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Brief communication

Monitoring efficiency of humoral rejection episode therapy in liver transplantation: any role for complement binding Luminex Single Antigen assays?

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ABSTRACT

Humoral rejection and its relationship with anti HLA antibodies have been extensively studied in organ transplantation with the exception of liver transplantation (LT). Recently, association between donor specific anti HLA antibodies (DSA) and increased risk of rejection and graft loss has been suggested in LT. When such antibodies appear, adequate treatment and monitoring are needed to avoid or delay allograft loss. We report here three cases of probable antibody-mediated rejection developed after pregnancy in liver transplanted women. Sera at the time of rejection and during follow-up have been retrospectively tested for the ability of DSA to bind complement components. These cases display different outcomes depending on the complement binding DSA capacity and titers after treatment of the rejection episodes. Thus, they highlight the potential interest of complement binding Luminex Single Antigen assays to monitor the efficiency of anti-rejection therapy.

1. Introduction

Humoral rejection is related to donor specific anti-HLA antibodies (DSA) and has been extensively studied in organ transplantation with the exception of liver transplantation (LT). Nevertheless, it has been suggested that positive crossmatch (XM) could increase risk of early rejection and allograft loss after LT [1–3]. Similarly, several studies have shown that post-LT DSA may play a role in acute and chronic rejection [4–7]. Risk factors for development of anti-HLA antibodies include blood transfusion, organ transplantation and pregnancy. When sensitization and rejection happens, the major goal is to have an adequate treatment to avoid or delay allograft loss. Tools that can help to closely monitor the effectiveness of those treatments are in need. We report herein three cases of probable severe AMR developed after pregnancy in young liver transplanted women, as a basis for discussion about the potential helpfulness of complement binding single antigen assays in monitoring rejection episode therapy.

2. Cases presentation

2.1. Case 1

A 6-year-old girl (HLA-A11, 23; B49, 51; DR1, 11; DR52, DQ5, 7) received a first cadaveric LT (HLA-A1, 30; B14, 50; DR7, −; DR53; DQ2, −) in June 1992 for the treatment of a liver failure related to biliary atresia. All donor's antigens were mismatched antigens. As she had no previous sensitizing events, CDC XM was negative for T and B cells at the time of LT. Initial immunosuppressive regimen consisted in anti-thymocyte globulins, azathioprine, cyclosporine and steroids. Follow-up after transplantation (Fig. 3) was uneventful and protocol liver biopsy was normal in 2007. Maintenance immunosuppressive regimen consisted in tacrolimus from July 2008, in replacement of cyclosporine. In November 2009, the patient became pregnant for the first time and an induced abortion was done. Liver biopsy performed in 2012 was normal. In March 2013, the patient became pregnant for the second time and presented a spontaneous abortion after 3 months. In July 2013, she presented a severe biopsy proven AR episode, c4d staining showed slight sinusoidal c4d deposition into the graft. At the same time, LSA assay revealed the presence of class II anti HLA antibodies, including DSA anti DR53 (MFI = 13,800).

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C3d LSA assay showed that anti DR53 antibody was complement activating (MFI = 1800). We concluded that the patient developed an AMR of her graft due to the presence of *de novo* anti-HLA antibodies. Unfortunately, HLA type of both potential fathers was unavailable. Thus, we cannot confirm that they shared HLA antigens with the donor. Treatment of this rejection episode was based on steroid boluses and increasing dosage of tacrolimus. Liver function tests did not improve and immunosuppression was majored using anti-thymocyte globulins without any significant effect. Then the patient received rituximab, plasmapheresis, IVIG and steroids. Unless the constant positivity of LSA assay for anti-DR53 DSA (MFI between 3000 and 6600), the C3d LSA assay showed the negativation of anti-DR53 DSA. Outcome was favorable.

2.2. Case 2

A 26-year-old woman (HLA-A30, 34; B8, 35; DR4, 11; DQ5, 8) received a first LT from a living donor, her husband (HLA-A2, 31; B50, 51; DR12, 13; DQ6, 7) in January 2009 for the treatment of liver failure related to Wilson's disease. All husband's antigens were mismatched antigens. As previous sensitizing events she had given birth to 2 children in 2004 and 2006. The recipient was not sensitized at the time of LT. Flow cell-XM was negative for T and B cells. Initial immunosuppressive regimen consisted in MMF, tacrolimus, steroids and basiliximab. In May 2009, she presented a first episode of mild biopsy proven AR. Treatment of this episode was based on increasing dosage of tacrolimus and outcome was favorable. In November 2009, she presented an episode of severe biopsy proven AR. Treatment was initially based on steroid boluses and increasing dosage of tacrolimus. As liver function tests did not improve, immunosuppression was majored using anti-thymocyte globulins and the outcome was slowly favorable. LSA assays remained negative during all this period (Fig. 2). In April 2010, the patient became pregnant. MMF was then suspended and reintroduced when she gave birth to her third child in January 2011. A retrospective analysis showed that in June 2011, LSA assay turned positive and showed class II circulating anti-HLA antibodies including anti-DQ7 DSA (MFI = 16,300). In October 2011, she presented a new severe biopsy proven AR episode. C4d staining showed only slight sinusoidal c4d deposition into the graft. At time of rejection, LSA assay showed anti-DQ6 (MFI = 1000) and DQ7 DSA (MFI = 17,500) and C3d LSA assay revealed that anti-DQ7 antibody was a complement activating one (MFI = 2800). We concluded that the patient developed AMR due to the development of *de novo* anti-HLA antibodies. This episode was treated with steroid boluses, increasing dosage of tacrolimus and introduction of everolimus. During treatment, DSA MFI with standard LSA assay remained stable, but MFI with C3d LSA assay decreased significantly and turned negative. In May 2012, she presented a new severe biopsy proven AR episode. Treatment was based on steroid boluses, increasing dosage of tacrolimus and everolimus. Outcome was favorable. In January 2013, she presented a new severe biopsy proven AR episode. Therapy was initiated with steroid, IVIG, plasmapheresis and rituximab with long-term maintenance IVIG. During follow-up of this episode, LSA assay showed high DSA titers. C3d LSA assay remained positive but showing low DSA titers. Outcome was favorable.

2.3. Case 3

A 28-year-old woman (HLA-A2, 24; B39, 50; C*06, *07; DR8, 15; DR51; DQ4, DQ6; DPB1*03, *04) received a first LT from a deceased donor (HLA-A2, 29; B8, 35; C*04, *07; DR17, 4; DR52, DR53; DQ2, 8; DPB1*02, *04) in April 2007 for the treatment of liver failure related to primary biliary cirrhosis. Then, mismatched antigens were A29, B8, B35, C*04, DR17, DR4, DR52, DR53, DQ2, DQ8, DPB1*02. At the time of transplantation, the complement-dependant cytotoxicity (CDC) XM was positive for T and B cells because of preformed DSA directed against A29 (Median Fluorescence Intensity (MFI) = 1900), DR4 (MFI = 19,400), DR53 (MFI = 9300) and DQ8 (MFI = 7300) mismatched antigens. As previous sensitizing events, the patient had given birth to 2 children in 2004 and 2006. Her husband was retrospectively HLA typed and showed HLA-A30, 68; B27, 53; C*02, *04; DR4, −; DR53; DQ8, −; DPB1*01, *104. He shared 4 HLA antigens with the liver donor: C*04, DR4, DR53, DQ8. This HLA type could explain the patient's presensitization against DR4, DR53 and DQ8 antigens. Initial immunosuppressive regimen consisted in mycophenolate mofetil (MMF), tacrolimus and steroids. Follow-up after transplantation (Fig. 1) was complicated by the occurrence of biopsy proven acute rejection (AR) episodes in November 2008, April 2009 and July 2010. C4d staining of liver biopsy at the time of rejection episodes showed only slight sinusoidal c4d deposition into the graft. At the time of the first rejection episode, Luminex Single Antigen (LSA) assay (Immucor, Norcross, GA) revealed the presence of anti-HLA class I and II antibodies including DSA directed against DR4 (MFI = 3300), DR53 (MFI = 17,000) and DQ8 (MFI = 7000) antigens, already present at the time of transplantation. The ability of these DSA to bind complement was retrospectively assessed using the C3d LSA assay (Immucor, Norcross, GA) and only anti-DR53 was found to be complement activating (MFI = 7600). We hypothesized that the patient developed antibody mediated rejection (AMR) of her graft due to the presence of preformed antibodies able to target donor antigens. Treatment of these rejection episodes was based on steroid boluses, increasing dosage of tacrolimus and MMF in 2008, steroid boluses and adjunction of everolimus in 2009 and steroid boluses and anti-thymocyte globulins (10 mg/kg total dose) in 2010. As the treatment was not efficient, the patient finally received plasmapheresis and rituximab in 2010. Outcome was then favorable. At the end of rejection episodes LSA assay remained positive and showed *de novo* DQ2 DSA (MFI = 2300), all preformed DSA had disappeared. The C3d-LSA assay performed on the same serum showed no complement fixing DSA. The patient became pregnant again in May 2011. Initial immunosuppressive regimen was then lightened to everolimus and corticosteroids. Patient gave birth to her third child in February 2012. She presented a new episode of AR in May 2012. Liver biopsy disclosed features of extensive fibrosis associated with severe rejection. Concomitant LSA assay identified the preformed anti-DR4 (MFI = 7500), DQ8 (MFI = 8500), DR53 (MFI = 17,100) DSA associated with *de novo* anti-DR52 (MFI = 4200), DR17 (MFI = 1600) and DQ2 (MFI = 15,500) antibodies. Using the C3d LSA assay on the same serum, we figured that anti-DR53 DSA (C3d MFI = 4500) was a complement activating antibody. Anti-rejection therapy was then re-initiated with steroids, intravenous immunoglobulins (IVIG), plasmapheresis and rituximab, with long-term maintenance IVIG. The treatment maintained stable levels of DSA MFI, but liver function kept deteriorating. During follow-up post-treatment, DSA were tested with both standard and C3d LSA assays. DSA directed against DR4, DQ2 and DQ8 appeared to be complement fixing as well. C3d LSA assay remained positive with high DSA titers (at least one DSA with MFI over 5000) unless the anti-rejection therapy. Because improvement of that rejection episode was only partial, the patient was listed for retransplantation in May 2013. She died from bacterial pneumopathy in February 2014.

3. Discussion

Considering LT patients, Kaneku et al. reported in a recent and large study that, during the first year post-transplantation, 8.1% of LT recipients develop *de novo* DSA with a MFI over 5000. Almost all *de novo* DSA were directed against HLA class II antigens recalling the cases we present here. Several factors have been associated with *de novo* HLA sensitization after the LT. The use of cyclosporine and low calcineurin inhibitor levels seem to increase the risk *de novo* DSA formation. On the other hand, a calculated MELD score > 15 at the time of LT and recipient age > 60 years old are associated with lower risk [7]. More generally, pregnancy is known to induce anti-HLA sensitization with transfusion and transplantation. Rebibou et al., reported that about 50% of women develop anti-HLA antibodies after 3 pregnancies [8].