Transplant International

Transplant International ISSN 0934-0874

CASE REPORT

Humoral and cellular rejection after combined liver–kidney transplantation in low immunologic risk recipients

Karine Hadaya,[1,2] Sylvie Ferrari-Lacraz,[3,4] Emiliano Giostra,[2] Pietro Majno,[2] Solange Moll,[5] Laura Rubbia-Brandt,[5] Nicola Marangon,[1,2] Jean-Pierre Venetz,[6] Jean-François Bolle,[2] Gilles Mentha[2] and Jean Villard[4]

1 Division of Nephrology, Geneva University Hospital, Geneva, Switzerland
2 Division of Transplantation, Geneva University Hospital, Geneva, Switzerland
3 Division of Laboratory Medicine, Geneva University Hospital, Geneva, Switzerland
4 Transplantation Immunology Unit, Geneva University Hospital and Medical School, Geneva, Switzerland
5 Division of Pathology, Geneva University Hospital, Geneva, Switzerland
6 Division of Transplantation, Lausanne University Hospital, Lausanne, Switzerland

Keywords
anti-HLA antibody, C4d, humoral rejection, liver–kidney transplantation.

Correspondence
Jean Villard MD, PhD, Transplantation Immunology Unit, Division of Immunology and Allergy, Geneva University Hospital, 24 rue Micheli-du-Crest, 1211 Geneva 14, Switzerland. Tel.: +41 22 372 9394; fax: +41 22 372 9390; e-mail: jean.villard@hcuge.ch

Received: 16 July 2008
Revision requested: 4 August 2008
Accepted: 16 September 2008

doi:10.1111/j.1432-2277.2008.00775.x

Summary

Combined liver–kidney transplantation is considered a low risk for immunologic complication. We report an unusual case of identical ABO liver–kidney recipient without preformed anti-human leukocyte antigen (HLA) antibodies, transplanted across a T- and B-cell-negative cross-match and complicated by early acute humoral and cellular rejection, first in the liver then in the kidney. While analyzing the immunologic complications in our cohort of 12 low-risk combined liver–kidney recipients, only one recipient experienced a rejection episode without detection of anti-HLA antibody over time. Although humoral or cellular rejection is rare after combined kidney-liver transplantation, our data suggest that even in low-risk recipients, the liver does not always systematically protect the kidney from acute rejection. Indeed, the detection of C4d in the liver should be carefully followed after combined liver–kidney transplantation.

Introduction

Acute humoral or antibody-mediated rejection in ABO-identical transplantation is usually related to the presence of preformed or acquired anti-human leukocyte antigen (HLA) donor-specific antibodies (DSA) [1]. This entity is well recognized in kidney- and heart transplantation and often associated with poor graft survival [2,3]. Although the liver has long been regarded as resistant to antibody-mediated rejection, several reports have shown that preformed DSA and a positive cross-match are associated with rejection and graft loss [4–6]. In case of combined liver–kidney transplantation, it is accepted that the liver provides some immune protection to the kidney leading to less acute rejection episodes [7] and several reports of successful combined liver–kidney transplantation across a positive cross-match have been published [8,9]. However, the presence of *de novo* DSA and their role in rejection after ABO-identical combined liver–kidney transplantation has not been extensively studied.

We report here an ABO-identical combined liver–kidney transplantation complicated by early acute cellular and humoral rejection of both organs in an unsensitized recipient. The immunologic complications of our cohort of 12 unsensitized ABO-compatible/identical recipients who underwent a combined liver and kidney transplantation were also analyzed.

Methods

Patients

Twelve adult unsensitized recipients (seven male and five female patients) underwent an ABO-identical combined liver and kidney transplantation between 1997 and 2007 with at least three HLA mismatches with the donor. All patients were transplanted with a T- and B-cell-negative CDC cross-match.

All recipients received an immunosuppressive regimen based on corticosteroids, a calcineurin inhibitor and an antimetabolite (mycophenolate mofetil or azathioprine) with an induction therapy of either anti-IL-2 receptor inhibitors (8/12) or thymoglobulin (1/12).

Immunologic testing

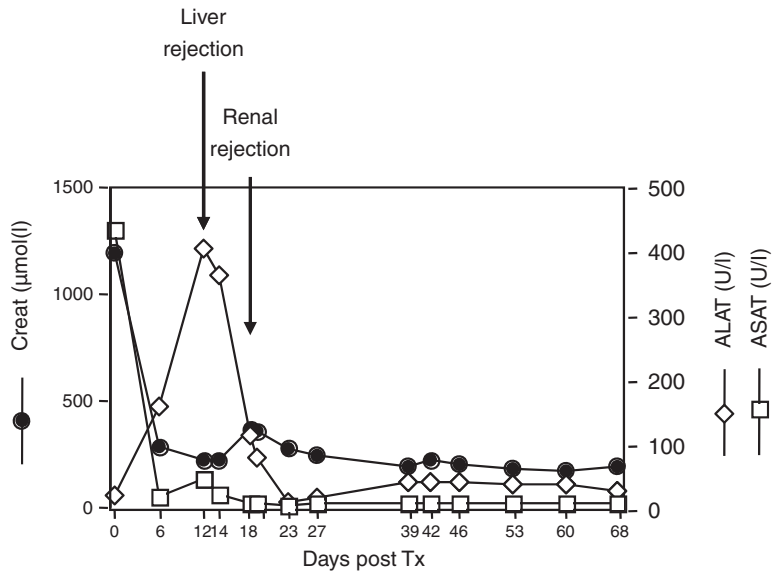
The panel-reactive antibody (PRA) and the T- and B-cell cross-match were tested by complement-dependent-cytotoxicity (CDC) on a HLA-typed lymphocyte panel of 30 individuals. The presence of anti-HLA class I and class II antibodies was first determined by ELISA (One Lambda, Inc. Canoga Park, CA, USA), and then by Luminex single antigen (LABScreen® single antigen class I and class II; One Lambda, Inc).

Histology

Liver and kidney tissues were fixed in 10% buffered formalin. Sections 3-μ thick were cut from paraffin-embedded tissue cores, and stained with hematoxylin and eosin as well as periodic acid Schiff reaction for morphologic examination. Liver and kidney formalin-fixed tissue was subjected to immunohistochemistry using a C4d rabbit polyclonal antibody (Biomedica, Vienna, Austria). Liver and kidney biopsies were performed at the time a rejection episode was suspected and 3 and 6 months after the transplantation.

Case report and results

A 25-year-old woman with type I primary hyperoxaluria presented with end-stage renal disease and received an ABO-identical orthotopic combined liver–kidney transplantation from a three-antigen mismatch, deceased donor. The patient had never received blood transfusions and had never undergone pregnancies. On the day of transplantation, the PRA was at 0% and class I and class II anti-HLA immunoglobulin IgG were negative by ELISA and Luminex single antigen. Immunosuppression consisted of induction with basiliximab (days 0 and 4) and of methylprednisolone, tacrolimus and mycophenolate mofetil as of day 0. The immediate postoperative course was uneventful with a regular decrease in liver test parameters and serum creatinine. On postoperative day 11, all liver test parameters were elevated, particularly aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) which reached three times the normal levels (Fig. 1). A liver biopsy revealed acute rejection with



	Day 0	Day 18	Day 60
CDC crossmatch T/B	neg/neg	neg/pos	neg/neg
anti-HLA class I	0	DSA anti-A26	No DSA
anti-HLA class II	0	DSA anti-DR4	No DSA

Figure 1 Postoperative clinical course. The clinical course covers the time points of liver and kidney rejection, serum creatinine levels (creat), ASAT and ALAT, T- and B-cell cross-match by CDC, anti-HLA antibody screening by ELISA and DSA by Luminex single antigen over time.

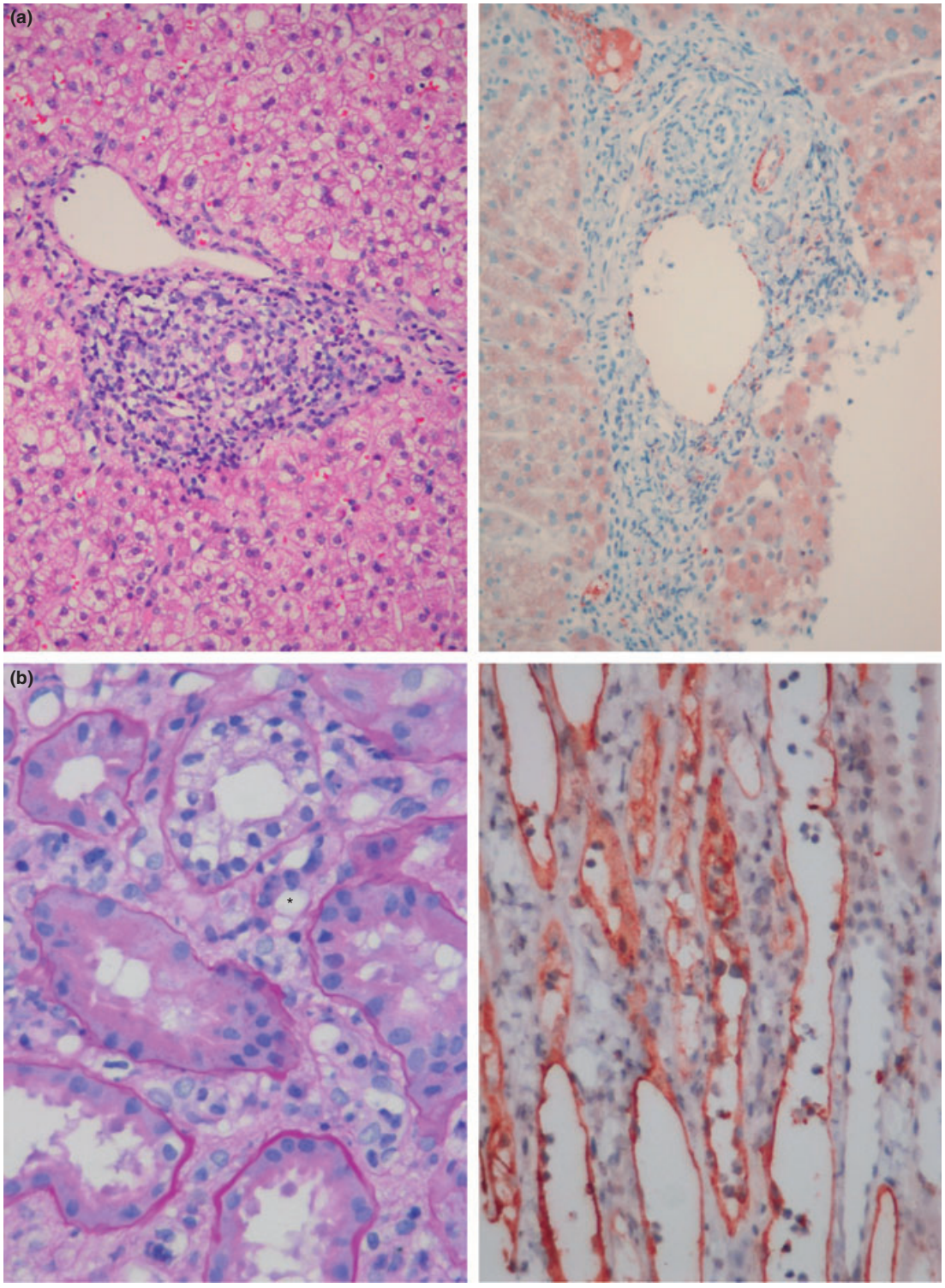


Figure 2 Hematoxylin and eosin and C4d-staining of kidney and liver transplant biopsies. (a) Hematoxylin and eosin and C4d-staining of liver biopsies. The structure of the liver is preserved without significant fibrosis. The portal space contains a moderate inflammatory infiltrate composed of mature lymphocytes, neutrophils and eosinophils (left). We also observed portal and centrolobular endothelitiis with C4d deposition by immunohistochemistry (right). (b) Periodic acid Schiff (PAS) staining and C4d immunohistochemistry of kidney biopsy. Aggregates of mononuclear inflammatory cells in peritubular capillaries (*) (PAS, original magnification ×400) (left). Diffuse C4d positivity along peritubular capillaries by immunohistochemistry characteristic of antibody-mediated rejection (original magnification ×400) (right).

Banff activity index of 5, mixed inflammatory cells in the portal triad, cholangitis and endothelitis. C4d-staining by immunochemistry carried out on formol-embedded liver biopsy was positive in the portal triad (Fig. 2a). ASAT and ALAT serum levels began to decrease without modification of the immunosuppressive drugs. At day 15, the patient was discharged home under a triple immunosuppressive therapy of tacrolimus (through levels at 12 ng/ml), mycophenolate mofetil 2 g/day and prednisone 15 mg/day. Three days later, serum creatinine rose to128 μmol/l while liver functions were still improving (Fig. 1). Kidney graft biopsy showed acute tubulo-interstitial rejection grade Ia and acute humoral rejection grade II with diffuse peritubular capillaries C4d positive by immunochemistry (Fig. 2b). The patient was treated with plasmapheresis, anti-rabbit thymoglobulin, five pulses of methylprednisolone and 2 g/kg IVIG (Fig. 1). As a result of this treatment, serum creatinine level returned to normal and the patient was discharged home under an immunosuppressive therapy of tacrolimus (targeted through levels 12 ng/ml), mycophenolate mofetil 2 g/day and prednisone 15 mg/day.

Luminex single antigen test demonstrated the presence of DSA to HLA class I and II donor antigens: anti-A26 and anti-DR4. B-cell CDC cross-match was strongly positive with the serum sample taken on the day of acute renal rejection. Three and 6 months after the transplantation, renal and liver functions were normal, no DSA were detected by Luminex single antigen and T- and B-cross-matches were negative. Biopsies of liver and renal grafts showed complete resolution of the acute rejection episodes.

We have reviewed 12 adult unsensitized patients, recipients of an ABO-identical combined liver and kidney transplantation between 1997 and 2007. The PRA was at 0% and the detection of anti-HLA antibody class I and class II by ELISA and Luminex was negative for all recipients at the time of the transplantation.

During the follow-up period of 5–132 months, a single patient experienced an acute cellular rejection episode of the liver only (at day 15), which resolved after a temporary increase of tacrolimus for 10 days. All organs were functional and one patient died from a cause unrelated to the transplantation. No other patient developed anti-HLA antibody over time.

Discussion

In ABO-identical/compatible liver transplantation, the liver has previously been premised to be resistant to humoral rejection [10]. More recently, C4d deposition in the portal capillaries has been proposed to be a hallmark of antibody-mediated rejection with either positive or negative cross-match, but the presence of DSA was usually not reported [11–14]. Periportal C4d-staining resembles nonspecific C4d-staining in kidney arterioles and may result from the activation of cellular infiltrate because of cellular rejection, bacterial infection or viral infection. C4d-staining could also help to differentiate between rejection and recurrent hepatitis C infection after transplantation [14,15]. However, there is currently a lack of consensus in the pathogenesis and therapeutic implication of C4d-staining in acute liver rejection [14–17].

To our knowledge, only two cases reported in the literature fulfilled all criteria for antibody-mediated rejection in ABO-compatible/identical liver allograft recipients as described in renal transplantation. The first one was a hyper-acute isolated antibody-mediated rejection. This report demonstrated histologic tissue injury, circulating donor-specific HLA antibodies, and linear C4d-staining throughout the dominant capillary bed leading to the diagnosis of antibody-mediated rejection in a liver transplant recipient with a preoperative positive T-cell cross-match [18]. In the second case, humoral and cellular rejections occurred in a preoperative unsensitized and negative cross-match patient 4 years post-transplantation. C4d-staining was positive in small portal vessels [19].

Although quadruple immunosuppression was administered to our patient, early acute rejection was diagnosed in both allografts, first the liver - demonstrating that in this case the liver did not provide immune protection to the transplanted kidney. In addition to acute cellular rejection of the liver graft, we observed C4d deposits in the portal triad. Few days later, the patient attained a condition that fulfilled all the criteria for acute antibody-mediated kidney rejection: acute renal dysfunction, histologic tissue injury with acute tubular necrosis and peritubular capillaries mononuclear infiltration, diffuse C4d immunofluorescence with monoclonal antibody and detection of *de novo* DSA [20]. The simultaneous rejection of liver and kidney suggests that positive C4d-staining of the liver allograft, as does that of the kidney allograft, reflects humoral rejection. Liver functions improved spontaneously before treatment of acute rejection, but this situation arises when recipient of liver transplant with acute rejection was already receiving immunosuppressive drugs at high doses, as was the case with our patient. Interestingly, in none of the 11 other patients transplanted with the same low immunologic risk, anti-HLA antibody was detected during follow up.

Even if the development of *de novo* anti-HLA antibody is uncommon after combined liver–kidney transplantation, we believe that systematic C4d-staining should be applied to liver biopsies when acute cellular liver rejection is diagnosed, in addition to the determination of anti-HLA antibody if humoral rejection is suspected. This analysis could help to assess the prevalence of antibody-mediated rejections and the potential need for early and

specific treatment leading to a favorable outcome after liver transplantation. Moreover, this case report demonstrates that after combined liver–kidney transplantations, the liver does not always protect the kidney from rejection and liver rejection episodes that could precede kidney rejection should be carefully followed.

Authorship

KH and SF-L: performed the study and wrote the paper. EG, PM, and J-PV: collected the data. SM, LR-B, and J-FB: contributed important data. NM: performed the study. GM: supervised the study. JV: supervised the study and wrote the paper.

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