Original Article

Prevalence of risk factors for platelet transfusion refractoriness in multitransfused hemato-oncological patients at tertiary care center in North India

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Abstract:

Background: This study was designed to determine the prevalence and assess the risk factors responsible for platelet transfusion refractoriness in hemato-oncological patients. **Materials and Methods:** The study included 30 patients. Twelve were clinically diagnosed as aplastic anemia and the 18 were of acute myeloid leukemia. A prospective 3 months follow-up was planned to monitor the response of platelet transfusion therapy, based on their posttransfusion corrected count increment at 1st and 24th h. Based on the observations, patients were categorized into refractory and nonrefractory groups. Common nonimmunological causes such as fever, sepsis, bleeding, disseminated intravascular coagulation, chemotherapy, splenomegaly, ABO mismatch, and antithymocyte globulin therapy were monitored. Among the immunological causes, presence of antihuman leukocyte antigen (HLA) class I antibodies and platelet glycoprotein antibodies in patient's serum were monitored. **Results:** During the study period, 17 (56.66%) patients did not show desired platelet count increment. Transfusion requirements of refractory group for both red cell and platelet product were significantly higher ($P < 0.05$) in comparison to nonrefractory group. Among immunological causes, anti HLA class I antibodies ($P < 0.013$), antihuman platelet antigen-5b antibodies ($P < 0.033$) were significantly associated with refractoriness. Among nonimmunological causes, bleeding ($P < 0.019$, odd ratio 8.7), fever ($P < 0.08$, odd ratio 5.2), and infection ($P < 0.07$, odd ratio 5.4) were found to associated with refractoriness. **Conclusion:** Platelet refractoriness should be suspected in multitransfused patients not showing expected increment in platelet counts and thoroughly investigated to frame further guidelines in order to ensure proper management of these kind of patients.

Key words:

Alloimmunization, acute myeloid leukaemia, aplastic anemia, multitransfused, platelet transfusion, refractoriness

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Introduction

Platelet transfusion has become the standard practice in the management of thrombocytopenia due to bone marrow failure. It is usually provided on prophylactic basis to maintain platelet count above a level that is considered safe (≥20 × 10^9/L). The repeated failure to obtain satisfactory or desired responses to platelet transfusions or platelet refractoriness is a well-recognized problem in patients with bone marrow failure that requires repeated multiple transfusions of blood and blood components.[1,2] It is reported to occur in 30-70% of multitransfused patients.[1,2] Platelet count increment 1 h after transfusion is used to monitor response.[3] Presently platelet refractoriness is defined as a 1 h corrected count increment (CCI) of <5 × 10^9/L on two sequential occasions, using ABO identical fresh platelets.[4] This has been attributed to immune causes,[5,6] such as the presence of platelet alloantibodies and platelet autoantibodies and nonimmune consumption associated with clinical factors such as fever,[7] infection/septicemia,[8] bleeding,[9,10] disseminated intravascular coagulation (DIC), and splenomegaly. A prospective follow-up study was planned to assess the prevalence of risk factors responsible for platelet transfusion refractoriness in our multitransfused hemato-oncological patients in a tertiary care referral institute.

Materials and Methods

This study was conducted on 30 consecutive patients, 15 males and 15 females admitted on follow-up in the hemato-oncology unit of the Department of Internal Medicine at our institute. Twelve patients (7 males and 5 females) had aplastic anemia (AA) and 18 patients (8 males and 10 females) had acute myeloid leukemia (AML). This study was approved by the Ethics Committee of the institute. All the patients were prospectively followed-up for 3 months after registration to the hemato-oncology clinic of the institute for following parameters.

Transfusion triggers

The institute transfusion policy recommends red cell transfusion to these patients at or below

hemoglobin 8 g/dl and platelet transfusion at or below 10×10^9/L platelet count. All the cellular blood components are routinely irradiated immediately as per institutional policy prior to transfusion to prevent graft versus host disease.

Evaluation of response to platelet transfusions

All patients were monitored during each platelet transfusion and the response was assessed by estimating CCI at 1 h and 24 h, as described by Davis *et al*.[11] Patients having CCI ≤5000 were labeled as refractory and those who showed desired increment (CCI ≥ 5000) were labeled as nonrefractory. The nonimmunological factors such as fever ≥38°C, infection, bleeding, splenomegaly, DIC, antibiotic usage, chemotherapy were also evaluated for their association with refractoriness.

Serological investigation

Alloimmunization against human leukocyte antigen (HLA) class I and platelet glycoproteins Ib/IX and V and against IIb/IIIa and Ia/IIa was tested through commercial ELISA kit (Pakplus, GT diagnostic, USA) at three occasions (first at the time of enrolment, second after 3 weeks or 4 transfusions whichever was earlier and third at the end of 3 months).

Inclusion criteria

The consecutive 30 patients of idiopathic aplastic anemia ($n = 12$) and AML ($n = 18$, excluding acute promyelocytic leukemia or M3) admitted to hemato-oncology unit of the hospital. All patients were diagnosed on bone marrow biopsy and flow cytometry and were willing to have a full course of treatment.

Exclusion criteria

Patient not willing to participate in the study or not willing to have a full course of treatment.

Results

The median age of the patients was 45 years (range 17-67 years). It was 27.5 years (range 17-67 years) for AA patients and was 49 years (range 18-64 years) for AML patients.

Previous transfusion profile of patients before enrolment in the study

All the 30 patients had received transfusion prior to inclusion in the study in the range of 1-60 units with a median of 11 units. Aplastic anemia patients received a median of 18 units (range 6-57 units) while AML patients received a median of eight units of transfusion (range 1-29 units). The majority of blood component transfused were random donor platelets. Prior to enrolment in the study, no data could be obtained for platelet transfusion triggers outside institute.

Transfusion profile of patients and their response to hemo-therapy

Patients during the study period received a median of 46 units of transfusion (range 10-103). The refractory group required significantly higher number of red cell as well as platelet transfusions than nonrefractory group and this was found to be statistically significant during the entire duration of study as shown in Table 1.

Association between platelet transfusion refractoriness and possible causative factors

At the onset of study, 23.2% enrolled patients (7 out of 30) were refractory to platelet transfusion therapy. Amongst them, 2 (1 AA, 1 AML) were refractory due to alloimmunization, 2 (1 AA, 1 AML) had nonimmune causes, whereas in remaining 3 (1 AA, 2 AML) both factors were present. During the course of study, 56.7% (17 out of 30 patients) became refractory to the platelet transfusion therapy. Among 15 patients, both immune and nonimmune factors were implicated, whereas alloimmunization and nonimmune factors alone contributed to one case each. Overall, during the course of study, 83.33% (25 out of 30) patients showed evidence of alloimmunization, of which 6.7% (5 of 30) were refractory at the onset and 53.4% (16 of 30) became refractory during the course of study, whereas 13.3% (4 of 30) remained nonrefractory despite being alloimmunized.

Prevalence of alloimmunization and nonimmune factors

Association of HLA class I antibody, human platelet antigen (HPA)-5b/5b antibody, HPA-1b/1b; HPA-3b/3b; HPA-4 antibody to platelet transfusion refractoriness was found significantly associated with refractoriness [Table 2]. Nonimmunological causes which were associated significantly with refractoriness were bleeding, infection, and fever.

Both nonimmune and immune causes

The presence of both the factors (immune + nonimmune) together in a patient was a strong determinant of poor platelet transfusion response both at the onset ($P = 0.021$) and during the course of the study period ($P \leq 0.000$).

In summary, 30.7% (4 out of 13) nonrefractory patients were having anti HLA antibodies whereas 82.3% (14 out of 17) refractory

Table 1: Transfusion requirements in patients during the study period

Days of follow-up	Blood components	Refractory		Nonrefractory		*P* value (a vs. b)
		Number of patients	Median number of units (a)	Number of patients	Median number of units (b)	
Day 1-30	Red cells	11	8	19	4	0.012*
	Platelets		14		7	0.023*
	Total transfusions		21		13	0.016*
Day 31-60	Red cells	15	8	14	4	0.001**
	Platelets		16		9	0.006**
	Total transfusions		12		21.5	0.008**
Day 61-90	Red cells	9	6	14	4.5	0.149#
	Platelets		16		8.5	0.018*
	Total transfusions		27		13.5	0.005**

**Highly significant; *Significant; #Nonsignificant. Day 1-30: 30 patients received transfusion; Day 31-60: 29 patients received transfusion; Day 61-90: 23 patients received transfusion

Table 2: Prevalence of immune and non immune factors

Non immune factors	At onset					During study period				
	Refractory (*n* = 7) (a) (%)	Non refractory (*n* = 23) (b) (%)	Overall (*n* = 30) (%)	Odd ratio	*P* value (a v/s b)	Refractory (*n* = 17) (c) (%)	Non refractory (*n* = 13) (d) (%)	Overall (*n* = 30) (%)	Odd ratio	*P* value (c v/s d)
Bleeding	5 (71.4)	4 (17.4)	9(30)	11.9	0.01**	15 (88)	6 (46.2)	21 (70)	8.7	0.019**
fever	3 (42.9)	5 (21.7)	8(26.7)	1.5	0.67#	13 (76.5)	5 (38.5)	18 (60)	5.2	0.08*
Infection	Nil	1 (4.3)	1(3.3)	0	1.0#	12 (70.5)	4 (30.8)	16 (53.3)	5.4	0.07*
HLA	4 (57.2)	12 (52.2)	16(53.3)	1.22	1.0#	14 (82.4)	4 (30.8)	18 (60)	10.5	0.013**
HPA-5b/5b	1 (14.3)	8 (34.8)	9(30)	0.31	0.39#	13 (76.5)	4 (30.8)	17 (56.7)	7.31	0.033*
HPA-5a/5a	2 (28.6)	10 (43.5)	12(40)	0.52	0.66#	12 (70.5)	7 (53.8)	19(63.3)	2.06	0.345#
HPA-1b/1b; HPA-3b/3b; HPA-4a	1 (14.3)	9 (39.1)	10(33.3)	0.26	0.37#	11 (64.7)	3 (23.1)	14 (46.7)	6.11	0.058*

**Highly significant; *Significant; #Non significant

patients were having anti HLA antibodies during the study period. This difference was statistically significant ($P \leq 0.05$). Anti HPA-5b was statistically significantly associated with refractoriness ($P = 0.033$). Among nonimmune factors, bleeding ($P = 0.019$) was significantly associated with refractoriness. Other nonimmune factors, for example, fever ($P = 0.08$), infection/sepsis ($P = 0.07$) were associated with refractoriness although we could not find statistically significant association ($P \leq 0.05$).

Discussion

Platelet transfusion is considered vital in keeping the patient platelet count above the chosen level in order to prevent hemorrhagic complications. It is generally accepted that prophylactic platelet transfusion reduces the risk of bleeding in thrombocytopenic hemato-oncological patients.

By definition "refractoriness" refers to the repeated demonstration of poor posttransfusion platelet increments.[12] Platelet refractoriness occurs in approximately 30-70% of patients who receive multiple transfusions.[13,14] Main reasons are either clinical causes, for example, fever, sepsis, splenomegaly, DIC, drugs (e.g. vancomycin, amphotericin B) or immune mediated destruction of platelets.[15,16] In most of the studies, which evaluated the presence of antiplatelet antibodies for platelet transfusion refractoriness, antibodies against HLA class 1 determinant were found;[17,18] and only rarely are they directed against platelet specific antigens. HLA class II antigen exposure is essential for the development of alloimmunization to HLA class I antigens, and it usually results from multiple transfusions or maternal-fetal incompatibility during pregnancy. The prevalence of HLA antibodies in multitransfused hemato-oncological patients has been reported from 25% to 93% by various workers. The frequency of HPA antibodies is much lower as compared to HLA antibodies as pure platelet transfusions are much less immunogenic, since platelets only express HLA class I antigens.[4] In a multicenter study (The Trial to Reduce Alloimmunization to Platelets [TRAP], 1997) the incidence of HPA antibodies was reported to be 8%;[4] in most cases these antibodies coexist with HLA antibodies.[19-22]

We found almost similar frequency of refractoriness in our population as reported by various workers. Klingemann *et al.* had reported 34% (71 of 210) aplastic anemia patients, refractory to nonleucoreduced pooled random donor platelet transfusions.[23] Slichter *et al.* have reported 27% patients (143 of 528) refractory to platelet transfusion.[24] Trial to reduce alloimmunization to platelets study group (TRAP) reported refractoriness to 16% in patients receiving nonleucoreduced blood components.[4] Mathew has reported incidence of 40% in preleucoreduction and 23% in postleukoreduction group respectively.[25]

Similar association of anti HLA antibodies with platelet refractoriness in hemato-oncology patients had been reported previously by several authors with alloimmunization ranging from 13% to 54% to platelets.[3,4,12,21,26] Bierling had described antiHPA-5b antibody causing refractoriness in one patient.[27] Kiefel *et al.* had also reported high prevalence of antiHPA-5b antibody in multitransfused hemato-oncologic patients, but could not establish its relation to refractoriness.[20]

Slichter *et al.*,[24] McFarland *et al.*,[9] and Doughty *et al.*[16] found a significant association ($P \leq 0.05$) between fever and nonresponsiveness to platelet transfusion. Slichter *et al.*,[24] McFarland *et al.*,[9] and Alcorta *et al.*[12] found a significant association ($P \leq 0.05$) between infection and nonresponsiveness to platelet transfusion. Slichter *et al.* in their longitudinal linear regression analysis of patients and platelet related variables affecting posttransfusion platelet increments showed that bleeding was independently associated with poor platelet transfusion outcome ($P \leq 0.001$).[24] Spleen is the biggest factor affecting posttransfusion platelet count increment.[24] In this study, we had just only one patient with splenomegaly, hence could not establish any correlation.

Doughty *et al.* in 1994 showed that nonimmune factors when associated with immune factors significantly lower platelet transfusion response in multiply transfused patients.[16] Thus, issue of platelet transfusion refractoriness should be dealt in a comprehensive manner taking into consideration both immune and nonimmune factors for appropriate patient management.

Conclusion

Provision of effective platelet transfusion support for patients with malignancy receiving chemotherapy warrants constant reassessment of the clinical state of the patient and *in vitro* antibody screening tests.

Platelet transfusion response in hemato-oncology patients is dependent on both clinical and immunological factors. In the management of platelet transfusion refractoriness, issues related

to underlying clinical factors and patient's clinical status need to be addressed first followed by the assessment of immunological factors. The current strategy to avoid immunological platelet refractoriness involves the recruitment of HLA class I-compatible platelet donors. This is both expensive and labor intensive. Where refractoriness is attributable to antibodies to a few epitopes, a strategy of "epitope avoidance" could be invoked as that would be more cost-effective. Antibodies would be analyzed with respect to their actual HLA specificity, and only donors that typed positive for the relevant epitope(s) would be excluded from the panel. This would substantially increase the proportion of acceptable donations.

In a country like India, provision of cross-match compatible platelets to the refractory patients would be a promising option, but all available strategies need to be evaluated in a prospective multicenter randomized trial to identify the factors, which are important in our patient population and based on this best suitable strategy can be adopted in Indian set up.

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