Antibody-mediated rejection with detection of de novo donor-specific anti-human leucocyte antigen Class II antibodies 3 years after heart transplantation: a case report

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Received 9 August 2019; first decision 16 September 2019; accepted 19 December 2019; online publish-ahead-of-print 25 January 2020

Background
Antibody-mediated rejection (AMR) in cardiac transplantation may manifest early within the first weeks after transplantation but also late after months to years following transplantation resulting in mild heart failure to cardiogenic shock. While patients with early cardiac AMR are less affected and seem to have survival rates comparable to transplant recipients without AMR, late cardiac AMR is frequently associated with graft dysfunction, fulminant forms of cardiac allograft vasculopathy, and a high mortality rate. Nevertheless, AMR of cardiac allografts remains difficult to diagnose and to treat.

Case summary
We report the case of a 47-year-old male patient with late AMR of the cardiac allograft 3 years after heart transplantation. Antibody-mediated rejection was confirmed by endomyocardial biopsy and the presence of donor-specific antibodies (DSA). The patient was treated with high dose of prednisolone, plasmapheresis, intravenous Gamma Globulin, rituximab, immunoabsorption, and bortezomib. Under this treatment regimen left ventricular ejection fraction and pro B-type natriuretic peptide recovered, and the patient improved to New York Heart Association Class I. Currently, 3 years after the diagnosis of cardiac AMR, graft function continues to be nearly normal, and there is no evidence for transplant vasculopathy.

Discussion
This case illustrates that AMR can occur at any time after transplantation. Although graft function fully recovered after treatment in our patient, the level of DSA remained high, suggesting that DSA may not be a reliable parameter to determine the intensity and duration of the therapy.

Keywords
Case report • Heart transplantation • Antibody-mediated rejection • Donor-specific antibodies

Learning points
• Antibody-mediated rejections after heart transplantation can occur at any time.
• Early diagnosis and intensive treatment are necessary (‘Hit early and hard’).
• Donor-specific antibodies may remain at high levels despite clinical improvement.
**Introduction**

Antibody-mediated rejection (AMR) is a rare condition, but serious in patients with organ transplantation. It is caused by the formation of antibodies against donor human leucocyte antigens (HLAs) on the endothelial layer of the cardiac allograft. Cardiac AMR may manifest early within the first weeks after heart transplantation, but also later after months to years following transplantation. Clinical presentation varies from mild heart failure to cardiogenic shock. Because the clinical symptoms are not specific, AMR of cardiac allograft is difficult to diagnose, but even more difficult to treat, as there are no Class 1 recommendations for standardized treatment.

**Timeline**

| Date Range            | Event Description                                                                                                                                                                                                 |
|-----------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| October 2014          | Patient with a history of severe ischaemic cardiomyopathy undergoes successful heart transplantation.                                                                                                             |
| October 2014 to December 2017 | Routine post-transplant surveillance examinations every 3–6 months show no signs of rejection with a stable left ventricular ejection fraction (LVEF) of 55%. Immunosuppressive regimen includes cyclosporine A and everolimus. |
| December 2017 to March 2018 | Extensive medical treatment includes steroids, extracorporeal procedures (plasmapheresis/immunoadsorption), intravenous Gamma Globulin, rituximab, and bortezomib (see also Figure 1). Left ventricular ejection fraction improves to 50% and proBNP level decreases to 1200 pg/mL approximately 1 month after treatment initiation. Donor-specific antibodies remain at high levels. Clinical improvement to NYHA Class I after 2–3 months. |
| May 2018 to July 2019 | Patient has no clinical symptoms (NYHA Class I), cardiac allograft function is nearly normal (LVEF 50%) and proBNP levels continuously decreases to 400–600 ng/mL, while DSA remain at a high level. |

**Case presentation**

A 47-year-old male patient was admitted to our clinic in December 2017 for routine heart catheter. He had a history of severe ischaemic cardiomyopathy since 2012 and had been supported by the left ventricular assist device (LVAD) Heart Mate II for 2 years. Due to recurrent driveline infections, he underwent heart transplantation with an HLA-compatible heart in October 2014. Since then, he has been regularly examined according to our routine post-transplant surveillance protocol every 3–6 months. The left ventricular ejection fraction (LVEF) remained stable at 55% since transplantation. His immunosuppressive regimen included cyclosporine A and everolimus, and he had no signs of rejections since transplantation. The current hospital admission was scheduled for evaluation of cardiac allograft vasculopathy.

On admission, the patient reported of new-onset of fatigue and dyspnoea during ordinary physical exertion [New York Heart Association (NYHA) Class II] a few weeks before. Physical examination at rest revealed a heart rate of 88 b.p.m. and blood pressure of 105/75 mmHg. Lung auscultation did not present rales, and there were no peripheral edema. However, echocardiographic assessment showed worsening of the LVEF to 30–35%. Serum pro B-type natriuretic peptide (proBNP) was increased up to 10 000 pg/mL (normal range < 125 pg/mL) in contrast to 250 pg/mL 6 months earlier. Cardiac allograft vasculopathy was excluded by coronary angiography. On suspicion of rejection, myocardial biopsy was performed and donor-specific antibodies (DSA) were evaluated.

In contrast to 3 months earlier, where no DSA were detected, serological testing was now positive for the HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 genes (Figure 1A), suggesting de novo DSA. Accordingly, endomyocardial biopsy showed histological and immunopathologic evidence for AMR of the cardiac allograft with a high number of CD68 macrophages and C4d positive deposits as well as MHC Class II molecules on capillary endothelial cells of the allograft. The biopsy did not show any evidence for acute cellular rejection (ISHLT classification: Grade 0R). Based on these findings, the diagnosis of late AMR of the cardiac allograft was made.

In order to prevent further deterioration of the allograft function, high-dose prednisolone was administered immediately after biopsy. Additionally, plasmapheresis was initiated for 5 days combined with intravenous Gamma Globulin (IV Ig). The prior maintenance immunosuppression regimen with cyclosporine A and everolimus was continued. However, LVEF estimated by echocardiography remained 30–35%. Therefore, rituximab (375 mg/m² weekly) was added for 4 weeks (Figure 1B). Unfortunately, DSA levels remained at a high level, and we replaced plasmapheresis by immunoadsorption, assuming that this procedure may be more effective in removing DSA. Additionally, we administered another round of IV Ig. Notably, under this strategy, and 35 days after initiating the treatment, LVEF improved to 50% and proBNP decreased to 1200 pg/mL (Figure 1C). However, there was no significant decline of DSA levels (Figure 1A). Therefore, we decided to add bortezomib. Over the next weeks, LVEF and proBNP remained stable, and the patient improved to NYHA Class I. However, even with additional immunoadsorption three times a week, DSA levels remained high. Following 8 rounds of...
plasmapheresis and 31 rounds of immunoadsorption, we stopped the extracorporeal procedures.

Currently, 3 years after the diagnosis of cardiac AMR, graft function continues to be nearly normal, while DSA levels remain at a constant elevated level. There is no evidence for transplant vasculopathy.

Discussion

Antibody-mediated rejection in cardiac transplantation is caused by the formation of antibodies against donor HLAs on the endothelial layer of the cardiac allograft. Antibodies induce activation and deposition of complement components, resulting in activation of the innate and adaptive immune system, inflammatory processes, and eventually allograft dysfunction. For yet unknown reasons, patients undergoing heart transplantation from LVAD (bridge-to-transplant), as was the case in our patient, are at increased risk to develop cardiac AMR.

Cardiac AMR may manifest early within the first weeks after transplantation but also late after months to years following transplantation resulting in mild heart failure to cardiogenic shock. While patients with early cardiac AMR are less affected and seem to have survival rates comparable to transplant recipients without AMR, late cardiac AMR is frequently associated with graft dysfunction, fulminant forms of cardiac allograft vasculopathy, and a high mortality rate. Nevertheless, AMR of cardiac allografts remains difficult to diagnose and to treat.

The general principles in the treatment of AMR consist of four pillars: removing circulating alloantibodies, reducing production of additional alloantibodies, suppressing T-cell and B-cell responses, and inhibiting of complement. However, Class 1 recommendations for standardized treatment protocol or treatment duration of cardiac AMR still do not exist.

In our case, we applied corticosteroid pulse regimen as the first approach. In order to remove circulating DSA as quickly as possible, plasmapheresis and subsequently immunoadsorption were initiated to mechanically remove circulating antibodies. Additionally, we added IVIg, although its effectiveness in the treatment of acute AMR has never been systematically studied. However, we assumed that it could inhibit the complement system, neutralize autoantibodies and cytokines and down-regulate B-cell receptors. The treatment was then escalated by rituximab to deplete B cells and to inhibit de novo production of donor-specific HLA antibodies. Multiple case reports have previously demonstrated the successful use of rituximab as a ‘salvage therapy’ for refractory AMR, and in a series of eight patients with AMR treated with rituximab monotherapy, left ventricular function recovered to normal in all patients. Although only limited experience existed with bortezomib in cardiac AMR, we decided to apply it to our patient, as DSA remained high despite the aggressive therapy. Interestingly, this did not affect the DSA level, which remained at a moderate elevated level after an initial fall. This phenomenon has been previously described as ‘accommodation’, which refers to an acquired resistance of an organ graft to humoral injury and rejection.

In summary, we presented an effective treatment protocol for late AMR with severe allograft dysfunction. Notably, despite our strategy to ‘hit hard and early’, DSA levels remained at a high level, suggesting that continuous monitoring of DSA may not be a suitable parameter.
to determine the intensity and duration of the therapy. Retrospectively, proBNP level and echocardiography together with clinical assessment seem to be the better parameters to evaluate treatment response.

**Lead author biography**

Bernd Ludwig attended medical school at the University of Freiburg, Germany and obtained his medical degree in 2013. He completed his clinical fellowship in internal medicine at the University Hospital Freiburg in 2019 and is currently a fellow in the Department of Cardiology at the Heart Center, University Freiburg.

**Supplementary material**

Supplementary material is available at European Heart Journal - Case Reports online.

**Acknowledgements**

We thank Dr Sebastian Grundmann and Dr Mohacsi for their advices and support.

**Funding**

The article processing charge was funded by the German Research Foundation (DFG) and the Albert Ludwigs University Freiburg in the funding programme Open Access Publishing.

**Slide sets**: A fully edited slide set detailing this case and suitable for local presentation is available online as Supplementary data.

**Consent**: The author/s confirm that written consent for submission and publication of this case report including image(s) and associated text has been obtained from the patient in line with COPE guidance.

**Conflict of interest**: none declared.

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