Severe and delayed immune-mediated hemolysis post-liver transplantation

To the Editor:

Hemolysis, which is caused by a variety of immune and non-immune mechanisms, is a well-recognized complication of solid organ transplantation.[1] Hemolysis post-liver transplantation can be induced by drug, infection, autoimmune disorders, blood-group incompatible transplantation or passenger lymphocyte syndrome (PLS), which mostly is a quick and non-fatal complication and usually recovered easily. However, immune-mediated hemolysis post-liver transplantation is rare. The antibodies can be of donor and/or recipient origin.[2] Recently, we encountered a case of severe and delayed hemolysis post-liver transplantation caused by anti-e autoantibody. It did not response by all the regular treatments. In addition to ABO and Rh blood systems, there are also some other blood systems in human beings, such as Lewis, MNSs or Kell blood groups.[3] Although those irregular blood phenotypes are not regularly tested, we still need understand comprehensively.

A 61-year-old male patient diagnosed as autoimmune liver cirrhosis was admitted to our center because of liver failure with large amounts of ascites. He had a history of multiple times transfusion owing to gastrointestinal hemorrhage. The preoperative laboratory results were as follows: hemoglobin (Hb) 99 g/L (120-160), alanine aminotransferase (ALT) 54 U/L (9-50), aspartate aminotransferase (AST) 64 U/L (15-40), total bilirubin (TBil) 64.8 μmol/L (0-22.0), serum creatinine 157 μmol/L (54-106) and international normalized ratio (INR) 2.06 (0.8-1.5), sodium 133.5 mmol/L (137-147). His Child-Pugh class was C and the score of model for end-stage liver disease (MELD)-Na was 27. On July 21, 2016, the patient (blood group: AB+) underwent the orthotopic liver transplantation from a donation after cardiac death (DCD) donor with blood group B+. The consent for organ donation was obtained from his family. This was also approved by the Ethics Committee of the Third Xiangya Hospital, and the liver was attributed by the China Organ Transplant Response System. Before transplantation, his blood and HLA antibodies were all negative. He received 500 mL fresh frozen plasma, 6 U red blood cells (RBC) and no platelet during the operation. The operation was successful, and the postoperative course was uneventful. His immunosuppressant regimen consisted of steroids, tacrolimus (initiated with 2 mg every 12 hours, adjusted by drug concentration ranging from 6-10 ng/mL) and mycophenolate mofetil (500 mg every 12 hours). He discharged with good liver function and normal Hb on postoperative day 20, and followed up once a week regularly.

On postoperative day 72, he was readmitted with severe anemia (Hb: 44 g/L) (Fig. A) and mild abnormal liver function (ALT: 41 U/L, AST: 42 U/L, TBil: 47.6 μmol/L, DBil: 16.4 μmol/L) (Fig. B and C). With AB+ RBC transfusion and intravenous steroid application (250 mg+125 mg +125 mg, once a day, for three days), the recipient discharged with Hb remission (around 80 g/L) and recovered liver function a week later. An acute rejection possibility was excluded by liver biopsy.

On postoperative day 117, his Hb decreased to 18 g/L (Fig. A) without any causes. Indirect/direct antiglobulin tests were both positive, reticulocyte count went up from 3.94% to 26.71% gradually. Cross matches were all positive even when mixing with RBC of reduced white blood cell. Flow cytometry test showed a high B cell (CD19+) percentage (25%-35%) in peripheral lymphocytes for several times. The Epstein-Barr virus (EBV) was negative in the peripheral blood, and no *de novo* enlarged lymph nodes were found. The bone marrow aspiration solely revealed an increase number of immature blood cells in all

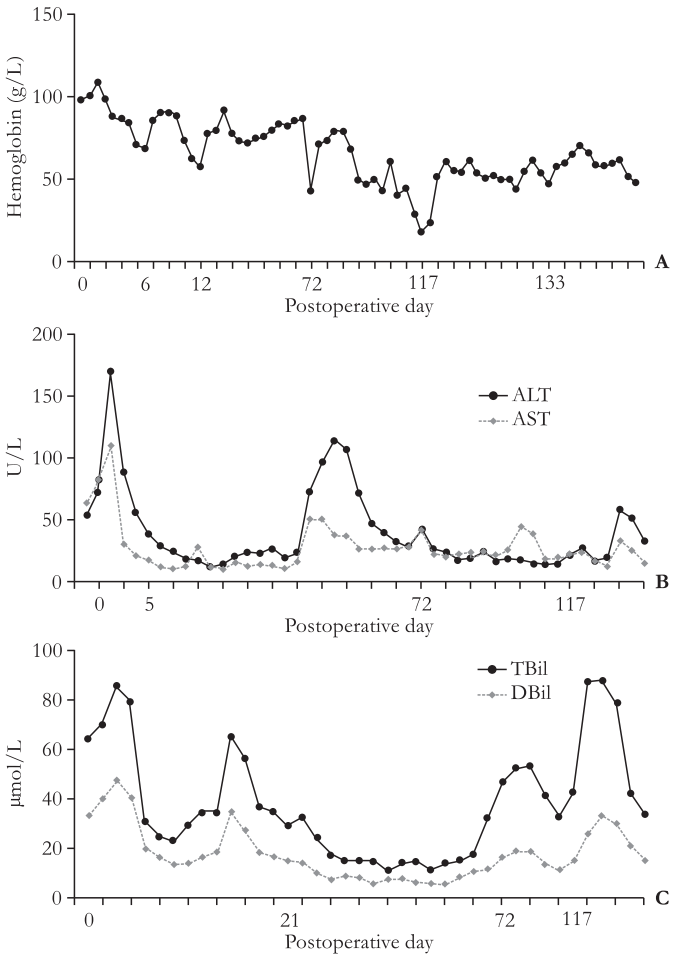

Fig. The regular tests of patient post-liver transplantation. **A**: hemoglobin; **B**: ALT and AST; **C**: TBil and DBil.

stages, but not any feature of post-transplant lymphoproliferative disorders (PTLD). The blood smear showed a slightly increased number of spherocytes. To screen the anti-RBC antibodies, no anti-A and anti-B antibodies were found. To further screen irregular anti-RBC antibodies by an elution test, anti-e IgG antibodies (IgG+, IgM-, C3c-, C3d-) were found strongly positive in either room temperature or 37 ℃. However, hemolysis and anti-e antibody were not found in the other two kidney recipients from the same DCD donor. We also investigated the Rh blood antigens. The Rh blood phenotypes of the liver and two kidney recipients from the same donor were D+C+c+E+e+, D+C+c-E-e+ and D+C-c+E+e+, respectively. The chimerism rates of the peripheral blood were 0.023% in liver transplant recipient, and 0.001% and 0 in the two kidney recipients, respectively. It was very difficult to give Rh (e) negative RBC to this patient in an emergent time, because of severe shortage of Rh (e) negative RBC in our region. Instead, RBC of reduced white blood cell transfusion, intravenous immunoglobulin, high-dose methylprednisolone, and plasmapheresis were used for treatment, but all did not response well. Additionally, tacrolimus was withdrawn, and sirolimus (0.5 mg/day) was applied. It was dangerous to use rituximab because of the weak immune condition of the patient (the immune function test showed CD4/CD8 T-cell ratio: 0.29). The chest CT scan indicated pulmonary infection. Laboratory results were as follows: procalcitonin 1.5 ng/mL (<0.05), C-response protein >5 mg/L (0-1), endotoxin 0.4368 EU/mL (0-0.035), GM test 178.4 pg/mL (0-100.5), cytomegalovirus-DNA (urine) 10^6/mL (<500). Finally, he died of bad nutrition, respiratory failure and severe infection several days later with stable liver function (Fig. B and C).

Hemolysis post-liver transplantation could result from immune and non-immune reasons. The non-immune reasons involved drug, infection and hypersplenism destruction. The immune reasons involved autoimmunity, PLS, passive transfusion of antibody, or major blood group incompatibility transplantation.[2] Autoimmune hemolytic anemia has been attributed to infections, immunosuppressive medications or is a manifestation of PTLD. The mechanism of immune-mediated hemolysis post-liver transplantation caused by autoimmunity is generally considered that anti-RBC antibodies are produced by host lymphocytes against recipient RBC. Because the use of calcineurin inhibitors, mainly cyclosporine A and tacrolimus are applied after liver transplantation, dysfunction of host T-cell immunity results in unbalanced B- and T-cell lymphopoiesis.[4] Under this circumstance, if the recipient has autoimmune primary diseases, he or she would be in highly sensitive status. Taken together, it favors to make host B cells produce either anti-RBC autoantibodies or alloantibodies when stimulated. The degree of this hemolysis varies from mild and transient to severe and chronic.[5] Additionally, as a consequence of immunosuppression, PTLD is usually derived from B cells with preferential presentation as lymphoma. Autoimmune hemolytic anemia, when PTLD is diagnosed, is thought to occur because of impairment of EBV specific, cytotoxic T-cell function by systemic immunosuppression that permits the expansion of recipient origin B cells latently infected with EBV.[6] The clinical manifestations vary from nonspecific symptoms in the form of fever, sweats, malaise, weight loss, and features of primary EBV infection in some patients, to sudden enlargement of tonsils, lymph nodes, or other extranodal lymphoid organs. Other organs such as the central nervous system, bone marrow, spleen, lung, small intestine, liver, and kidney may also be affected.[7] In our case, neither phenomena nor tests indicated EBV infection, and no *de novo* enlarged lymph nodes were found. Also, the bone marrow aspiration report did not show almost any abnormality and the blood smear showed a slightly increased number of spherocytes. These would indicate the high possibility to exclude PTLD. The most reported reason for immune-mediated hemolysis post-liver transplantation is PLS. It is the result of RBC alloantibody production from passenger lymphocytes accompanying the allograft against the recipient's RBC. This condition is believed to result from calcineurin inhibitors mediated T-cell suppression and upregulated B-cell proliferation;[8] the syndrome can be regarded as a type of graft-versus-host disease. This hemolysis usually has an abrupt onset, tends to be self-limiting and varies in severity, and it is occasionally fatal. Not all patients who develop antibodies experience RBC destruction. Additionally, this hemolysis is mostly common with ABO blood system when there is a minor mismatch between the donor and recipient, although it can also occur with other blood systems, such as Rh, Kidd and Lewis.[9]

In our case, hemolysis did not occur either during the transfusion process of liver transplantation or in the early course after liver transplantation, which suggested that anti-e antibody were not passively transfused both pre- and within-operatively. Hemolysis and anti-e antibody were not found in the other two kidney recipients from the same DCD donor, which indicated that anti-e antibody was not from the donor. In liver allograft, donor lymphocytes could not be washed off by organ douche, and there were still approximately $(5\text{-}6)\times10^9$/L donor lymphocytes in the graft after douching. Chimerism caused by PLS could not be detectable 3 months after liver transplantation.[10] However, in our case, chi-