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CASE REPORT

Catastrophic graft-versus-host disease after lung transplantation proven by PCR-based chimerism analysis

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Summary

Acute graft-versus-host disease (GvHD) is a rare complication after solid organ transplantation. We describe a 52-year-old female developing neutropenia and fever 48 days after single lung transplantation for chronic obstructive pulmonary disease. Bone marrow (BM) biopsy suggested drug-induced marrow failure, so immunosuppression was reduced. Five days later a maculopapular skin rash was observed, progressing to a generalized erythema with desquamation. Skin biopsy was suspectable for GvHD, so immunosuppression was re-initiated. PCR-based chimerism analysis of BM revealed 78% donor cells. Intensified immunosuppression resulted in temporary improvement, but BM aplasia recurred and the patient experienced severe GvHD of gut and liver. Despite extensive immunosuppression the patient died from multi-organ failure 99 days after transplantation. This report describes the occurrence of neutropenia as an early presenting sign of acute GvHD after lung transplantation. We therefore recommend incorporating GvHD in the differential diagnosis of neutropenia after solid organ transplantation, calling for early chimerism analyses.

Introduction

Graft-versus-host disease (GvHD) occurs when immunocompetent lymphocytes are transfused into a recipient who is unable to mount a host response to the cells because of human leucocyte antigen (HLA)-one way compatibility and/or immunosuppression. This allows engraftment of these cells leading to a rejection of the host because of immunological differences. GvHD mainly occurs after haematopoietic stem cell transplantation (HSCT) but can also be seen after transfusion of blood components containing viable T lymphocytes (transfusion-associated GvHD, TA-GvHD) and after solid organ transplantation. After HSCT, GvHD is a common complication with an incidence of 50–90%, but is rare and often fatal following solid organ transplantation [1–6]. Acute GvHD primarily affects skin, gastrointestinal tract and liver and is graded clinically on a scale of I to IV based on the severity and number of organ systems involved [7]. In contrast to GvHD following HSCT, in solid organ transplantation often additional bone marrow (BM) aplasia – induced by passenger leucocytes from organ allografts – can occur [4,8]. Normally, diagnosis of GvHD depends on clinical suspicion, supported by histology of appropriate biopsies, and demonstration of donor lymphocyte chimerism in the host tissue.

We describe, in this article, a case of fatal GvHD following lung transplantation primarily presenting with pancytopenia, fever and maculopapular skin rash. GvHD was confirmed by detection of donor leucocytes using a PCR-base technique.

Case report

In December 2007, a 52-year-old woman with end-stage chronic obstructive pulmonary disease underwent single lung transplantation from a 19-year-old male donor. HLA class I and class II genes were typed at the high resolution level by sequence-based typing. HLA-type of the donor was A*02 A*30, B*18 B*44, DRB1*11 DRB1*13 and of the patient A*02 A*68, B*18 B*39, DRB1*11 DRB1*13 respectively. During surgery, extracorporeal membrane oxygenation was not required and peri-operatively no blood products were given. Lymphocytotoxic antibody screening from the patient was negative 1 day before transplantation. Postoperative immunosuppressive therapy consisted of a triple regimen of tacrolimus (FK 506, target level 18 ng/ml), mycophenolate mofetil (MMF) and prednisone. Because of seizures and cerebral microangiopathy, proven by magnetic resonance imaging, on day 15 after transplant (TX), tacrolimus was switched to cyclosporine A (CsA, target level 300–350 ng/ml). The patient was discharged on day 25 after TX with cotrimoxazol and valaciclovir prophylaxis.

Three weeks later (day 50 after TX) she was readmitted with pyrexia, neutropenia (0.1×10^9/l), thrombocytopenia (47×10^9/l) and anaemia (reticulocytes <30 G/l). Suspecting infectious complication or drug-induced marrow failure, MMF was promptly discontinued and therapy with granulocyte colony-stimulating factor (G-CSF) was initiated. Polymerase chain reaction (PCR) for cytomegalovirus and Parvovirus B19 were negative. Leucocyte counts did not rise during G-CSF treatment. BM biopsy on day 53 after TX revealed hypocellularity suggesting toxic marrow failure, thus CsA was stopped on day 62 after TX. Molecular diagnostic of BM and peripheral blood with regard to post-TX lymphoma were negative. Unfortunately, at this time, chimerism analyses were not performed. Five days after withdrawal of CsA, the patient developed a maculopapular skin rash with subsequent desquamation, starting on the palmo-plantar surfaces and rapidly progressing to >75% of the body surface (Fig. 1). Skin biopsy showed a lichenoid reaction pattern with basal vacuolar changes and dyskeratosis consistent with either a drug eruption or GvHD. Because of haemodynamic deterioration, the patient was admitted to the intensive care unit on day 77 after TX. Suggesting acute GvHD IV, immunosuppressive treatment was intensified according to the experience in HSCT [9].

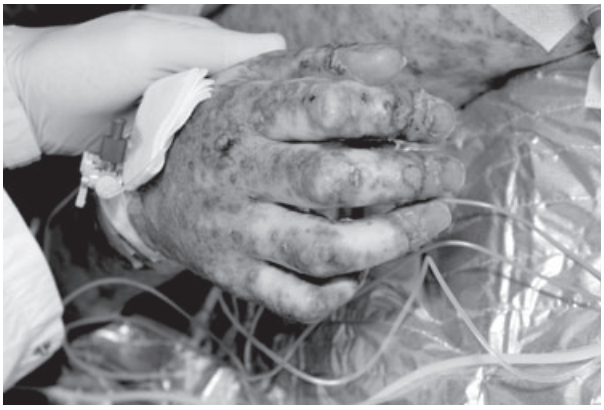

Figure 1 Graft-versus-host disease i.v. of the skin on day 77.

CsA was re-administered; prednisone increased to 2 mg/kg body weight, and additionally extracorporeal photochemotherapy (ECP) was initiated.

In order to substantiate the diagnosis of GvHD, we retrospectively analyzed retained BM and peripheral blood cells and BM biopsy obtained on day 53 after TX, with regard to chimerism.

Results of PCR-based chimerism studies revealed a recipient:donor lymphocyte ratio of 22:78% already present on day 53 after TX. In contrast, fluorescent *in situ* hybridization (FISH) analysis of retained BM biopsy (50 cells analyzed) and fresh skin biopsy (31 cells analyzed) only demonstrated female (recipient) cells. Intensified immunosuppressive therapy resulted in a remarkable attenuation of the skin lesions, and peripheral leucocyte counts increased up to 2.3×10^9/l. On day 82 post-TX, massive diarrhoea and severe gastrointestinal bleeding occurred; endoscopy revealed the presence of intestinal GvHD, so additionally basiliximab (anti-interleukin-2 receptor monoclonal antibody) at a dose of 20 mg i.v. on days 82, 89 and 96 after TX was given to intensify immunosuppression. However, the patient developed hyperbilirubinaemia suggesting additional GvHD of the liver, and BM aplasia recurred.

Chimerism analysis of sorted peripheral blood leucocytes on day 89 after TX revealed a donor cell ratio of >95% in CD33 and CD3 positive cell fraction. Because of severe gastrointestinal bleeding, ammonia levels increased to >2000 μmol/l (normal value <50 μmol/l) consequently leading to generalized cerebral oedema. The patient subsequently died from multi-organ failure 99 days after transplantation. FISH analysis of the postmortem-related BM biopsy showed 67 out of 74 (91%) cells to be positive for X- and Y-chromosomes (donor cells).

Details on clinical course and immunosuppressive therapy are shown in Fig. 2.

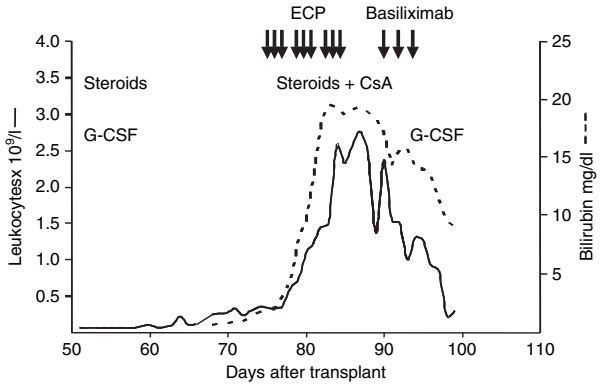

Figure 2 Clinical course and immunosuppressive therapy. ECP, extracorporeal photochemotherapy; G-CSF, granulocyte colony stimulating factor; CsA, cyclosporine A.

Chimerism analysis

Chimerism analysis was based on amplification of the STR loci D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820, and the gender marker Amelogenin with the AmpFlSTR®Profiler™ PCR Amplification Kit as recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). Fragment analysis was performed using the ABI PRISM 3100 Genetic Analyzer with the ABI PRISM GENESCAN ANALYSIS Software (Applied Biosystems). A minor cell population representing 5% of total cells was reproducibly detected in several independent dilution experiments [10].

Fluorescent *in situ* hybridization study

Fluorescent *in situ* hybridization analysis was carried out on the initial BM smear and skin biopsy and on postmortem-related BM biopsy using directly labelled centromeric probes for the X- and Y- chromosomes according to the manufactures instructions (Abbott Laboratories. Abbott Park, IL, USA). FISH images were captured using Isis Metasystems (Metasystems, Altusheim, Germany).

Discussion

Graft-versus-host disease in solid organ transplantation is related to the lymphoid characteristic of the transplanted organ. The highest number of GvHD after solid organ TX is reported in liver transplantation, thus supporting the theory that the pathogenesis of GvHD is dependent on the transfer of a high number of donor lymphocytes [5,11,12]. Lung allograft recipients might therefore also be at risk for GvHD because of the high amount of lymphoid tissue transplanted with the graft [5,13]. However, as up to this stage, more than 21 000 lung transplantations have been performed worldwide but less then 10 cases of GvHD in lung allograft recipients have been reported [4,5,13,14]. As the clinical and pathological diagnosis of GvHD is difficult and the histological features are often nonspecific, the initiation of treatment is sometimes delayed, thus contributing to the poor outcome of patients. In order to establish a firm diagnosis, it is therefore essential to perform appropriate biopsies and chimerism analysis of the involved tissue, including BM and peripheral blood in case of neutropenia.

Recently, the documentation of GvHD by FISH and HLA typing has been reported [4,15,16]. In our case, chimerism analysis was based on amplification of the different STR loci and the gender marker amelogenin, whereas initial interphase FISH analysis of retained BM biopsy was, interestingly, not able to confirm the existence of donor cells. However, different samples were analyzed: BM aspirate and peripheral blood by a PCR-based technique and BM biopsy in paraffin by FISH.

Although the prognosis of GvHD after solid organ transplantation is unfavourable, early diagnosis and intensive immunosuppressive treatment may increase survival in these patients [17]. The major problem in this case was that chimerism analyses were not performed earlier from the initial BM biopsy on day 53. In addition, retrospective analyses from stored samples were not available until day 82 post-TX.

Several immunosuppressive agents such as steroids, calcineurin inhibitors (CsA, FK506), MMF, monoclonal antibodies (anti-IL-2 receptors, anti-CD23) anti-thymocyte globulin or ECP have been used for the treatment of acute GvHD after HSCT [9,18–20]. However, there are no controlled trials or guidelines as to the best treatment modality for GvHD related to solid organ transplantation. Therefore, patients normally are treated according to the experience in HSCT. Favourable results in patients not responding to steroids and CsA have been reported with the use of ECP or basiliximab (anti-IL-2 antibody) resulting in up to 86% and 82.5% response in HSCT respectively [9,20]. Because of profound pancytopenia, our experience in treatment of GvHD after HSCT and the patient's deleterious condition, we decided to augment baseline immunosuppression by ECP and cytokine blocking agents instead of additional lymphocytotoxic treatment.

Unfortunately, the clinical condition of our patient did not improve despite administration of steroids, CsA, ECP and basiliximab, probably because of delayed treatment.

By way of summarizing the present literature, diagnosis of solid organ TX-related GvHD should be suspected when a patient develops skin rash, fever, pancytopenia,

diarrhoea or a combination of these symptoms several weeks after transplantation. Early chimerism analysis of appropriate specimens (e.g. skin biopsy, BM aspirate) and peripheral blood using a PCR-based technique or FISH analysis – to identify the existence of donor DNA or sex chromosomal differences – serves as a valuable diagnostic test in suspected GvHD. In case of sudden pancytopenia after solid organ transplantation and supposed toxic marrow failure, GvHD has to be excluded before taper of immunosuppression.

Although GvHD after solid organ transplantation is rare, it is life-threatening, early identification is essential for improving the prognosis of this devastating disease.

Authorship

NW: wrote manuscript. AB: collected data. MB: analyzed skin biopsy. PJ: collected data. GM: perform PCR based chimerism analyses. BS: performed FISH analyses. FT: collected data. TS: analyzed data. KFL, GJL: contributed to manuscript.

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