Transfusion Associated Graft *versus* Host Disease

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Transfusion-associated graft-versus-host disease (TA-GVHD) is a dreaded complication in immuno-compromized hosts. The diagnosis is often delayed because of lack of awareness and the non-specific clinical features. More than 90% patients succumb to refractory infections. The only effective preventive measure is administration of irradiated blood products, which must be made available in centers managing immunocompromized patients. We report three cases and discuss pathophysiology and preventive strategies in this communication.

Key words: *Graft versus host disease, Transfusion.*

Graft-versus-host disease (GVHD) is the clinical syndrome ascribed to the inflammatory reaction mounted by the donor cells against the host organs(1). It was first described in humans after marrow transplantation (BMT) in 1959(2). Since then, it has been described in solid organ transplantation(3), blood transfusion(4) and maternal-fetal transfer of leukocytes(5). Transfusion-associated GVHD (TA-GVHD) is a dreadful, albeit infrequent complication of blood transfusion(6), first reported in 1955(7). Inspite of GVHD being a well defined syndrome, the diagnosis of TA-GVHD is often delayed because of lack of awareness and the seemingly non-specific manifestations. The relative rarity of this syndrome prompted us to share our experience of managing three children with TA-GVHD.

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Case Reports

Case 1: A five-year-old girl, a case of acute lymphoblastic leukemia (L1) presented with fever and lethargy a fortnight after receiving the late intensification chemotherapy consist-ing of vincristine, daunorubicin, cytarabine, etoposide, thioguanine and oral dexametha-sone over a period of five days (UK ALL X protocol)(8). She had been in a sustained first remission for nearly 18 weeks. Examination revealed a febrile child with no localizing features. Investigations revealed a hemoglobin (Hb) of 95 g/L, a while blood cell (WBC) count of 0.04 10^9/L and a platelet count of 8×10^9/L. The very severe leuco-penia precluded a differential count. The serum biochemistry, including liver and renal function tests, was within normal limits. In accordance with the protocol for febrile neutropenia, she was started on intravenous cefotaxime and amikacin. A packet red-cell transfusion was administered on day 3 of admission for anemia (Hb level of 69 g/L). The blood culture grew *Escherchia coli* and *Streptococcus pneumoniae* that were sensitive to the antimicrobials being administered. Fever subsided on day 4 of admission. The child appeared to be recovering till day 9, when she had high-grade fever. It was followed by an erythematous maculopapular rash, which was first noticed on the trunk and spread to involve the palms and soles, with periungual and auricular erythema. Simultaneously, she had loose stools and was detected to be icteric. Repeat investigations revealed a Hb of 82 g/L, a WBC count of

0.1× 10^9/L and a platelet count of 11× 10^9/L. The serum bilirubin level was 8.4 mg/dL, with a conjugated fraction of 7 mg/dL. Serum transaminases and alkaline phosphatase levels were within the normal range. In view of the clinical spectrum of skin rash, diarrhea, jaundice and fever in an immunocompromised patient, a possibility of TA-GVHD was entertained. A skin biopsy showed focal vacuolation of basal epithelial cells. There was lymphocytic infiltration in the stratum malphigi, along with scattered necrotic keratinocytes. Mild to moderate perivascular infiltration was seen in the upper dermis. The findings were consistent with GVHD grade II (*Fig. 1*). Intravenous infusion of methylprednisolone in a dose of 30 mg/kg/day for 3 days was administered. The recovery was dramatic with subsidence of fever and loose stools within 48 hours. The serum bilirubin declined progressively to recovery in 12 days. Oral prednisolone was given for 3 months and then tapered gradually. The child is in a sustained first remission for nearly a year.

Case 2: A six-year-old boy was diagnosed to have acquired very severe aplastic anemia. He was treated with antithymocyte globulin (ATG) followed by oral cyclosporine for six months. He had remained transfusion-free after the administration of ATG. The while cell counts had recovered. He presented, on an unscheduled visit, with fever, pallor and gum bleeds. Examination revealed severe pallor with widespread skin and mucosal bleeds. Investigations revealed a Hb of 62 g/L, WBC count of 2.5 × 10^9/L and a platelet count of 18 × 10^9/L. Absolute neutrophil count was 0.625 × 10^9/L. He was transfused two units of packed red cells and a single unit of platelet concentrate. Febrile neutropenia was managed with cefotaxime and amikacin, as per standard guidelines. On day 8 of admission, he had high grade fever and a generalized erythematous maculopapular rash, which involved the palms and soles. He developed diarrhea two days later. Repeat investigations revealed a persisting pancytopenia. The total serum bilirubin was 3.5 mg/dL, with a

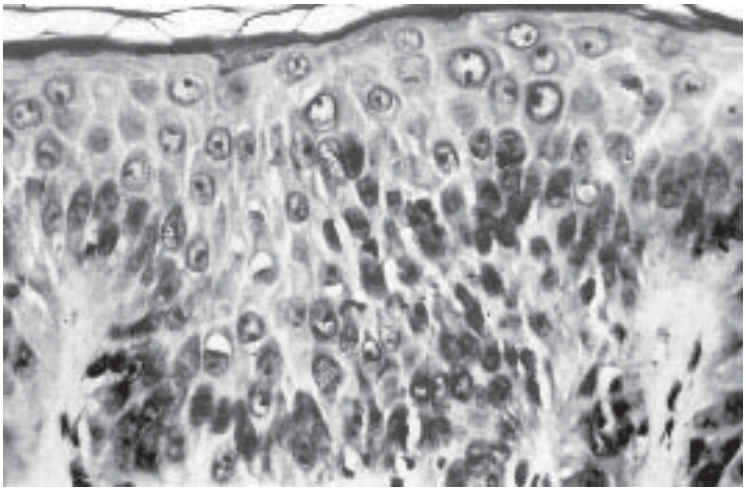

Fig. 1. Photomicrograph of skin showing mild lymphocytic infiltrate with occasional apoptotic bodies in the epidermis (H&E, 20X).

conjugated fraction of 2.1 mg/dL. Serum transminases and alkaline phosphatase levels were in the normal range. Blood culture was sterile. The skin biopsy findings were consistent with a diagnosis of grade II GVHD. He was administered pulse methylprednisolone therapy, followed by oral prednisolone. However, his illness deteriorated progressively and he died a fortnight later, with complications of infections and bleeds.

Case 3: A four-year-old boy was a diagnosed case of acquired, very-severe aplastic anemia. Financial constraints precluded the use of ATG. The blood counts, after six weeks of monotherapy with cyclosporine A (12 mg/kg/day) were still in the range designated as very severe(9). During this period, he received four units of packed red-cells besides multiple units of platelet concentrates for ongoing bleeding manifestations. A week after the last red-cell transfusion, he developed high grade fever and a maculopapular rash on the trunk with progression to all parts of the body over a period of 48 hours. There were no gastrointestinal symptoms. The results of investigations were a Hb of 35 g/L; WBC count of 1.8 10^9/L and a platelet count of 7 × 10^9/L. The absolute neutrophil count was 0.072 × 10^9/L. Serum bilirubin was 8.4 mg/dL with a conjugated fraction of 50%. There was no transaminitis; the serum alkaline phosphatase level was 22 KA units/L (normal 0-13). The skin biopsy showed features consistent with grade II GVHD. He was started on pulse methyl-prednisolone. There was no response. Alpha-hemolytic Streptococcus and *Staphylococcus aureus* were isolated from the blood. Antibiotics and antifungals were administered as per protocol guidelines for the management of febrile neutropenia. His condition progressively deteriorated and culminated in death about three weeks after the onset of GVHD.

Discussion

TA-GVHD is an infrequent complication of blood transfusion(6). The true incidence of this disorder is not known, as the manifestations are often mistaken for a viral exanthem or a durg reaction(6). Two cases of TA-GVHD in neonates, following exchange transfusion have been reported earlier from our center(10). A thorough literature search did not yield any other report of TA-GVHD from India.

Immunologically competent cells in the graft, transplantation alloantigens in the host and an immunosuppressed host are the three important prerequisites for the development of GVHD(6). All cellular blood products contain mature T cells, which act as immunocompetent cells in the graft and mount GVHD. Routine blood transfusion are not matched for the major histocompatibility complex. There are certain alloantigens in the host, which are lacking in the graft. These foreign antigens stimulate immunocompetent cells of the graft to produce GVHD. Under normal circumstances, an immunocompetent host destroys the donor cells, thereby preventing them to mount a graft-versus-host response. However, in immunodeficient states, the host cannot reject the foreign T cells, which proliferate, resulting in GVHD. Rarely TA-GVHD has been described in an immunocompetent host, when the host and the graft share HLA haplotype(6). In such a condition, the host does not recognize the transfused donor cells as foreign and cannot reject them.

The patients at risk of developing TA-GVHD include those with (*i*) congenital immunodeficiencies (*ii*) acquired immunodeficient states (*iii*) lymphoreticular malignancies (*iv*) intensive chemotherapy/radiotherapy and (*v*) preterm babies and

infants who have received an exchange transfusion(6).

All cellular blood products have been implicated in TA-GVHD. Transfusions from blood relatives and 'fresh blood' components have a large number of viable T cells, thereby enhancing the risk of TA-GVHD(12).

Pathophysiologically, TA-GVHD is quite similar to GVHD associated with bone marrow transplantation. However, the clinical course in TA-GVHD is more fulminant with marrow aplasia and consequent complication of infections(6). Marrow involvement does not occur in GVHD associated with BMT, as antigenically, marrow is similar to the graft. In TA-GVHD, the marrow is antigenically similar to the host tissue, against which the graft T cells react.

The dominant clinical manifestations in TA-GVHD are fever and rash as seen in our patients. The median interval between the transfusion and the onset of fever, which is usually the first symptom is 10 days, though it can occur as early as 4 days(6). An erythematous maculopapular rash is observed on the trunk. It then spreads to involve the extremities, including the palms and soles. It may progress to generalized erythroderma or bullae formation. Involvement of the liver is variable. Usually there is mild to moderate elevation of serum billirubin; predominantly conjugated. The liver enzymes may be mildly elevated. The serum alkaline phosphatase is generally raised. Some cases have anorexia, nausea and occasionally massive diarrhea. Pancytopenia due to bone marrow aplasia is a late manifestation; occurring after a median interval of 16 days. Uncontrolled infections are the most common cause of death which frequently occurs within three weeks of the onset of GVHD(6). Overall mortality is reported to be more than 90%(6).

A constellation of clinical features related to skin, gastrointestinal tract, liver and the bone marrow, in an appropriate setting, must arouse suspicion of TA-GVHD. A lower threshold for performing skin biopsy aids in supporting the diagnosis; the findings are however supportive and not pathognomonic. The histological features in skin biopsy are graded as: (*i*) epidermal basal cell vacuolization (grade 1), (*ii*) mononuclear cell infiltration and degeneration of epidermal basal layer (grade II), (*iii*) bulla formation (grade III), and (*iv*) ulceration of the skin (grade IV). Similar findings have been described in drug reactions. Typical clinical manifestations along with the findings in skin biopsy are generally sufficient for the diagnosis. Hepatocellular and cholangiolar cholestasis is associated with degeneration of small bile ducts. The bone marrow is usually hypocellular with a lymphocytic or histiocytic infiltration. Demonstration of donor lymphocytes in the recipient's circulation or in the cellular infiltrates by HLA typing, sex chromatin or DNA analysis is the diagnostic investigation in an appropriate setting, however is not routinely available(6).

Mortality in TA-GVHD is high and is attributed to the complications of acquired bone marrow failure. Various drugs including high-dose steroids, ATG, cyclosporine, anti-CD3 monoclonal antibodies, serine protease inhibitors and growth factors have been tried with no proven benefit(6). There are however, isolated case reports of successful outcome with one or more of these drugs(13,14). Pulse methylprednisolone was effective in only one of our patients. As treatment is largely ineffective, prevention of TA-GVHD is of paramount importance. Irradiation is the best method to inhibit proliferation of immunocompetent cells in the donor blood products(6). Leucocyte depletion and photo-

inactivation are the other not-so-effective methods.

In India, facilities to irradiate blood products are not available even in tertiary care centers. Directed blood donations by relative are frequent. These observations suggest that TA-GVHD is probably more frequent, but under-reported. The features are often interpreted as sepsis, drug reactions or viral exanthem in an immunosuppressed host, who is often on multiple drugs. A strong clinical suspicion in an appropriate setting is required for clinching the diagnosis. Prevention of TA-GVHD is of utmost importance; the only effective preventive measure being blood irradiation. It is imperative that blood irradiation be made available in medical centers managing immunocompromized patients.

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