CASE REPORT

Fluorescence in situ hybridization-based confirmation of acute graft-vs-host disease diagnosis following liver transplantation: A case report

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Abstract

BACKGROUND

Although acute graft-vs-host disease (aGvHD) is a rare complication of liver transplantation, it is poorly understood and has an extremely high mortality rate. No standardized diagnostic criteria or treatment regimens currently exist.

CASE SUMMARY

The present study investigated the etiology, diagnosis, and treatment of aGvHD following liver transplantation. Presentation, diagnosis, disease course, histology, and treatment of an aGvHD case are reported, and associated literature is reviewed. A 64-year-old female required LTx due to primary biliary cirrhosis. The donor was a 12-year-old male. Three weeks following liver transplantation, the recipient developed pyrexia, diarrhea, rashes, and antibiotic-unresponsive pancytopenia. Clinical symptoms together with laboratory investigations suggested a diagnosis of aGvHD, which was confirmed via peripheral blood fluorescent in situ hybridization. Donor XY chromosome fluorescent in situ hybridization indicating early chimerism achieved 93% sensitivity in the detection of GvHD. Existing immunosuppressants were discontinued, and high-dose intravenous methylprednisolone was initiated along with antibiotics. While diarrhea resolved, the patient’s general condition continued to deteriorate until demise due to multi-system organ failure at 37 d post-liver transplantation. This case illustrates the life-threatening nature of aGvHD.

CONCLUSION
The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

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Herein, we have summarized a post-LTx aGvHD case and reviewed associated literature in order to increase awareness and provide potentially risk-mitigating recommendations.

**Key Words:** Liver transplantation; Graft-vs-host disease; Fluorescence in situ hybridization cytogenetics; Chimerism; Diagnosis; Case report

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**Core Tip:** At present, the risk factors, pathogenesis, optimal treatment, and prognosis associated with acute graft-vs-host disease following liver transplantation are unclear. Currently, the most reliable diagnostic method is specific immunostaining for donor-specific antigens. If the donor is male and the recipient is female, fluorescent in situ hybridization-based detection of the Y chromosome is a diagnostic option. In the present case, acute graft-vs-host disease was confirmed via fluorescent in situ hybridization, demonstrating the presence of male donor DNA.

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**INTRODUCTION**

Acute graft-vs-host disease (aGvHD) is one of the most dangerous complications following liver transplantation (LTx)[1]. It involves overactivation of donor helper T lymphocytes by recipient antigen-presenting cells, leading to a local inflammatory reaction against recipient tissue. Although the rate of aGvHD incidence after LTx is low (1%-2%), the mortality rate is extremely high (85%-90%)[2]. Skin rash and pyrexia are the most frequently noted early signs, followed by leukopenia. Although aGvHD was first proposed as a clinical entity in 1988, its mechanisms and optimal treatment strategies remain controversial[3]. Modification of the post-transplant treatment plan, including incorporation of more effective immunosuppressants, has a limited effect on the course of aGvHD[4,5]. In most cases, death results from overwhelming sepsis or gastrointestinal hemorrhage as a consequence of bone marrow involvement[6]. Due to the low incidence (but high mortality) of aGvHD following LTx, analysis of the present case with respect to existing literature is worthwhile in order to raise awareness regarding the condition, which may assist in the early diagnosis of suspected cases. It will also help improve diagnostic criteria and establish standardized evidence-based treatment regimens. Moreover, we wish to draw attention to the diagnostic utility of sex chromosome fluorescent in situ hybridization (FISH) when the donor and recipient are of different chromosomal sexes.

**CASE PRESENTATION**

**Chief complaints**

The patient was a 64-year-old female with primary biliary cirrhosis, esophageal-fundal variceal hemorrhages, and decompensated hepatocirrhosis in September 2017.

**History of present illness**

A 64-year-old female received a liver from an ABO-matched (A-positive) 12-year-old male cadaveric donor. The donor and recipient details are shown in Table 1. The donor was a 12-year-old male. Three weeks following liver transplantation, the recipient developed pyrexia, diarrhea, rashes, and antibiotic-unresponsive pancytopenia.

| Table 1 | 
| --- | 
| **Recipient** | **Donor** |
| Age | 64 | 12 |
| Gender | Female | Male |
| ABO Type | A-positive | A-positive |
| Primary Diagnosis | Primary biliary cirrhosis | 

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Table 1 Recipient and donor demographic, clinical, and typing data

| Recipient | Donor |
|-----------|-------|
| Age       | 64    | 12 |
| Sex       | Female | Male |
| Primary complaint | PBC | Hypoxic-ischemic encephalopathy |
| Special history | Low-dose glucocorticoids | NA |
| Blood group | A | A |
| HLA | NA | NA |

PBC: Primary biliary cirrhosis; HLA: Human leukocyte antigen; NA: Not applicable.

History of past illness
A 64-year-old female with primary biliary cirrhosis, esophageal-fundal variceal hemorrhages, and decompensated hepatocirrhosis.

Personal and family history
The patient grew up in her locality, denies any contact with contaminated water or radiation exposure, and denies smoking and alcohol consumption.

Physical examination
On physical examination, we found her poor nutritional status, the abdomen was moderately distended with mild tenderness, and there was moderately yellow staining of the skin and mucous membranes. The rest of the physical examination revealed no abnormal findings.

Laboratory examinations
The following timeline of events refers to post-operative days. On day 22, the patient developed pyrexia of unknown origin, fluctuating between 38.2 °C and 39.3 °C. On day 26, sex chromosome FISH was performed on peripheral venous blood samples. No gastrointestinal tract lesions were apparent, and no evidence of aGvHD was noted on gastrointestinal endoscopic biopsy (histologically normal esophagus, stomach, and ileum). On day 31, the presumptive diagnosis of GvHD was made based on the following clinical ground observations: Generalized maculopapular eruption (largely involving the back, neck, and face), pyrexia, pancytopenia, low blood pressure, and watery diarrhea (Figure 1 and Table 2). FISH revealed chimerism (presence of the fluorescently stained donor XY chromosome) consistent with aGvHD (Figure 2).

Two days following the development of thrombocytopenia, a bone marrow biopsy revealed marked hypocellularity. No skin rash was yet apparent. The findings of detailed post-operative laboratory investigation are summarized in Table 3. Because no sample of indwelling peripheral blood from the donor prior to LTx was available, donor lymphocytes could not be identified in recipient peripheral blood using short tandem repeat sequencing or human leukocyte antigen (HLA) typing.

Imaging examinations
Abdominal computed tomography and color ultrasound findings suggested laminar portal vein, inferior vena cava, hepatic artery, and hepatic venous flow (Figure 3).

FINAL DIAGNOSIS
aGvHD, primary biliary cirrhosis, esophageal-fundal variceal hemorrhages, and decompensated hepatocirrhosis.

TREATMENT
Initial treatment involved tapering the dosage of immunosuppressants to allow the recipient immune system to reject donor lymphocytes. Due to the inefficacy of this
Table 2 Clinical manifestation and treatment timeline

| PO day | Temperature (°C) | Skin rash | Diarrhea | Myelosuppression | Tacrolimus (mg/d) | MMF (g/d) | MP | IgG (g/d) | Antibiotics |
|--------|-----------------|-----------|----------|------------------|------------------|-------------|-----|-----------|-------------|
| 22     | 38.3            | Palm      | 2        | NA               | 3                | 0.25        | 500 | 10        | Yes         |
| 24     | 38.6            | Neck      | 3        | NA               | 2                | 0           | 500 | 10        | Yes         |
| 26     | 38.5            | Face      | 6        | Yes              | 2                | 0           | 120 | NA        | Yes         |
| 28     | 38.2            | Trunk     | 7        | Yes              | 2                | 0           | 40  | NA        | Yes         |
| 30     | 39              | > 35%     | 6        | Yes              | 2                | 0           | 20  | NA        | Yes         |
| 32     | 38.6            | > 50%     | 5        | Yes              | 1.5              | 0           | 20  | 10        | Yes         |
| 34     | 38.7            | > 55%     | 4        | Yes              | 1.5              | 0           | 20  | 10        | NA          |
| 36     | Demise          |           |          |                  |                  |             |          |           |             |

Table 3 Post-operative laboratory investigation timeline

| Value/PO day | 0 | 4 | 8 | 12 | 16 | 20 | 24 | 26 | 30 | 34 | 36 |
|--------------|---|---|---|----|----|----|----|----|----|----|----|
| AST (U/L)    | 643 | 47.6 | 56 | 48 | 64 | 75 | 63 | 56 | 44 | 52 | 74 |
| ALT (U/L)    | 772 | 88.1 | 96 | 86 | 107.3 | 62 | 59 | 64 | 66 | 71 | 83 |
| Total bilirubin (mg/dL) | 231.4 | 123.5 | 119.5 | 76.9 | 65.5 | 23.7 | 25.8 | 24.2 | 35.1 | 45.6 | 48.7 |
| Direct bilirubin (mg/dL) | 146.1 | 63.2 | 59.3 | 43.2 | 38.1 | 13.3 | 15.6 | 16.5 | 24.7 | 28.5 | 31.2 |
| Leukocyte count × 10^9/L | 17.5 | 8.7 | 12.4 | 17.3 | 7.2 | 6.7 | 1.3 | 0.39 | 0.24 | 0.12 | 0.08 |
| Neutrophil % | 93 | 79 | 86 | 92 | 81 | 80 | 63 | 17.9 | 0 | 0 | 0 |
| Hemoglobin (g/L) | 89 | 92 | 176 | 113 | 92 | 85 | 75 | 63 | 58 | 53 | 47 |
| Hematocrit % | 42 | 46 | 50 | 32 | 26.5 | 23.3 | 22 | 17.6 | 16.5 | 15.6 | 14.8 |
| Platelets × 10^9/L | 21 | 26 | 44 | 58 | 77 | 73 | 71 | 56 | 47 | 46 | 41 |
| Prothrombin time (s) | 17.9 | 19.9 | 16.5 | 22.4 | 13.5 | 13.1 | 12.7 | 13.1 | 12.8 | 13.2 | 13.6 |
| INR | 1.82 | 1.7 | 1.34 | 1.98 | 1.05 | 1.01 | 0.97 | 0.99 | 0.98 | 1.02 | 1.07 |
| Sodium (mmol/L) | 147 | 145 | 142 | 139 | 136 | 134 | 143 | 138 | 139 | 143 |
| Potassium (mmol/L) | 3.8 | 4.5 | 3.1 | 3.4 | 3.6 | 3.8 | 3.9 | 4.2 | 4.1 | 3.9 | 3.7 |
| Urea (mmol/L) | 32.52 | 29.8 | 16.42 | 4.55 | 4.77 | 4.46 | 4.13 | 3.8 | 4.17 | 3.74 | 4.02 |
| Creatinine (μmol/L) | 89.73 | 85.64 | 64.59 | 43.78 | 58.44 | 53.76 | 49.19 | 46.52 | 27.26 | 24.54 | 30.45 |
| PCT (ng/mL) | 5.73 | 3.86 | 11.5 | 5.1 | 1.86 | 2.65 | 2.58 | 2.45 | 2.18 | 3.65 | 4.53 |

PO: Post-operative; NA: Not applicable; MMF: Two oral formulations of mycophenolate mofetil; MP: Methylprednisolone; IgG: Immunoglobulin G. Antibiotics: Melophenan (1 g every 8 h) + carpophennet (50 mg per day) + vancomycin (0.5 g every 6 h).

OUTCOME AND FOLLOW-UP

Severe inflammation induced multi-system organ failure, which led to the patient’s demise on post-operative day 37.
DISCUSSION

At present, the risk factors, pathogenesis, optimal treatment, and prognosis associated with aGvHD following LTx are unclear. Current (incomplete) understanding of aGvHD pathogenesis may be summarized as follows. The conditioning regimen induces initial recipient tissue damage, followed by auto- and alloantigen denudation in the recipient concomitant with antigen-presenting cell activation and massive inflammatory cytokine release (a “cytokine storm”). If a sufficient number of donor lymphocytes, especially T lymphocytes, of the correct specificity are present, direct recognition of and activation by antigen-presenting cell (either locally or within secondary lymphoid tissues) results in T lymphocyte interleukin (IL)-2 and IL-2R expression. Activated T-cells then stimulate donor monocytes to produce significant levels of myeloid cytokines (e.g., IL-1 and tumor necrosis factor) and also trigger a cascade of cytotoxic signal transduction pathways, such as the perforin/granzyme B or Fas/FasL pathways (although direct cytokine-mediated injury is also possible). Finally, inflammatory infiltration in the digestive tract, skin, and bone marrow leads to severe clinical presentations[7]. In the present case, abnormally high numbers of CD8+ T lymphocytes were present during the acute phase of GvHD, while the CD4+/CD8+ T lymphocyte ratio was less than 0.1. This indicates that perhaps cytotoxic T lymphocytes (with a minor contribution by helper T lymphocytes) are the cells primarily involved in GvHD pathogenesis. In summary, the necessary conditions for the occurrence of aGvHD[8-10] include the presence of donor immunoreactive cells within graft tissue, presence of recipient tissue antigens not present in donor organ
Figure 2 Twelve erythrocytes analyzed, 11 showed an XY signal pattern, while one showed an XX signal pattern (91.7% showed one X and one Y signal, and 8.3% showed two X signals). Y is the red fluorescent signal; X is the green fluorescent signal.

Figure 3 The portal vein, inferior vena cava, hepatic artery, and hepatic venous blood flow were smooth.

Tissue, and inability of the recipient immune system to eliminate effectively donor leukocytes.

Triulzi et al[9] have described the diagnostic criteria for aGvHD following LTx in the following three requirements: (1) Characteristic clinical symptoms affecting related organ systems (e.g., skin, gastrointestinal tract, and bone marrow), including rash, diarrhea, and pancytopenia, among others; (2) Abnormal skin or digestive tract histology; and (3) HLA or DNA evidence of donor immunoreactive lymphocytes in involved organs or peripheral blood of the recipient. In addition to the above criteria, T lymphocyte counts and cytokine quantitation provide clear diagnostic support. Currently, the most reliable diagnostic method is specific immunostaining for donor-specific antigens. If the donor is male and the recipient is female, FISH-based detection of the Y chromosome is a diagnostic option[9,11,12]. At present, no false negatives have been reported for this method. In the present case, aGvHD was confirmed via
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CONCLUSION

In the present case, aGvHD was confirmed via FISH, demonstrating the presence of male donor DNA. If the donor is male and the recipient is female, FISH-based detection of the Y chromosome is a diagnostic option.
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