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Panel Reactive Antibodies in Patients with Neoplasia and Haematological Disorders

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ABSTRACT Characterization of HLA antibody is important for donor selection for HLA matched or crossmatched blood components. 304 multiple transfused patients with neoplastic or haematological disorders were screened for HLA antibodies by the lymphocytotoxicity assay. The panel reactive antibodies (PRA) were determined against a panel of 100 lymphocytes of known HLA antigens in order to evaluate the degree of sensitization. 35 (16.7%) of the 210 male patients and 29 (30.9%) of the 94 female patients were positive for HLA antibodies. Out of the 29 positive female patients, seven patients had a history of abortion and one full term normal delivery, which suggests an anamnestic response to antigen exposure. PRA ranged from 10% to more than 90%. The specificities of identified antibodies were anti-A1, A2, A11, A24 and B55. 8 patients had multiple specificities and in 47 patients HLA specificities could not be defined.

INTRODUCTION

The highly polymorphic human leukocyte (HLA) class I antigens are widely distributed on all nucleated cells and platelets. HLA antibodies are acquired through alloimmunization by transfusion of blood products, by pregnancy or by previous transplants. HLA alloimmunization often results in Febrile Non-Hemolytic Transfusion Reaction (FNHTR) and / or refractoriness to platelet transfusions upon re-exposure to HLA antigens (Walker 1986; Kickler 1990). About 20% to 70% of multiple transfused patients become alloimmunized to HLA antigens and no longer respond to platelet transfusions (Lee and Schiffer 1987; MacGrath et al. 1988; Slichter 1990; Hogge et al. 1995). HLA alloimmunization can be delayed and/or prevented by transfusion of leukocyte depleted and ultraviolet irradiated blood components (The Trial to Reduce Alloimmuni-zation to Platelets Study Group 1997; Schiffer 2001).

Preformed circulating HLA alloantibodies are a contraindication to allotransplantation and result in graft rejection if directed against donor HLA antigens (Kerman 1991). The degree of HLA sensitization is determined as the percentage of positive reactions against a known panel of lymphocytes termed as Panel Reactive Antibodies (PRA) (Hogge et al. 1995). The present study was undertaken to determine the degree of HLA sensitization, to characterize the HLA antibody and to evolve strategies for transfusion management in positive patients.

MATERIALS AND METHODS

Three hundred and four multitransfused patients suffering from various neoplastic and hematological disorders referred for HLA typing were tested for HLA antibodies against the HLA matched prospective donor. A panel of 100 lymphocytes of known HLA antigens by the two-stage microlymphocytotoxicity test using extended incubation times (Terasaki et al. 1978). *Statistical Analysis:* HLA antibody was determined by using the 2x2 chi-square table while the PRA was determined as a percentage (%) as follows:

Number of positive reactions /Cell panel × 100

RESULTS

Out of a total of 304 multitransfused patients, 210 patients were male and 94 patients were female. 35/210 (16.7%) male patients and 29/94 (30.9%) female patients were positive for HLA antibodies. Table 1 depicts the disease-wise distribution of cases. All the patients tested negative with the HLA matched prospective bone marrow donor.

Among 29 female patients with HLA antibody, 9 patients were less than 10 years of

Table 1: Patients tested for HLA antibodies

Diagnosis	*No. of patients tested (N=304)*		*No. of patients positive for HLA antibodies (N=64)*	
	Male	*Female*	*Male*	*Female*
AML	80	43	9	12
Aplastic anemia	54	20	8	4
Thalassemia	36	16	9	5
ALL	29	13	3	6
CLL	2	0	0	0
MDS	5	0	4	0
CML	4	2	2	2
Total	210	94	35 (16.70%)	29 (30.90%)

age, 5 patients were between 11-20 years of age, 9 patients were between 21-30 years of age and 6 patients were between 31-40 years of age. Out of the 29 positive female patients, 7 patients had a history of abortion and one full term normal delivery (Table 2). The percent PRA in positive patients is depicted in Table 3. The percent PRA was less than 10% in one patient, between 11% to 20% in 3 patients, between 21% to 30% in 6 patients, between 31% to 50% in 14 patients, between 51% to 70% in 7 patients, between 71% to 90% in 10 patients and more than 91% in 13 patients respectively. Nine patients had one antibody, 6 patients had two antibodies, 2 patients had three antibodies whereas 47 patients had more than three antibodies (Table 4).

DISCUSSION

Chronic transfusion therapy, multigravida and a rejected allograft result in HLA alloimmunization due to recipient exposure to major histocompatibility complex (MHC) antigens. HLA antibodies are usually detected by the lymphocytotoxicity assay. PRA is used to screen patients receiving multiple blood transfusion and increases in the PRA levels over time are used to predict alloimmune refractoriness (Lee and Schiffer 1987; Hogge et al. 1995). In our study sixty-four (21%) patients were sensitized to HLA antigens and is in accordance with various investigators who reported 20% to 70% incidence of HLA antibodies (Lee and Schiffer 1987; MacGrath et al. 1988; Slichter 1990; Hogge et al. 1995). The percentage positivity in female patients (30.9%) was almost twice that in male patients (16.7%). Patients with previous exposure to HLA antigens due to multiparity are at a high risk to develop HLA alloimmunization. Seven (7.5%) female patients in the series had a history of abortion and one full term normal delivery which suggests a pre-existing or an anamnestic response to antigen exposure. Taanig and Skibsted (1990) reported that in the absence of a previous blood

Table 2: Age distribution in positive female patients (N=29)

Age (Years)	*Diagnosis*					
	AML	*Thalassemia*	*Aplastic Anemia*	*ALL*	*CML*	*Total*
<10	2	3	1	3	0	9
20-Nov	2	1	1	1	0	5
21-30	4	1	01+1*	2	0	9
31-40	04*	0	0	0	02*	6
Total	12	5	4	6	2	29

Key: * Patients with >1 pregnancy and history of abortion

Table 3: Percentage PRA in positive patients (N=64)

% PRA	*Number of patients*		
	Male (N=35)	*Female (N=29)*	*Total (N=64)*
<10	0	1	1
20-Nov	2	1	3
21-30	4	2	6
31-50	11	02+01*	14
51-70	6	07+04*	17
71-90	2	06+02*	10
>91	10	3	13

Key: * Patients with >1 pregnancy and history of abortion

Table 4: Identification of HLA antibody in positive patients

HLA antibody	*Number of patients (N=64)*
A1	5
A2	1
A11	1
A24	1
B55	1
A1+A24	1
A2+A28	2
A11+A1	2
B5+B35	1
A1+A11+A24	2
More than 3 specificities	47

transfusion, 4% of women develop HLA antibodies after one pregnancy and 26% after four pregnancies.

Preformed circulating antibodies present before the current course of transfusion are likely to persist (Murphy et al. 1987). It is imperative to regularly monitor patients every few weeks for the persistence of the HLA antibody, since HLA antibodies tend to decrease or disappear with time and may not reappear with subsequent transfusions (Lee and Schiffer 1987). Such patients can be supported with unmatched platelet transfusions. HLA antibodies may be specific for one antigen, more than one antigen or structurally similar epitopes called cross-reactive groups. A high proportion of patients is immunized to multiple HLA antigens (MacPherson et al. 1986). In the present study, 9 patients were positive for one antigen, 8 patients were positive for cross-reactive groups and 47 patients had multiple HLA antibodies.

Characterization of HLA antibodies helps in donor selection in patients who are less broadly immunized. Such patients are candidates for HLA matched or crossmatched blood products (Schiffer 2001). HLA crossmatched blood components have been reported to improve post transfusion platelet recovery in patients with platelet refractoriness due to HLA alloimmunization (Duquesnoy 1978). However in India since a repository of HLA matched donors is unavailable it is very difficult to find a compatible HLA matched donor. The next best option is leukocyte depleted blood components. Universal leukocyte reduction of cellular components to prevent HLA alloimmunization has emerged as a cornerstone of addressing the problem of platelet transfusion refractoriness due to HLA alloimmunization.

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