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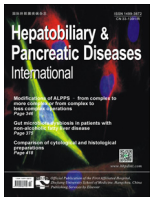

Letters to the Editor

Detecting chimerism contributes to diagnosis of graft versus host disease after orthotopic liver transplantation

To the Editor:

Graft versus host disease (GVHD) occurs when the immunocompetent cells from the donor mount an immune response against the tissues of the host [1]. It is commonly seen in the patients who received hematopoietic stem cell transplantation (HSCT). GVHD is a rare but lethal complication in orthotopic liver transplantation (OLT). The incidence varies from 0.1% to 2.0%, while the mortality rate is up to 75.0% [2–4]. Herein, we reported a successfully treated case of GVHD after OLT, who was diagnosed timely with the help of a novel chimerism test technique, and was treated with drug withdraw and basiliximab.

The patient, male, 65-year-old, was in poor nutrition with the body mass index (BMI) of 20.7 kg/m^2. He had a long history of hepatitis B virus (HBV) infection, and was diagnosed with hepatocellular carcinoma (single focus with the diameter of 4.3 cm, no distant metastasis, within the Milan Criteria) for three months. On April 27, 2016, he received allogeneic OLT from the donation after citizens' death. This was permitted by the Ethics Committee of the Third Xiangya Hospital, and the liver was attributed by the China Liver Transplant Registry. The donor, a 44-year-old man, was matched for ABO blood type (B positive), and shared two human leukocyte antigen (HLA) loci (B46 and DQ9) with the patient. The operation was well performed, and the postoperative course was uneventful. The immunosuppressive regimens consisted of steroids, tacrolimus [0.10 – 0.20 mg/(kg . d), adjusted by drug concentration ranging from 6 – 8 ng/mL] and mycophenolate mofetil (500 mg, twice per day). The liver function recovered to normal level in 10 days (Fig. 1A and 1B).

Unfortunately, on postoperative day (POD) 25, the patient manifested skin rashes, on the extremities first, and soon spread to the trunk and face (Fig. 2A). The pathological examination of the rash showed nonspecific manifestations including epidermal dyskeratosis, lymphocytic infiltrates, apoptotic bodies and cellular vacuolization of the epithelium. Moreover, the patient got fever progressively, ranging from 38 to 40 °C, and severe diarrhea (watery stool, > 10 times per day). Several days later, he developed pancytopenia (Fig. 1C, 1D and 1E), and complained of dysphagia due to oropharyngeal ulcers. The white blood cells (WBC) dropped to the minimum of 0.76×10^9/L on POD 30. Bone-marrow biopsy showed no meaningful result, and the test of cytomegalovirus (CMV) DNA was negative. Antiviral drugs and antibiotics were used empirically, but the symptoms were not mitigated.

Given all the symptoms and the biopsy results above, we suspected that the patient had GVHD. The essential of GVHD is the alloreaction to the recipient's tissues caused by the immunocompetent cells from the donor, thus detecting donor lymphocytes in the recipient's peripheral blood or tissues involved, namely chimerism, could help make a definitive diagnosis of GVHD [3,5]. Because the chimerism rate might be quite low, a novel technique involving quantitative real-time polymerase chain reaction (qPCR) with the markers of insertion-depletion polymorphisms (InDels) was applied (Shanghai Tissuebank Diagnostics Co., Ltd., Shanghai, China). Briefly, the DNA samples extracted from the peripheral blood of the donor and from epithelia cells of the recipient's pharynx (sampled by throat swab, avoiding from ulcers and blood of the recipient, regarded as the specimen pre-transplantation) were screened with 30 InDels markers respectively. Markers that were specific in the donor were determined as the "fingerprinting" for detecting chimerism in the recipient's samples post-transplantation, and chimerism rate could be calculated by the delta-delta cycle threshold method [6].

Two donor-specific InDels markers were identified in the screening test, and were used for calculating the chimerism rate. On POD 31, when the patient suffered from severe symptoms, the mean chimerism rate in peripheral blood was 1.8%, and 6.3% in skin tissue. The typical clinical symptoms and pathological manifestations as well as the existence of chimerism confirmed the diagnosis of GVHD.

The treatment strategies were immediately adjusted. Tacrolimus and mycophenolate mofetil were stopped on POD 28. Oral prednisone was switched to injected methylprednisolone, 120 mg for 3 days from POD 28, and reduced gradually (80 mg for 5 days, 40 mg for the next 5 days and then switched back to oral prednisone). Basiliximab was administrated on POD 30 and 32, 20 mg per day. Combined anti-infection therapies were applied, and strict isolation was enforced in intensive care unit. Supportive therapies, including administration of colony stimulation factor, strengthened nutrition and intensive oral care, were also given. During this period, the liver function remained normal all the time.

With all the treatments above, the patient's condition showed improvement. The temperature was controlled, and the diarrhea was relieved gradually. Nearly one month after the onset, all the skin rashes disappeared (Fig. 2B), and pancytopenia was almost recovered. The follow-up test of chimerism on POD 58 showed that the mean rate of chimerism in peripheral blood was merely 0.007%. The patient was discharged on POD 96 and kept follow-up with a good condition.

Although GVHD after HSCT has been well illustrated and has a relatively sound prognosis, the pathogenesis of GVHD after OLT has not been fully clarified, and the mortality remains high. The prerequisites for the development of GVHD after OLT are considered the same as those in HSCT: the graft must contain sufficient immunocompetent cells; the host must be recognized as foreign by the cells from graft; the host must be unable to reject the graft before it mounts allogeneic immune response [1]. It is estimated that about 10^9 to 10^{10} donor lymphocytes remain in the portal

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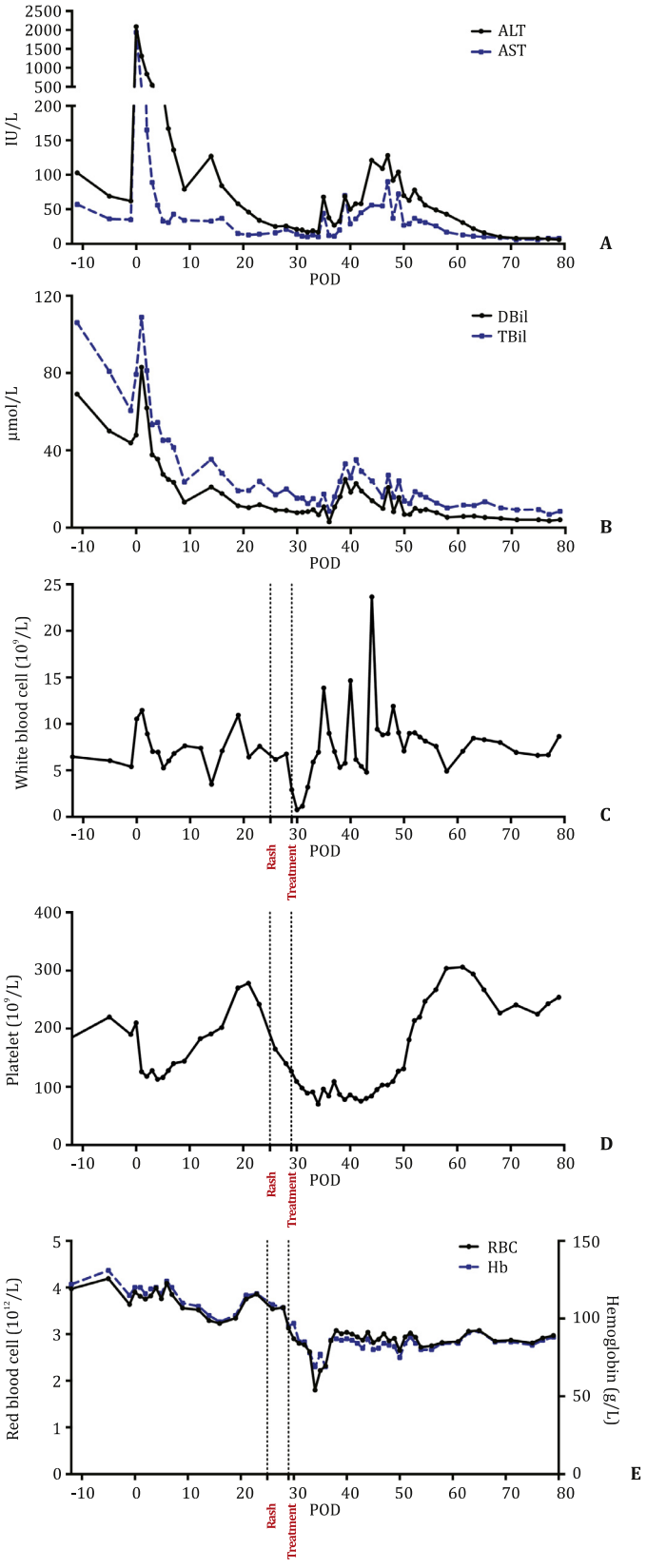

Fig. 1. The liver function and blood routine of the patient post liver transplantation. **A:** Alanine aminotransferase (ALT) and aspartate aminotransferase (AST); **B**: The direct bilirubin (DBil) and total bilirubin (TBil); **C**: The white blood cells; **D**: The platelets; **E**: The red blood cells and hemoglobin.

tracts and parenchyma of the liver graft, even if the graft has been flushed with cold preservation, and the remaining engrafted lymphocytes are enough to mount an immune response to the recipient [7]. Factors that permit patients to lapse into an immunocompromised state, such as immunosuppressive treatment before transplantation and recipients aged over 65 (especially giant age gap between young donors and old recipients), were reported as risk factors for development of GVHD after OLT, which supported the prerequisites talked above [2,4,8]. Interestingly, some patients experienced acute cellular rejection to the donor liver before the onset of GVHD, which was also reported as a risk factor [2,5,7]. This makes it more complicated for GVHD after OLT.

There is no standard diagnostic criterion for GVHD after OLT. The manifestations of GVHD usually present at two to eight weeks after OLT, including rash, fever, diarrhea, pancytopenia, and a normal liver function [1,2,7]. These symptoms can be a clue for GVHD, but they are more commonly seen with drug reaction or infections, which easily leads to misdiagnosis or the delay of exact diagnosis and proper treatment. For patients highly suspected GVHD, it is advocated that biopsy should be done to the involved tissues. The typical finding of skin biopsy is epidermal necrolysis, but it is also seen in drug-induced rash; the intestinal biopsy with apoptosis of crypt cells may be more specific, but it is invasive and not suitable for all patients [7]. Thus, pathological evidence could support the diagnosis of GVHD, but still not specific enough for confirmation.

Although transient chimerism appears in the majority of patients in the early stage post OLT, which peaks within the first week and usually disappears three to four weeks postoperation, persistent high level of chimerism supports the definitive diagnosis of GVHD [1,3,5,8,9]. Short tandem repeats (STR) tested by PCR and capillary electrophoresis is currently the most widely used technique, in which the chimerism rate is calculated by the fluorescence intensity of the STR markers. However, the sensitivity is relatively low (positive when the chimerism rate is over 1% to 5%) and the interpretation of results is difficult due to the existence of stutter bands, which may cause false negative when diagnosing GVHD [6,10]. Compared with STR-PCR, the InDels technique has an informative marker in $>$ 99.9% of unrelated individuals, a much higher sensitivity (positive when the chimerism rate is 0.01% to 0.001%) and a quantitative result. In HSCT patients, the InDels assay showed an excellent correlation with those from STR-PCR assay, which further proved its reliability [6,10]. The feasibility and enhanced sensitivity make the InDels technique suitable for detecting chimerism in patients after OLT, which contributes to the early diagnosis of GVHD.

The treatment to GVHD after OLT is largely empirical. Although extensive literatures on managing GVHD after HSCT provide guidance, the effect of the treatments, such as high dose of corticosteroids and use of monoclonal antibody, is not ideal [2,8]. The principle for adjustment of immunosuppressive regimens (whether to decrease or to increase, or even to switch drugs) is still confusing, and the effects varied enormously in reported literatures [1–3,5,7,8]. Therefore, no definitive treatment has been advised. In the light of our experience, the treatment should be personalized for a given patient, based on the respective immunological status. For this successful case, the core treatment strategy consists of basiliximab, drug withdraw (calcineurin inhibitors and antimetabolites) and steroids. Within the limited cases reported, this strategy has a relatively better prognosis [8]. At the early stage, IL-2 receptor monoclonal antibody is helpful to control the expansion of the lymphocytes from the donor, which are IL-2-dependent. Drug withdraw contributes to the restoration of the recipient's immune system so that it could clear the donor's cells itself. Steroids should be maintained at a certain level for its effects of anti-inflammation and prophylaxis of acute rejection. Benefiting from early diagnosis