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MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

CASE REPORT

CASE REPORT: PRE-TRANSPLANT TESTING AND POST-TRANSPLANT MONITORING FOR AN HLA-IMMUNIZED RECIPIENT IN HEART TRANSPLANTATION

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Abstract: We report the workflow of immunogenetic pre-transplant testing and post-transplant monitoring in the case of a recipient immunized to human leucocyte antigens (HLA) who was waitlisted for heart transplantation. The recipient underwent heart transplantation across preformed HLA class I Donor Specific Antibodies (DSAs) detected by solid phase Luminex screening method but not by complement dependent cytotoxicity (CDC) screening method. The CDC lymphocyte crossmatch, which was performed retrospectively, was a weak positive. Post-transplant DSA monitoring by Luminex method revealed the decrease of HLA-A1, A25 and B57 DSAs with, at the same time, an increase of HLA-B8 DSA, as well as weak transient non-DSA HLA-DP antibodies. This case presents the importance of extensive immunogenetic testing and monitoring for identifying recipients with increased immunological risk for successful heart transplantation.

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INTRODUCTION

Heart transplantation is considered standard therapy for patients with end-stage heart failure when other medical treatments are not effective. While there has been much improvement in survival rates during the last five decades of heart transplantation, allograft rejection still remains a major cause of morbidity and mortality in the post-transplant period.[1] Rejection of a transplanted heart can be caused by the presence of donor specific antibodies (DSAs), recipient's antibodies against the donor's HLA antigens. They can be persistent in a recipient that was sensitized before transplantation through prior transplantation, transfusion or, in case of female recipients, pregnancy, or they can be formed after transplantation as de novo DSAs. Sensitized recipients have traditionally had high waitlist mortality due to unacceptable antigens that limit the available donor pool and increase wait time.[2] In case of kidney and bone marrow transplantation, the importance of HLA matching to avoid sensitization has been widely accepted; however, it is still disputed in heart transplantations. Currently, HLA matching is not widely accepted as a selection criterion for heart recipient choice for a few reasons. Firstly, there is not enough research with uniform results depicting clinical relevance of HLA matching in heart transplanting. Secondly, potential heart recipients usually have bad short-term prognosis, and waiting for a heart transplant with a high degree of HLA histocompatibility could be fatal for them. Furthermore, HLA matching of a potential heart donor with a recipient is limited by the small pool of heart donors, organ maintenance and preservation techniques and the short time before graft ischemia.[3]
Croatia is one of the eight member states of Eurotransplant, the biggest European international organization for organ exchange, participating in the

heart transplantation program with two transplant centres for which our centre is a full-service histocompatibility laboratory. Heart allocation within Eurotransplant is based on waiting time and urgency. Histocompatibility in terms of HLA matching is not considered, HLA typing and antibody screening are not mandatory for active status of the recipient on the waiting list. Thus, a local clinical protocol is established taking histocompatibility testing into account. Our centre policy includes mandatory HLA-A, B, DR molecular typing, CDC screening and the Luminex HLA antibody detection screening test at the time of the recipient registration on the waiting list. In case of an immunized (sensitized) recipients, Luminex screening is expanded with single antigen beads (SAB) testing in order to precisely define HLA class I and class II antibody specificities present in the recipient's serum. Based on overall results, donor HLA antigens that should be avoided in a case of an organ offer are reported as a recommendation to the transplant clinicians.

Measure of sensitization is expressed through percentage of Panel Reactive Antibodies (%PRA) and is calculated either based on the percentage of positive reactions in a panel of 50-100 individuals in Complement Dependent Cytotoxicity (CDC) test or calculated (cPRA; virtual PRA) by taking in the count frequency of the recipient's unacceptable HLA alleles in the Eurotransplant donor population. The higher the PRA, the higher the probability of a recipient reacting immunologically against the donor population and giving a positive CDC lymphocyte crossmatch (CM), which can even in the case of heart recipient be considered as a contraindication for transplantation.[4] Wait time on the waitlist of sensitized heart transplant recipients can therefore be shortened by transplanting across positive DSAs and positive CM but at the cost of an increased risk of post-transplant antibody-mediated rejection (AMR; humoral rejection), which presents a major threat to graft survival after heart transplantation, especially during the first 1-2 months after transplantation. AMR is a process where recipient antibodies "attack" donor HLA antigens on the surface of the transplanted organ, which leads to the activation of the complement cascade and the activation of innate and adaptive immune responses. This results in an inflammatory process that manifests itself as allograft dysfunction and possibly allograft rejection. For that reason, it is highly important to monitor levels of DSAs in sensitized recipients before and after transplantation in order to predict possible graft rejection events and to adapt immunosuppression therapy.[5, 6]

Thresholds of Mean Fluorescence Intensity (MFI) in Luminex SAB testing of approximately 5000 for DSA against class I antibodies, 2000 against class II antibodies or an overall cut-off of 5000-6000 for any DSAs are taken to be predictive values for AMR.[7]

Here, we report the results of immunogenetic pre-transplant testing and post-transplant monitoring of a sensitized heart transplant recipient that was transplanted across Luminex detected DSAs and a weak positive CM.

CASE REPORT

A 67-year-old recipient with B positive blood type was registered in April 2017 on the Eurotransplant elective waiting list for heart transplantation. Prior to wait-listing, immunogenetic testing was done according to standard protocol which includes molecular HLA typing and HLA antibody determination and identification by CDC and Luminex SAB. HLA-A, B, C, DR, DQ typing was performed by polymerase chain reaction - sequence specific primers (PCR-SSP) method (CareDx, Olerup SSP AB, Sweden). CDC antibody screening both before and after dithiothreitol (DTT) treatment was performed using a local panel of 50 HLA-A, B, C, DR typed donors. Commercial Immucor's LIFECODES LSA class I and class II Single Antigens tests (SA1 and SA2; Immucor Transplant Diagnostics Inc., Stamford, Connecticut, USA) were used for screening by Luminex method.

Patient's HLA typing was: HLA-A*11, *68; B*35, -; C*04, -; DRB1*08, *13; DQB1*03, *06. CDC screening was positive, the percentage of PRA was 24 without DTT and 20% with DTT, pointing to the presence of IgG subclass of cytotoxic complement binding HLA antibodies of HLA-B7, B60 and B61 specificity. The Luminex SA1 and SA2 test results showed the presence of HLA class I antibodies with MFI in the range 1000-16000. HLA-B7 antibody specificity had the highest MFI value, confirming the CDC antibody identification result. Complete HLA class I antibody Luminex profile pointed to the presence of antibodies specific for 163EW+73TE, 113HN, 24T and 82LR epitopes. HLA class II antibodies were negative.

Recipient received a heart offer in September 2018 from a cadaveric donor, blood type B, with HLA typing HLA-A*01, *25; B*08, *57; C*06, *07; DRB1*11, *13; DQB1*03, *06.

Antibody profile was analyzed for mismatched donor antigens HLA-A1, A25, B8, B57, Cw6, Cw7 and DR11, revealing positive reactions in Luminex for HLA-A1, A25, B8 and B57 specificities, with MFI values in the range 1100 (HLA-A1) to 2500 (HLA-B57). The analysis of pre-transplant CDC screening results indicated that cytotoxic antibodies are not present for any of the donor's HLA-A and HLA-B MM antigens. Due to logistic conditions, CDC CM was carried out retrospectively, on post-transplant day 1. The recipient's pre-transplant serum was tested against T and B lymphocytes of the donor in a crossmatch reaction, following the same procedure as the one used for CDC screening. The result of CM was a "weak positive" meaning that cytotoxic antibodies present in the patient's serum were reacting to 10-20% of donor lymphocytes. Taking into consideration pre-transplant results of weak positive DSAs in Luminex screening and weak positive CDC CM, extensive protocoled post-transplant monitoring, as well as monitoring by indication, was introduced. CDC antibody monitoring showed an increase of %PRA shortly after the transplantation, with a result of PRA 62% at post-transplant day 30 and day 60, while afterwards steadily

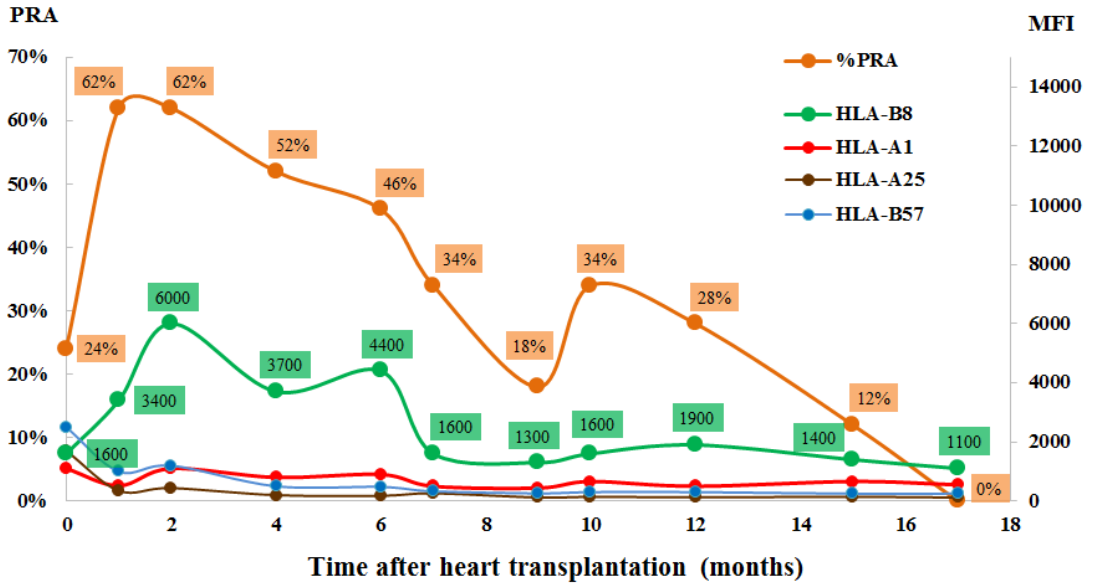

Figure 1. Results of CDC screening reported as %PRA throughout the post-transplant follow-up period and Luminex single antigen beads test results showing Mean Fluorescence Intensity (MFI) values for Donor Specific Antibodies in recipient serum after transplantation. Cut-off MFI value is 1000.

decreasing, with the latest result of PRA 0% 18 months after the transplantation (Figure 1). Luminex screening showed a MFI decrease for HLA-A1, A25 and B57 antibodies at 1 month after the transplantation, remaining either low positive or negative in all further testings up to 18 months post-transplant. In contrast, HLA-B8 antibody reactions in SAB assay were increasing in the early post-transplant period, reaching a peak value of MFI 6000 two months after the transplantation. MFI values at four and six months post-transplant were still high (3700 and 4400) but subsequently started to steadily decrease, but never reaching negative result (Figure 1, Figure 2). Class II antibodies that were negative pre-transplant, turned to be weak positive for DP18 antibodies at four months post-transplant. HLA-DP typing of both the recipient and the donor was urgently performed, the antibodies turned out not to be DSAs, as the recipient and the donor were HLA-DP identical. HLA-DP antibody positivity remained in three further testing, and subsequently the reactions turned negative.

DISCUSSION

HLA sensitization prior to heart transplantation is a well-established risk factor for a higher incidence of rejection as well as for worse organ and recipient survival. Thus, precise and timely detection of HLA antibody profile prior to waitlisting, definition of DSAs at the time of organ offer and subsequent DSA monitoring in case of organ acceptance across positive DSAs is vital for evaluating the recipient's humoral immune status pre- and post-transplantation. The recipient presented in this paper was sent for pre-transplant immunogenetic testing as a heart transplant candidate two months prior to registration on the Eurotransplant waiting list. CDC screening result revealed that the patient has cytotoxic HLA antibodies belonging to HLA-B7 cross-reactive group (CREG). Luminex SAB screening confirmed serologically obtained results, with an extension of antibody profile to 163EW+73TE, 113HN, 24T and 82LR epitopes. We assume blood transfusions as probable historical immunizing event(s), as this was a male recipient without previous transplantations and without a left ventricular assist device, which is also reported to be the cause of HLA sensitization in heart recipients.[8] Screening results obtained with the serum sample taken immediately before the transplantation were concordant with these historical screening results.

Avoiding DSA at transplant is a desirable objective, although not always possible to attain, particularly in highly sensitized (HS) recipients or in recipients in clinically high urgent need of transplantation. With a cPRA of 98%, DSA negative transplantation was hardly feasible for this recipient, and all the efforts were directed towards avoiding transplantation with CDC identified DSAs, above all antigens from the HLA-B7 CREG group. The heart offer that was accepted and the heart transplantation performed fulfilled these criteria. However, the transplantation was not immunologically ideal, as it was performed across weak positive reactions for both donor's mismatched HLA-A and HLA-B antigens. Even more, retrospectively performed CDC CM was also a weak positive, although it was not an expected result, as Luminex reactions were not in the range that is

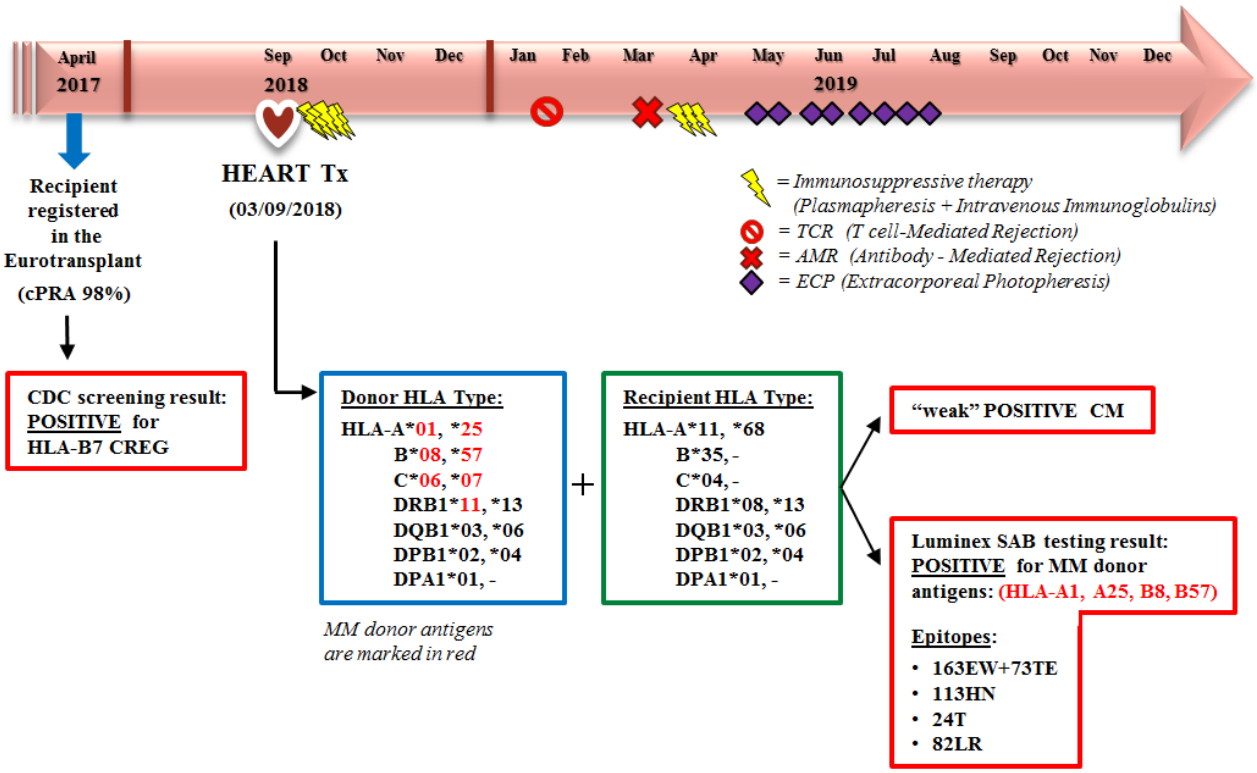

Figure 2. Patient's timeline with main immunological events and results of testings before and after transplantation (Recipient and donor HLA typing, mismatched alleles and antibody specificities in CDC screening and solid phase Luminex screening method). DSA - Donor-Specific Antibody, MM - Mismatch, MFI - Mean Fluorescence Intensity, CREG - Cross-Reactive Group.

proven to correlate with CM positivity. Namely, literature data,[9] as well as our own validation results, show that an MFI range 2000-8500 for DSAs towards HLA-A and/or HLA-B is a predictor for a negative CDC CM. In our recipient, all four DSAs had MFI falling into this range; moreover, the values were mostly below MFI 2000. One possible explanation can be that weak reactions were against the Bw4 public epitope present in the donors' cells, as anti-Bw4 antibodies, proven to be present in the recipient serum by Luminex assay, might not recognize all Bw4 positive molecules, resulting in weak positive reactions in cytotoxicity testing. Intensive post-transplantation monitoring revealed different behavior of pre-transplant DSAs. Three DSAs, HLA-A1, A25 and B57, decreased in the mean of MFI values shortly after the transplantation, never again reaching pre-transplant level, and they all turned to be steadily negative 6 months after transplantation until the last check-up in February 2020. In contrast, HLA-B8 antibody increased shortly after transplantation, and the increase was correlated with the increase of overall positive reactions, suggesting that the recipient is at immunological risk for AMR. The recipient was treated with plasmapheresis and intravenous immunoglobulins (IVIg), as well as with four cycles of extracorporeal photopheresis, which was accompanied with the decrease of HLA-B8 antibody level, but the reactions never reached a stable low or negative value, remaining prone to MFI variability (Figure 2).

The transient appearance of HLA class II antibodies of HLA-DP specificities that turned out to be non-DSA, were detected shortly after the overall increase of HLA class I antibodies and might be the reappearance of previous pre-transplant HLA-DP sensitization.

This case emphasizes the importance of careful and well-timed immunogenetic testing and monitoring before and after transplantation as new and preformed HLA DSAs and non-DSAs need to be adequately interpreted in terms of increased immunological risk, which might result in worse outcome for heart transplant recipients.

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