishbein MC, Kobashigawa J. Biopsy-negative cardiac transplant rejection: etiology, diagnosis, and therapy. Curr Opin Cardiol 2004;19:166–9.

[3] O'Boyle PJ, Smith JD, Danskine AJ, Lyster HS, Burke MM, Banner NR. De novo HLA sensitization and antibody mediated rejection following pregnancy in a heart transplant recipient. Am J Transplant 2010;10:180–3.

[4] Fredrich R, Toyoda M, Czer LS, Galfayan K, Galera O, Trento A *et al*. The clinical significance of antibodies to human vascular endothelial cells after cardiac transplantation. Transplantation 1999;67:385–91.

[5] Nowak G, Karrar A, Holmén C, Nava S, Uzunel M, Hultenby K *et al*. Expression of vascular endothelial growth factor receptor-2 or Tie-2 on peripheral blood cells defines functionally competent cell populations capable of reendothelialization. Circulation 2004;110:3699–707.

[6] Breimer ME, Rydberg L, Jackson AM, Lucas DP, Zachary AA, Melancon JK *et al*. Multicenter evaluation of a novel endothelial cell crossmatch test in kidney transplantation. Transplantation 2009;87:549–56.

[7] Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A *et al*. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol 2005;16:2804–12.

[8] Jurcevic S, Ainsworth ME, Pomerance A, Smith JD, Robinson DR, Dunn MJ *et al*. Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation. Transplantation 2001;71:886–92.