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Amplified fragment length polymorphism analysis in the evaluation of posttransfusion graft-versus-host disease in a child undergoing extracorporeal membrane oxygenation

To the Editor:

Posttransfusion graft-versus-host disease (GVHD) is an infrequent but serious complication of blood transfusion with a mortality rate of 84% or greater.¹ Posttransfusion GVHD has been described in preterm and term neonates, in children with congenital immune deficiency, in patients with hematologic and nonhematologic malignant diseases, in both autologous and allogeneic bone marrow transplant recipients, and in an infant undergoing extracorporeal membrane oxygenation (ECMO).² A very high degree of suspicion is required to make the diagnosis of posttransfusion GVHD. Both underreporting and overreporting of posttransfusion GVHD are likely occurring because of the lack of uniform confirmatory criteria in establishing the diagnosis. We report the case of a child presumed to have posttransfusion GVHD on the basis of very strong clinical and histopathologic evidence. Amplified fragment length polymorphism (FLP) analysis was successfully used to rule out what appeared to be a bonafide case of posttransfusion GVHD.

A 2½-year-old boy with complex congenital heart disease was admitted to Children's Hospital for postoperative ECMO support. He underwent a Blalock-Taussig procedure in early infancy and 3 days before admission underwent a Glenn procedure. His postoperative course was complicated by pneumonia caused by *Hemophilus influenza* as well as adult respiratory distress syndrome. The patient was supported by ECMO because conventional ventilatory assistance had failed. During

Fig. 1. Skin biopsy. The epidermis shows a lymphocytic infiltrate with single cell necrosis and basal cell vacuolar degeneration. The papillary dermis is infiltrated by a mononuclear cell population.

the first 12 days of ECMO, the patient required 21 red cell transfusions and 64 units of platelets. Only those red cell transfusions derived from the nursery inventory for small volume transfusions of 25 ml or less were irradiated per a preexisting protocol. Six units of packed red cells and 64 platelet units were transfused and not irradiated for two cardiac bypass procedures and ECMO, as well as post-ECMO thrombocytopenia. On day 12, an erythematous maculopapular rash appeared on the abdomen and subsequently spread to the remainder of the face and body. Three days after the rash appeared, a significant gastrointestinal hemorrhage developed. Alanine aminotransferase and aspartate aminotransferase blood concentrations were mildly elevated. A skin biopsy suggested the diagnosis of GVHD. Amplified FLP and HLA antigen typing on peripheral blood and an abdominal skin biopsy specimen were obtained. Results were compared with peripheral blood specimens from both his biologic parents.

Blood cultures at this time were positive for *Candida tropicalis*. He subsequently had massive gastrointestinal hemorrhage and hemoperitoneum. Because of continued cardiac dysfunction and pulmonary failure, ECMO support was terminated. The patient died on day 22 of his hospitalization and an autopsy was performed.

High-molecular-weight deoxyribonucleic acid (DNA) was obtained from heparinized peripheral blood from the patient and both biologic parents. Skin fibroblasts from the patient were analyzed in a similar manner. Peripheral blood mononuclear

Fig. 2. FLP analysis of the Co12A1 region. Lane 1, Father's mononuclear cells; lane 2, mother's mononuclear cells; lane 3, patient's mononuclear cells; lane 4, patient's neutrophils; lane 5, patient's skin; lane 6, laboratory standard for sensitivity at 5%; lane 7, laboratory standard for sensitivity at 1%; lane 8, negative control (no DNA).

cells and neutrophils were separately isolated by Ficoll-Hypaque density gradient centrifugation. The blood and fibroblast DNA were then amplified in the D1S80, apolipoprotein B, Co12A1, and pYNZ22 regions using the polymerase chain reaction. Products from the reactions were separated by electrophoresis on polyacrylamide gels, stained with ethidium bromide to visualize the bands, and photographed. Because of the lack of a pretransfusion sample, laboratory standards of sensitivity consisted of diluting DNA from normal individuals at 1% and 5% final concentrations. Histopathologic analysis and HLA typing were performed by standard methods.

Microscopic examination of the skin biopsy obtained on day 12 of hospitalization revealed an intact epidermis with basal cell vacuolar degeneration (Fig. 1). Single cell necrosis (apoptosis) and intraepidermal lymphocytes surrounding the apoptotic cells (satellitosis) were also noted. The papillary dermis was infiltrated by a mononuclear cell population and an occasional colloid body was seen. The final autopsy diagnostic evaluation revealed that the patient had situs inversus totalis and complex congenital heart disease. Evidence of widespread disseminated intravascular coagulation and multiple pulmonary hemorrhages was present, likely caused by the patient's sepsis. No evidence for posttransfusion GVHD was seen in sections obtained from the gastrointestinal tract, liver, and bone marrow.

The patient's HLA typing revealed one inherited haplotype from each of his parents (A32, C5, B44, B4 and A24, C3, B62, B6). No evidence of additional HLA antigens was seen. Analysis of the D1S80, apolipoprotein B, Co12A1, and pYNZ22 regions revealed one inherited FLP from each of his parents. No evidence of additional FLP bands were noted, thus excluding the presence of circulating third-party allogeneic cells. Analysis of the Co12A1 region is shown in Fig. 2.

FLP analysis has been used recently in the diagnostic evaluation of posttransfusion GVHD.³ In these reports, the technique

was able to confirm the presence of third party-mediated GVHD in patients receiving blood transfusions, and it appeared to be a satisfactory alternative to HLA typing. We included amplified FLP analysis in the evaluation of a 2½ year old male child undergoing ECMO. Although infants undergoing ECMO do not appear to be immunosuppressed by the procedure, we⁴ have recently reported that they do not show any evidence of B-cell or T-cell activation after concomitant blood transfusions. This is in contrast to what is seen in adults undergoing cardiopulmonary bypass procedures.⁵ Therefore, our patient could have had an altered immune response to blood transfusion, possibly placing him at higher risk for posttransfusion GVHD. We were further concerned that posttransfusion GVHD had developed because the skin biopsy results were highly suggestive. The presence of single cell dyskeratosis, satellitosis, and basal cell vacuolar degeneration in an at-risk patient, although not pathognomonic of posttransfusion GVHD, is virtually diagnostic. However, distinction of GVHD from a drug reaction, erythema multiforme, spongiotic dermatitis, and viral eruptions may be impossible. One or more of the aforementioned disorders could have been responsible for the histopathologic changes seen in the skin biopsy. Determining the correct diagnosis in this setting is crucial because posttransfusion GVHD is not treatable and has a high mortality as compared with the other disease entities listed.

Sensitivity and accuracy of amplified FLP analysis is likely to be different with respect to HLA analysis; the latter technique may not always be useful. In fact, in addition to HLA analysis on peripheral blood lymphocytes, patient fibroblast cultures may be necessary for the possibility of posttransfusion GVHD to be fully evaluated. Our data suggest that amplified FLP analysis can be used as an alternative to HLA assays for the evaluation of posttransfusion GVHD. HLA analysis in this setting has been shown to have a lower sensitivity with respect to Southern blot analysis with FLP. FLP analysis alone had a sensitivity of 1% detection of allogeneic DNA. When used in conjunction with polymerase chain reaction for the detection of residual leukemic cells, sensitivity was in the range of 1 in a total of 10^5 cells. A sensitivity of 1% as illustrated in our investigation of posttransfusion GVHD appears to be an excellent detection limit, in that no evidence of posttransfusion GVHD was seen at autopsy. However, because the minimum number of circulating allogeneic lymphocytes necessary to elicit posttransfusion GVHD is currently not known, future studies are needed to establish optimum sensitivity of this analysis in this pathologic setting.

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Plasma elastase concentrations and pulmonary function after cardiopulmonary bypass

To the Editor:

Postbypass pulmonary dysfunction remains a relevant problem in patients undergoing heart operations with extracorporeal circulation.¹ Experimental data suggest that activated leukocytes contribute to lung injury after cardiopulmonary bypass.² Leukocyte depletion by mechanical filtration is therefore recommended during cardiopulmonary bypass.² To determine the role of activated leukocytes in a clinical setting, we measured pulmonary function and plasma elastase concentrations as a marker of leukocyte activation³ in patients undergoing aorta-coronary artery bypass operations.

Twenty-six patients undergoing elective, uncomplicated aorta-coronary bypass operations were investigated (age, 61.2 ± 7.0 years; mean $\pm$ standard deviation). In all patients, standard cardiopulmonary bypass techniques with moderate systemic hypothermia, aortic crossclamping, and cardioplegic arrest were used (bypass time, 117 ± 32.9 minutes; aortic crossclamp time, 59.4 ± 18.7 minutes; mean $\pm$ standard deviation). During aortic crossclamping, lungs were not ventilated and continuous positive airway pressure (8 mm Hg) was applied. Venous blood samples were drawn before anesthesia, before operation, before cardiopulmonary bypass (0 to 2, Fig. 1), with aortic unclamping, at 1, 2, 3, 4, 8, 12, 16, 20, and 24 hours later (3 to 12, Fig. 1), and daily thereafter for 7 days (13 to 19, Fig. 1). Elastase α_1 proteinase inhibitor complex concentrations were measured with an immunoassay (PMN-Elastase IMAC; Merck, Darmstadt, Germany). Postbypass pulmonary function was assessed by the quotient of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) 1 and 3 hours after aortic unclamping.⁴

Plasma elastase concentrations showed a marked increase after initiation of cardiopulmonary bypass ($p < 0.0001$, repeated-measures analysis of variance). Peak values occurred 1 to 4 hours after aortic unclamping, followed by a rapid subsequent decrease within 12 hours (Fig. 1). There were no close, significant correlations between elastase concentration and bypass time, aortic crossclamp time, or plasma free hemoglobin concentrations. Pulmonary function after cardiopulmonary bypass varied over a wide range ($\text{PaO}_2/\text{FiO}_2$ quotients ranging from 93 to 560). There was no significant close correlation between elastase concentration and $\text{PaO}_2/\text{FiO}_2$ quotients 1 and 3 hours after aortic unclamping. Eleven patients (group 1) had $\text{PaO}_2/$