Transfusion-associated graft-versus-host disease in immunocompetent patients: case series and review of the literature

Kemal Agbaht, Neriman Defne Altintas, Arzu Topeli, Ozay Gokoz, and Osman Ozcebe

BACKGROUND: Transfusion-associated graft-versus-host disease (TA-GVHD) is a fatal complication of transfusion of blood products that usually affects immunocompromised patients. Articles reporting this condition in immunocompetent recipients are usually from countries that still have problems in irradiation of blood products.

CASE REPORTS: This report presents fatal TA-GVHD in four immunocompetent patients referred from rural areas where blood irradiation is still not the routine procedure to our tertiary-care center between July 2004 and July 2005. A similar history and chronological order of events were observed: fresh whole-blood transfusion from relatives, fever, rash, liver dysfunction, diarrhea, and pancytopenia. Skin biopsies demonstrated Grade II to III GVHD involvement. Marrow biopsies showed hypoplasia. In two cases, HLA typing studies were performed. Donors were homozygous for a shared HLA haplotype in the patients. All cases were admitted to the intensive care unit within 3 weeks after transfusions with the diagnosis of sepsis, which rapidly progressed to septic shock and multiorgan failure. Another common observation was Candida albicans growth in blood cultures. Unfortunately, all died despite prompt and appropriate sepsis treatment, along with immunomodulatory therapy.

CONCLUSION: TA-GVHD is probably more prevalent than reported in the literature. It must be considered in the differential diagnosis, if the patient with a recent transfusion history admits with fever, skin rash, abnormal liver function tests, and pancytopenia associated with hypoplastic marrow. In rural areas where gamma irradiation is not possible, the overall policy of transfusion (e.g., restriction of transfusion indications and alternative methods for pathogen inactivation) should be reassessed.

ABBREVIATIONS: EBV = Epstein-Barr virus; HAV = hepatitis A virus; HSV = herpes simplex virus; TA-GVHD = transfusion-associated graft-versus-host disease.

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nonirradiated, fresh whole blood from blood relatives, and all died with *Candida albicans* fungemia.

**MATERIALS AND METHODS**

**HLA typings**

HLA typings were done by standard DNA testing methods with sequence-specific primers (Olerup SSP, Qiagen Vertriebs GmbH, Wien, Austria).

**Review of the literature**

We searched MEDLINE articles published between 1970 and September 2006 with the key word “transfusion-associated graft vs. host disease.” The search was limited to articles published in English. Pediatric cases were not included. Overall, 135 articles describing this entity were found, and 46 of them report 188 immunocompetent recipients with TA-GVHD.

**Case 1**

A 50-year-old man with known pancytopenia (with a hematocrit [Hct] value of 17.5 percent, hemoglobin [Hb] level of 6.3 g/dL, white blood cell [WBC] count of $2.1 \times 10^9$/L, platelet [PLT] count of $116 \times 10^9$/L, and mean cell volume of $121 \mu m^3$) due to vitamin B12 deficiency had received transfusion with 4 units of fresh whole blood from his brothers (1 unit from each brother) because of development of fatigue and pallor after an inguinal hernia operation in an urban hospital. Vitamin B12 replacement therapy was begun just after transfusions. Two weeks after the transfusion, he presented to his doctors with the complaints of fever, skin rash, and bloody diarrhea. He was referred to our center with the preliminary diagnosis of viral hemorrhagic fever in July 2004. On admission, he had widespread erythematous skin lesions and severe pancytopenia (Table 1). There was no history of medications other than vitamin B12 replacement injections. Other frequent causes of pancytopenia were excluded. Serologic studies for human immunodeficiency virus (HIV), parvovirus B19, Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), brucellosis, anti-nuclear antibody, anti-dsDNA antibody and studies for mycobacteria were unrevealing. Treatment with broad-spectrum antibiotics was started for febrile neutropenia. He received intravenous immune globulin (IVIG; 0.5 g/kg/day for 4 days), antithymocyte globulin (7.5 mg/kg/day for 2 days), methylprednisolone (1000 mg/day for 3 days), and granulocyte–colony-stimulating factor (G-CSF; 48 IU/day) with the possible diagnosis of TA-GVHD. Marrow aspiration biopsy revealed necrosis of the marrow, and skin punch biopsy obtained on the second day of admission revealed findings compatible with acute GVHD (Fig. 1). These findings were basal vacuolar degeneration, necrotic-apoptotic keratinocytes, and lymphocytic infiltration (Grade II). HLA typing studies revealed that one of his donors was homozygous for both Class I and Class II shared haplotype with the patient (Table 2). Potassium hydroxide (KOH) preparation of skin smear showed hyphae. Quantitative sampling from lower respiratory tract was positive for the presence of *C. albicans*, and blood culture was positive for the presence of both

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50</td>
<td>23</td>
<td>49</td>
<td>70</td>
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<tr>
<td>Sex</td>
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<td>Female</td>
<td>Male</td>
<td>Female</td>
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<tr>
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<td>Fresh whole blood</td>
<td>Fresh whole blood</td>
<td>Fresh whole blood</td>
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<td>Units of blood product</td>
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<td>5</td>
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<td>Fever onset (days after transfusion)</td>
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<td>8</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Rash onset (days after transfusion)</td>
<td>11</td>
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<td>14</td>
<td>15</td>
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<td>Bloody</td>
<td>Bloody</td>
<td>Not bloody</td>
<td>Not bloody</td>
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<tr>
<td>Duration to ICU admission (days after transfusion)</td>
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<td>15</td>
<td>20</td>
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<tr>
<td>On admission</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hct (%)</td>
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<td>32.7</td>
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<td>35</td>
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<tr>
<td>Hb (g/dL)</td>
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<td>ALT (IU/mL)</td>
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<td>737</td>
<td>333</td>
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<td>AST (IU/mL)</td>
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<td>10.9</td>
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<tr>
<td>Candidemia</td>
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<td>Present</td>
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<td>Length of ICU stay (days)</td>
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<td>9</td>
<td>3</td>
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<tr>
<td>Cause of death</td>
<td>Septic shock and MOF</td>
<td>Septic shock and MOF</td>
<td>Septic shock and MOF</td>
<td>Septic shock and MOF</td>
</tr>
</tbody>
</table>

ICU = intensive care unit; MOF = multiorgan failure.
Enterococcus faecalis and C. albicans. Fundoscopic examination also suggested retinal lesion due to candidiasis. He died on the fourth day of admission due to refractory septic shock. Autopsy study detected disseminated candidiasis as the cause of death.

Case 2
A 23-year-old woman in the postpartum period was admitted to our hospital in July 2004 with the development of maculopapular rash, fever, jaundice (indirect [un conjugated] and direct [conjugated] bilirubin levels were 2.5 and 8.4 mg/dL, respectively), and pancytopenia (Table 1). She was HIV-negative; immunoglobulin levels were normal; and autoimmune markers, EBV, and parvovirus B19 serologies were unrevealing. Folate and vitamin B12 levels were within reference ranges. There was no drug or chemical exposure and she was previously known to be healthy. The history revealed transfusion of 3 units of fresh whole blood from her cousins and brother on the 37th week of gestation because of a Hct level of 21.4 percent. She had an uncomplicated birth on the 39th week of gestation, her complaints started within the peripartum period. She was referred to our center with the preliminary diagnosis of a viral infection or systemic lupus erythematosus, based on aforementioned symptoms. Marrow aspiration and biopsy showed aplasia and skin biopsy revealed findings compatible with acute GVHD, Grade II (basal vacuolar degeneration, epidermal mononuclear infiltration, and basal membrane degeneration). KOH preparation of skin smear showed hyphae and blastospheres. Besides broad-spectrum antibiotics for febrile neutropenia, G-CSF (30 IU/day), IVIG (0.5 g/kg/day), lymphoglobulin (15 mg/kg/day), methylprednisolone (1000 mg/day), mycophenolate mofetil (1000 mg/day), and cyclosporine A (10 mg/kg/day) were started with the diagnosis of TA-GVHD. On the 3rd hospital day, HLA typing of peripheral-blood lymphocytes revealed HLA-A 11,6901, HLA-B 35,51, HLA-C 04,15, HLA-DRB1 1001,13, HLA-DQB1 03,05. Her blood-donor brother was found to be homozygous for a shared HLA Class I and Class II haplotype (Table 2). Marrow transplantation was planned but could not have been performed due to her deteriorating condition. She died on the 9th day of admission due to refractory septic shock and multiorgan failure. Blood culture obtained on the 2nd day of admission yielded Klebsiella pneumoniae, whereas another one obtained on the 6th day of admission yielded C. albicans growth.

![Fig. 1. Marrow and skin biopsy findings of Case 1. Marrow biopsy revealed necrosis and hypocellularity (A). Eosinophilic apoptotic keratinocytes, basal vacuolar degeneration in the epidermis, capillary endothelial degeneration, melanin incontinence, RBC extravasation, and a few mononuclear inflammatory cells are seen in the dermis (B).](image)
Case 3
A 49-year-old man was transferred to our hospital with the diagnosis of fever of unknown origin and septic shock in November 2004. He had received 9 units of fresh whole blood collected from different relatives (2 of them were his sons), because of massive duodenal bleeding 3 weeks before ICU admission. He had no other illness, and he had received no medication before onset of illness. Despite broad-spectrum antibiotics, his fever could not be controlled. Therefore, he was referred to our hospital for further investigation. Prior culture results were unrevealing. Upon arrival, he had to be intubated because of hypoxemia. The first laboratory studies revealed anemia and leukopenia and impaired liver functions (Table 1). The patient was apparently immunocompetent. Results of the laboratory tests were negative for the presence of HIV, parvovirus B19, EBV, CMV, HAV, HBV, HCV, and herpes simplex virus (HSV). There was a diffuse confluent, maculopapular, patchy-type erythematous rash on his skin. Skin biopsies were compatible with Grade II GVHD (vacuolar degeneration of basal keratinocytes, basal membrane degeneration, and intraepidermal lymphocytes). Hypocellularity was noticed in marrow biopsy, no malignant cells were observed. In addition to broad-spectrum antibiotics, methotrexate (30 mg/day), methylprednisolone (1000 mg/day), and cyclosporine A (300 mg/day) were started as immunomodulatory therapy with the diagnosis of GVHD. The patient died, however, on the third day of admission with septic shock. The blood culture yielded \textit{C. albicans} growth; no other growth was reported.

Case 4
A 70-year-old woman was admitted to the emergency department in July 2005 with deteriorating consciousness during the past few days and a rash that had started 5 days ago. The rash did not respond to antihistamines. Her past medical history was insignificant except for a coronary artery bypass graft surgery 2 weeks before the onset of her complaints. During surgery she had received 5 units of fresh blood donated by her different relatives (three of them were her descendant boys) at another center. She was hypotensive and hypoxemic. Physical examination revealed generalized macular erythematous rash and bilateral basilar rales. She was admitted to the intensive care unit with the diagnosis of severe septic shock and multiorgan failure. Laboratory examination revealed leukopenia and thrombocytopenia (Table 1). Serologic studies for HIV, parvovirus B19, EBV, CMV, HAV, HBV, HCV, HSV, and brucellosis revealed insignificant results. Creatinine level increased to 4.1 mg per dL, lactate dehydrogenase (LDH), and transaminases were elevated, which were all within normal limits before the surgery, as documented by the prior laboratory reports. Broad-spectrum antibiotic therapy consisting of ofloxacin, piperacillin-tazobactam, and vancomycin was started after obtaining samples for cultures. Prednisolone at a dose of 50 mg per day IV was added to vasopressors for refractory septic shock. She died within 36 hours of admission to the hospital, however. Skin biopsy was compatible with acute Grade III GVHD (basal vacuolization, mainly epidermal but also dermal mononuclear cell [MNC] infiltration, and bullae formation). Marrow biopsy showed hypocellularity without malignant cells. Blood culture revealed \textit{C. albicans} growth.

**DISCUSSION**
TA-GVHD is a fatal complication of transfusion of nonirradiated blood products. It is rarely encountered in everyday practice, probably because of the improvement in blood transfusion protocols. Therefore, it might not be in the list of differential diagnoses of clinicians for patients presenting with pancytopenia, skin rash, fever, or jaundice. Likewise, our patients were referred to our hospital with the preliminary diagnoses of viral infection or autoimmune diseases. The diagnoses were suspected from patient histories and chronology of the events and were confirmed by skin and marrow biopsies and additionally in two patients with HLA matching studies. Although TA-GVHD in the context of nonirradiated directed donations is a well-known phenomenon, the frequency of cases admitted with TA-GVHD to our institution in a short period of time is striking. Even though our tertiary care center admits patients from all around Turkey, our observation may suggest that TA-GVHD is underreported and misdiagnosed.

The etiology of pancytopenia can be simplified in two main categories: 1) causatives that result in hypocellular marrow (for example, toxins, medications, TA-GVHD, and virus-associated aplastic anemia) and 2) causatives that result in cellular marrow (for example, metastatic solid tumors, autoimmune diseases like systemic lupus erythematosus and Sjögren syndrome, vitamin B12 and folate deficiencies, overwhelming infection and/or sepsis, brucellosis, mycobacteria, storage disease as Gaucher and Niemann-Pick). Therefore, marrow aspirate helps clinicians in the differential diagnosis of pancytopenia. In all of our cases, marrow was hypoplastic and other potential causes of pancytopenia associated with hypoplastic marrow were excluded.

It was thought that TA-GVHD is a disease of immunocompromised states, such as bone marrow transplantation (BMT), and congenital immunodeficiency syndromes, but in 1988 a report proposed that this condition may also occur in immunocompetent recipients. Subsequent reports supported this opinion. Our patients had no evidence of immunosuppression, but all had received blood products from their relatives, like...
most of the patients in aforementioned previous reports. TA-GVHD occurs due to engraftment of immunocompetent donor lymphocytes into the recipient. The main pathology is the inability of the recipient to mount an immune response against donor lymphocytes. As might be expected, the greatest risk for developing TA-GVHD is the presence of immunosuppression. A similar result is observed when the donor and recipient possess similar HLA antigens, such as when donors are family members or in populations that have a high homogeneity in HLA types, even in the absence of immunosuppression.\textsuperscript{1,9} Haploidentical donor lymphocytes may escape the recipient’s immune surveillance, engraft, proliferate, and attack recipient tissues reacting against the second haplotype or HLAs that are not shared. The result is the catastrophic clinical picture of TA-GVHD.\textsuperscript{1} The minimum number of active T cells necessary to induce TA-GVHD is unknown. Even an amount of $10^4$ per kg lymphocytes, however, may be lethal for a severely immunocompromised patient.\textsuperscript{13} Definitive diagnosis of TA-GVHD entails the demonstration of donor-derived cells or DNA fragments in the blood or affected tissues of the recipient. Obtaining direct evidence of donor cells in the recipient’s blood may be difficult, however, because those patients are frequently leukopenic at the time of investigation.\textsuperscript{16} In the present cases, history of blood transfusion from relatives, followed by typical chronological events of TA-GVHD, histologic findings of skin, and marrow biopsies, and finally, in two patients, HLA studies, strongly supported our diagnosis.

Skin involvement of TA-GVHD is graded according to histopathologic findings in the epidermis: Grade I, epidermal basal vacuolization; Grade II, epidermal MNC infiltration and basal membrane degeneration; Grade III, bullae formation; and Grade IV, ulceration.\textsuperscript{17} The present Cases 1, 2, and 3 yielded Grade II changes with basal vacuolar degeneration and necrotic-apoptotic keratinocytes. Bullae formation was observed in Case 4. The inflammation in the dermis was minimal, and this observation is not specific for the diagnosis of any bacterial or fungal infections. In contrast, similar findings in the epidermis and the dermis can be observed in drug eruptions; but almost always, the inflammation contains eosinophils.\textsuperscript{18} Populations with a high degree of HLA homozygosity are known to be predisposed to TA-GVHD.\textsuperscript{13} Fresh whole-blood transfusion (that is, collected within 48 hr before transfusion), another risk factor for TA-GVHD, is a common practice in rural areas of Turkey. All four patients had received fresh whole blood. Consanguineous marriages in Turkey, a social tendency to transfuse fresh blood from relatives, and inadequate blood banking systems make the population vulnerable to TA-GVHD.\textsuperscript{8,19} Therefore, prevention should be the aim, because prognosis is very grave. In Japan, where there is a high degree of HLA homozygosity, irradiation of any blood product to be transfused within 3 days of collection is recommended.

The use of fresh blood and blood products is discouraged except for PLT transfusions.\textsuperscript{20} The number of viable lymphocytes that are capable of proliferation is inversely related to the storage duration of blood and blood components.\textsuperscript{21} Removal of the donor WBCs or their inhibition should be attempted. Gamma irradiation of the blood components at 25 Gy is the currently accepted method to prevent TA-GVHD.\textsuperscript{22} Proper maintenance and use of the instrument, however, requires education of the technicians and recognized standards to be followed. Leukoreduction is not adequate to prevent TA-GVHD, because there are remaining active cells.\textsuperscript{12} Photochemical treatment of the blood products is a promising alternative way. Both photochemical treatment with amotosalen combined with UVA light and dimethylmethylene blue photo inactivation has shown significant reduction of activation of lymphocytes.\textsuperscript{23-26}

The most commonly affected organ systems in TA-GVHD are skin, liver, and intestines. The classic symptoms are fever, rash, liver dysfunction, and diarrhea. Fever is the first symptom with median onset of 10 days after transfusion. After fever, an erythematous, maculopapular skin rash appears on the trunk and spreads through palms and soles. The degree of liver involvement is variable. Gastrointestinal complications may range from anorexia to bloody diarrhea. Pancytopenia develops in a median of 16 days and causes a more severe clinical picture. The most common cause of mortality is overwhelming infection that occurs approximately 3 weeks after transfusions. The other cause is bleeding due to thrombocytopenia. TA-GVHD is the most common cause of death resulting from transfusion.\textsuperscript{1,12} In the present cases, the clinical picture is the same in the literature (Table 1). All patients died because of septic shock. Moreover, interestingly all of them had candidemia, and the first patient’s autopsy revealed disseminated candidiasis. The patient reported by Triulzi and coworkers\textsuperscript{11} also had sepsis due to C. albicans, and the autopsy study had shown disseminated candidiasis.\textsuperscript{11} In Cases 1 and 2, the candidiasis was diagnosed in the premortem period by different organ involvement including skin, retina, and lower respiratory tract, but positive blood culture reports were available just after death. These observations may suggest earlier start of antifungal therapy in this particular subset of patients, because candidemia itself has attributable mortality.\textsuperscript{27} It can be speculated that impaired integrity of the intestinal mucosa in TA-GVHD may lead to translocation of yeast and this may cause candidemia.\textsuperscript{28}

Prognosis is reported to be very poor in overall TA-GVHD with few survivors in the literature.\textsuperscript{29-31} In these cases, treatment was attempted with autologous BMT\textsuperscript{29} with combination of cyclosporine and the anti-CD3 monoclonal antibody (OKT3).\textsuperscript{30} One patient had entered spontaneous remission.\textsuperscript{31} This patient had acute myelogenous leukemia and received transfusion after which she devel-
oped transient TA-GVHD symptoms and chimerism. There are some investigations with serine protease inhibitors, such as nafamostat mesilate and chloroquine, which were shown to have inhibitory effects on cytotoxic T cells. Their effects, however, were shown to be transient, and patients treated with these drugs died due to recurrences. In each of our cases, the diagnosis was very prompt, and treatment was started within 24 hours of admission. Despite currently concordant treatment and monitoring in the intensive care unit, however, all patients died because of severe septic shock and multiorgan failure.

All of the present cases were referred to our hospital from different rural areas, where irradiation of blood products was not possible. In such rural areas, transfusion indications should be strictly adhered to, because there may be many complications, and TA-GVHD is only one of them. Second, HLA-haploidentical donors, and therefore blood donation from relatives, should be avoided. Third, fresh blood and blood products should not be used except for PLTs. If HLA-haploidentical blood products are to be used, complete removal of T lymphocytes from donors’ blood or abolishing their proliferating potentials should be done. Currently this can be achieved effectively only by irradiating blood product with gamma or X-ray irradiation. It has been demonstrated that 25 Gy, whether delivered via X-ray or gamma irradiation, provides an equivalent effect on lymphocytes, so both are equally effective in reducing the risk of TA-GVHD. As well, it has been shown that neither the X-ray–irradiated nor gamma-irradiated units exceed the currently recommended limit of 0.8 percent hemolysis before 14 days of storage with 25 Gy. Therefore, X-ray irradiation may be an alternative to gamma irradiation for irradiating blood products in rural areas, because the X-ray irradiator is less expensive and does not have a radioactive source.

In conclusion, there is no definite treatment modality for this mostly fatal condition of TA-GVHD. Therefore, prevention of TA-GVHD either by irradiation of blood products or by inactivation of T cells is of utmost importance. Each institution should adopt its transfusion policy considering these prevention methods, keeping in mind that TA-GVHD is more prevalent than reported in the literature, but commonly misdiagnosed.

ACKNOWLEDGMENTS

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REFERENCES
