A PREVALENCE STUDY OF PLATELET ALLOANTIBODIES IN MULTIPLY TRANSFUSED THROMBOCYTOPENIC PATIENTS IN HUSM

by

Dr WAN HASLINDAWANI WAN MAHMOOD

M.D.(USM)

Dissertation Submitted In Partial Fullfillment Of The Requirements

For Degree Of Masters Of Pathology (Haematology)

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JABATAN HEMATOLOGI
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Tarikh : 17 Januari 2006

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Peruntukan 2005 (Tahun 2)	RM	10,968.00		
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304	21000	PPSP	6131312		870.00	918.00	(48.00)	1,800.00		1,800.00	(1,848.00)
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304	26000	PPSP	6131312		8,838.00	-	8,838.00	-	-	-	8,838.00
304	27000	PPSP	6131312		6,480.00	4,514.08	1,965.92	-	8,024.00	8,024.00	(6,058.08)
304	28000	PPSP	6131312		-	-	-	-	-	-	-
304	29000	PPSP	6131312		3,480.00	1,850.00	1,630.00	-	2,105.61	2,105.61	(475.61)
304	32000	PPSP	6131312		-	-	-	-	-	-	-
304	35000	PPSP	6131312		-	-	-	-	-	-	-
					19,968.00	7,282.08	12,685.92	1,800.00	10,129.61	11,929.61	756.31

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A Prevalence Study Of Platelet Alloantibodies in Multiply Transfused Thrombocytopenic Patients in HUSM

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Kerap menerima pemindahan darah	multiply transfused
alloantibodi kepada platelet	platelet alloantibody
kegagalan kepada transfusi platelet	platelet refractoriness
thrombocytopenia	thrombocytopenia

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28/2/06

Platelet alloantibody in multiply transfused thrombocytopenic patients.

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From Department of Hematology, Hospital Science of Malaysia.

Abstracts

BACKGROUND: Multiply transfused patients are frequently subjected to platelet alloimmunization.These platelet alloantibodies produced can result in refractoriness to platelet transfusion.

STUDY DESIGN AND METHODS: Ninety five thrombocytopenic (platelet count <100 x 10^9/l) and multilply transfused (more than 2 times transfusion of any blood component with last transfusion more than 2 weeks prior to the study) patients were recruited prospectively. The blood samples were tested using a Solid Phase system (Capture P).

RESULTS: There were 45 males (47.4%) and 50 females (52.6%) recruited with ages from 3 to 90 years. The frequency of transfusions ranged from 10-168. Seven patients (7.4%) were detected to have platelet alloantibodies, predominantly anti-HPA-5b in 4 patients (4.2%). Three of them (3.2%) showed a non-specific pattern. Six patients (6.3%) had received packed cells less than 20 units and another 1 (1.1%) received more than 20 units of packed cells. Four patients (4.2%) received platelet transfusion of less than 20 units and another 3 patients (3.2%) received more than 20 unit platelets.

CONCLUSION: The study may have future implications for the selection of platelet donors for alloimmunized recipients in HUSM.

Keywords : multiply transfused , thrombocytopenia , platelet alloantibody

INTRODUCTION

Alloimmunization is an immune response that happens when body reacts to foreign antigen (from donor's blood) and creates antibodies against them. Patients receiving leucocytes reduced blood product are at much lower risk for refractoriness to platelet transfusion than are recipients of blood that is not leucocytes reduced.[1]

Alloimmunization in polytransfused patient is a well known observation which very often results in refractoriness to platelet transfusions. Alloantibodies are usually directed against HLA antigens and their frequency is evaluated between 20 to 70 %. Alloantibodies may also be directed against platelet-specific antigens but their frequency is controversial.[2] Alloantibodies against platelet membrane glycoprotein are a common cause for febrile non hemolytic transfusion reactions.[3]

Patients who are given platelets over longer periods of time, such as those under treatment for hematologic or oncologic disorders, may develop a condition called refractoriness to platelet transfusions.[4] These refractory patients may become resistant to subsequent platelet transfusion thus increasing the risk of spontaneous bleeding.[1] A patient with previous platelet refractoriness secondary to alloimmunization may need HLA-matched or crossmatched platelets for transfusion support.[5]

MATERIALS AND METHODS

This was a cross sectional study conducted at Hospital Universiti Sains Malaysia from June 2003 to June 2004.This study was approved by the School of Medical Sciences Research and Ethical Committee. All subjects gave an informed written consent

Patient

Subjects were recruited from wards, medical clinic and transfusion records from blood bank. Subjects with history of multiple transfusions (more than twice of any of blood components, more than 2 weeks but not more than 2 years of their last transfusions) and thrombocytopenia (platelet count less than 100 x 10^9/l). Patients who were on heparin or with autoimmune diseases such as ITP, SLE or AIHA were excluded. Selected patients were then given written consent. 7 mls of peripheral blood were taken and collected in EDTA bottles. Two mls of the peripheral blood for FBP and 5 mls for antibody detection. The analysis was performed at the Haematology Laboratory, HUSM.

Laboratory tests

The laboratory tests performed were:

1. Full blood picture
2. Screening test for platelet antibodies.
3. Confirmation test.

Full blood picture was done as a screening to confirm thrombocytopenia. Screening was performed by using Capture –P Solid phase system from Immunocor Inc.. Donor platelets

were used as a source of platelets. Positive cases, were confirmed by the Capture-P Ready Screen test.

Statistical analysis

The data was analysed by using SPSS software, Descriptive analysis and Chi-square (to associate the presence of antibody with age, diagnosis, number of plasma and packed cells transfused).

RESULTS

Of the 95 studied patients, demographic data as shown in Table 1. Platelet alloantibodies were detected in 7 (7.4%) patients. Six of them were diagnosed to have haematological disorders (3 were diagnosed to have Aplastic Anaemia, 1 with AML, 1 with NHL and another 1 with Familial thrombocytopenia). Another 1 patient was involved in Motor Vehicle Accident. The age of the patients were between 12 years old to 75 years old. There were 2 males and 5 females. All of them were Malays. The number of packed cells that had been transfused was between 1 unit to 25 units. The number of platelet concentrate that had been transfused was between 1 unit to 139 units. Four (4.2%) patients were found to have anti-HPA 5b and another 3 (3.2%) patients were found to have non-specific pattern. Table 2

There was no significance relationship between presence of platelet alloantibody and age of patient (P value > 0.05). This is probably due to small number of patient recruited in this study.

There was also no significant relationship between presence of platelet alloantibody with sex and race of the patients. However all patients with platelet alloantibody were Malays and 2 of them were males and the rest were females.

The number of packed cells and platelet concentrate transfused also showed that it was not associated with the presence of platelet alloantibody. Table 3

DISCUSSIONS

The purpose of this prospective study was to determine the frequency and specificity of platelet alloantibody in these multiply transfused patients and also to correlate with any underlying disease, age of patients, sex of patients, race of patients, and also the numbers and types of blood components given to the patients with the development of platelet antibodies.

The majority of the patients were diagnosed as haematological disorders (66.3%) and the remaining 33.7% were diagnosed to have non-haematological disorders (end-stage renal failure, chronic liver disease and others). In these non-haematological patients, most of them were having fever and sepsis during platelet alloantibody tests was undertaken.
In this study, seven (7.4%) of the patients were found to have platelet alloantibody. The anti-HPA-5b was found to be positive in 4 (4.2%) of these patients and 3 (3.1%) of them were female and only 1 (1.1%) was male. Another 3.2% of patients were shown to have non-specific pattern of platelet alloantibody.

The finding of anti-HPA-5b as the highest frequency in this study was similar with the finding by Kiefel et al.[6] which had shown that the antibody specificity found with the highest frequency was anti-HPA-5b (Br^a). Other specificities found (in decreasing order of frequency) were anti-HPA-1b (Pl^{A2}), anti-HPA-5a (Br^b), anti-HPA-2b (Ko^a), and anti-HPA-1a (Pl^{A1}).

Reports about frequency and specificity of platelet-specific alloantibodies in multitransfused patients are rare. This is due to the fact that in such patients platelet-specific antibodies will occur rarely without HLA antibodies, thus complicating their identification and especially their specificities. Murphy et al. .[7]The possibility of having the non-specific pattern of platelet alloantibodies in this study (3.2%) may be due to the presence of anti-HLA especially anti-HLA 1 which is the commonest to be presence in leukaemia patients.

In the United States, HLA class 1 antibodies were involved in majority of the alloimmunized cases, whereas platelet specific antigens (e.g. HPA) were involved in approximately 10-20% of refractory cases. Both types of antibodies were involved in approximately 5% of cases. A single random RBC or platelet transfusion induces anti-HLA antibodies in fewer than 10% of recipients (most likely related to the tolerogenic effect of blood transfusions). If patients have more than 20 transfusions, they become sensitized in increasing proportions; after 50 transfusions, the majority (as many as 70%) of patients have anti-HLA antibodies. The presence of HLA antibodies showed better

correlation with platelet refractoriness than antibodies directed against platelet-specific antigens. The most common platelet-specific antibody was directed against the HPA-1a antigen present on glycoprotein 11a.[8]

Refractoriness to platelet transfusions due to platelet-specific antibodies is often difficult to assess since, if they developed anti-HLA antibodies, such patients must be transfused with HLA-matched platelets. Nevertheless, platelet-specific antibodies (HPA-1a, HPA-1b, HPA-2b, HPA-3a) have already been found to be responsible for platelet refractoriness.[9] Study done by Uhrynowska et al. [2], showed that the efficacy of platelet transfusions was difficult to assess due to other non haematological factors which could contribute to refractoriness.[10]

Alloantibody specificities encountered in multiply transfused patients differ considerably from those in patients with PTP and maternal alloantibodies in NAIT. Two latters conditions, anti-HPA-1a was the platelet antigen, most frequently encountered among white patients Kroll et al. [11], whereas anti-HPA-1b and anti-HPA-5b were prevalent among multiply transfused patients. In a large series of multiparous blood donor, anti-HPA-5b was the most common alloantibody Kalinowski et al.[12]. However, anti-HPA-1a was the second most frequent alloantibody in that donor group, which reflects the different mode of immunization in that study population (pregnancy).

The frequency of platelet-specific antibodies in multitransfused patients and their implication in platelet transfusion refractoriness has been a matter of controversy for years. The main reasons are the difficulties encountered in differentiating between HLA-

and platelet-specific antibodies and, within platelet-specific antibodies, between auto- or alloantibodies, using the available platelet antiglobulin test Schnaidt et al. [13]. Thus, data suggesting a frequency of platelet-specific antibodies in about 20% of patient were obtained indirectly, such as different reaction patterns of sera tested on lymphocytes and platelets of the same individual as well as transfusion failures with HLA-matched platelets Pegels et al.[14]. In the recent years, capture assays were developed, using immobilized platelet membrane glycoprotein (GP) carrying the antigens under investigation to avoid using whole platelets. These assays overcome the difficulties in differentiating platelet-reactive antibody mixtures and allow determination of antibody specificity.

It is known that previous pregnancies predispose to the production of multispecific HLA antibodies during transfusion therapy.[15] However, there is no evidence to suggest for pre-immunization due to pregnancies as a main cause for the presence of platelet-specific alloantibodies in patients presenting with multispecific HLA antibodies.[13]

Multispecific HLA antibodies are a prerequisite for transfusion-induced platelet-specific alloantibodies. Since only a small percentage of haematologic-oncologic patients undergoing platelet transfusion therapy develop such multispecific HLA-antibodies Brand et al.[15], Schnaidt et al. [13] have concluded that frequency of platelet-specific alloantibodies and their impact on platelet refractoriness with regard to all patients transfused was low. They also believed that, nonimmunological factors were more often implicated in platelet refractoriness than platelet-specific alloantibodies. For the group of

highly HLA-immunized patients in need for HLA-matched single donor platelets, however, there are only as many as 25% will have additional platelet-specific alloantibodies. These platelet-specific alloantibodies will prohibit satisfactory transfusion results if HLA-matched but HPA-incompatible platelets are transfused.

CONCLUSIONS

Alloimmunization in polytransfused patients is a well known observation which often results in decreased responses to subsequent platelet transfusions and failure to achieve haemostatic levels of platelets that may preclude these patients from important procedures, including bone marrow transplantation. Our data showed a significant rate of platelet alloimunization (7.4%) in multiply transfused thrombocytopenic patients. Perhaps more samples are needed to analyse the full spectrum of platelet alloimmunizations, especially platelet specific alloantibody.

From our data, the most frequent platelet alloantibody was anti-HPA-5b. For future transfusion, we recommend that patients receive compatible platelets after ruling out nonimmune, autoimmune and drug-related causes of platelet refractoriness since compatible platelets can significantly improve the platelet recovery.

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Table 1 Demographic data of patients with multiple transfused thrombocytopenia.

Demographic data	Number of patients (n)	(%)
Total Patients	95	
Diagnosis		
Haematological disorders	63	66.3%
Non-Haematological disorders	7	7.4%
-Chronic Liver Disease	11	11.6%
-ESRD	3	3.2%
-Others	11	11.6%
Gender		
Female	50	52.6%
Male	45	47.4%
Ethnic		
Malay	88	92.6%
Chinese	5	5.3%
Indian	1	1.1%
Others	1	1.1%
Age		
0-20 years	28	29.5%
21-40 years	15	15.8%
41-60 years	32	33.7%
61-80 years	17	17.9%
>80 years	3	3.2%

Table 2 Data of patients with platelet alloantibodies

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age	75	46	12	17	50	50	39
Sex	Male	Female	Female	Male	Female	Female	Female
Race	Malay	Malay	Malay	Malay	Malay	Malay	Malay
Diagnosis	NHL	Aplastic anaemia	Familial thrombocy-topenia	MVA	Aplastic anaemia	Aplastic anaemia	AML
Number of packed cells transfused	25	0	11	10	6	5	7
Number of platelet transfused	39	8	25	16	0	24	139
Type of alloantibody	Non specific pattern	HPA-5b	HPA-5b	HPA-5b	No specific pattern	No specific pattern	HPA-5b

Table 3 Association between presence of platelet alloantibody with age, sex, race, number of packed cells and plasma transfusion.

Independant	Absence of alloantibody	Presence of alloantibody	Statistical Tests	P value
Age				
0-40	40	3	0.06	0.807
>40	48	4		
Gender				
Female	45	5	1.19	0.275
Male	43	2		
Ethnic				
Malay	81	7	0.00	0.999
Non Malay	7	0		
Number of packed cells transfused			0.87	0.350
<20	83	6		
>20	5	1		
Number of platelet transfused			0.008	0.927
<20	50	4		
>20	38	3		

(Level of significance, $p < 0.05$)

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Dedicated to my lovely husband, Suhairi Ibrahim and my two daughters Nur Syazlin Amalina and Nur Sofea Amirah for supporting me and loving me unconditionally.

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LIST OF ABBREVIATIONS

Ab	Antibody
ACD	Acid-Citrate Dextrose
ACE	Angiotensin- Converting Enzyme
Ag	Antigen
AIHA	Auto-Immune Haemolytic Anaemia
BFU	Burst- Forming Unit
CD	Cluster of Differentiation
CPDA-1	Citrate- Phosphate-Dextrose- Adenine 1
CPD	Citrate-Phosphate-Dextrose
EDTA	Ethylenediamine Tetra-Acetic Acid
ELISA	Enzyme Linked Immuno-Sorbant Assay
FBP	Full Blood Picture
FFP	Fresh Frozen Plasma
FNHTR	Febrile Non-Haemolytic Transfusion Reaction
GP	Glycoprotein
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
HPA	Human Platelet Antigens
HTR	Haemolytic Transfusion Reaction
ITP	Idiopathic Thrombocytopenic Purpura

LISS	Low Ionic Strength Saline
NAIT	Neonatal Alloimmune Thrombocytopenia
PTP	Post-Transfusion Purpura
PTR	Post-Transfusion Refractoriness
SLE	Systemic Lupus Erythematosus
TAGvHD	Transfusion Associated Graft Versus Host Disease
TRALI	Transfusion Associated Lung Injury
RBC	Red Blood Cell
WBC	White Blood Cell

ABSTRAK

Pesakit yang kerap menerima pemindahan darah adalah terdedah kepada platelet alloimmunisasi. Alloantibodi kepada platelet akan menyebabkan kegagalan kepada transfusi platelet. Tujuan kajian ini dijalankan adalah untuk mengesan alloantibodi kepada platelet didalam pesakit yang kerap menerima transfusi darah di HUSM.

Sebanyak sembilan puluh lima pesakit thrombocytopenia (kiraan platelet < 100 x 100^9/l) yang kerap menerima transfusi darah (lebih dari dua transfusi darah dari sebarang komponen dan tarikh terakhir pemindahan sekurang-kurangnya dua minggu dari tarikh kajian) diambil secara prospektif. Sampel darah diuji dengan menggunakan "Solid Phase System" (Capture-P).

Seramai 45 lelaki (47.4%) dan 50 perempuan (52.6%) termasuk dalam kajian ini. Jarak umur adalah diantara 3 hingga 90 tahun. Kekerapan transfusi darah adalah diantara 10 hingga 168 kali. Tujuh pesakit (7.4%) didapati alloantibodi kepada platelet, terutamanya anti-HPA-5b (4.2%). Tiga pesakit (3.2%) daripada mereka menunjukkan paten yang tidak khusus. Enam pesakit (6.3%) telah menerima transfusi sel darah merah padat kurang daripada 20 unit dan seorang pesakit (1.1%) telah menerima transfusi lebih daripada 20 unit sel darah merah padat. Empat pesakit (4.2%) menerima transfusi platelet kurang dari 20 unit dan tiga pesakit (3.2%) menerima lebih dari 20 unit platelet.

Keputusan ini membuktikan implikasi di masa hadapan dalam memilih penderma platelet yang mempunyai alloimmunisasi.

ABSTRACT

Multiply transfused patients are frequently subjected to platelet alloimmunization.These platelet alloantibodies produced can result in refractoriness to platelet transfusion. The aim of this study is to detect the presence of anti-platelet alloantibodies in multiply transfused thrombocytopenic patients in HUSM

Ninety five thrombocytopenic (platelet count <100 x 10^9/l) and multilply transfused (more than 2 times transfusion of any blood component with last transfusion more than 2 weeks prior to the study) patients were recruited prospectively. The blood samples were tested using a Solid Phase system (Capture P).

There were 45 males (47.4%) and 50 females (52.6%) recruited with ages from 3 to 90 years. The frequency of transfusions ranged from 10-168. Seven patients (7.4%) were detected to have platelet alloantibodies, predominantly anti-HPA-5b in 4 patients (4.2%). Three of them (3.2%) showed a non-specific pattern. Six patients (6.3%) had received packed cells less than 20 units and another 1 (1.1%) received more than 20 units of packed cells. Four patients (4.2%) received platelet transfusion of less than 20 units and another 3 patients (3.2%) received more than 20 unit platelets.

The study may have future implications for the selection of platelet donors for alloimmunized recipients in HUSM.

Chapter 1

INTRODUCTION

1.0 INTRODUCTION

1.1. Platelets

1.1.1. Formation of blood platelets

Blood platelets are fragments of the cytoplasm of megakaryocytes, hence there are non nucleated and are formed chiefly in the bone marrow. Most of the available knowledge about thrombopoeisis is derived from bone marrow culture experiments, or from observations in humans and animals recovering from drug induced thrombocytopenia, which are conditions under which physiological mechanisms may not prevail.

Megakaryocytes are derived from pluripotential stem cells, as the earliest recognized platelet precursors is a burst-forming unit denoted BFU-Meg. Under the influence of thrombopoeitin (Tpo) and cytokines such as IL-3 and IL-11, the BFU-Meg develop into megakaryocyte colony-forming units (CFU-Meg).

The committed progenitor cells cease to undergo classical mitosis, developing instead by endomitotic reduplicative, where nuclei divide but not the cell, the morphological and biochemical features of megakaryoblasts and then megakaryocytes. Their ploidy states progress from 2N up to 32N or even 64N. Their diameters increase from 6-20μm (megakaryoblasts) to around 60μm (fully mature megakaryocyte) associated with proliferation of the characteristic platelet granules (α-granules and dense bodies) and

membrane glycoproteins, which are vital to platelet functions. The membrane glycoproteins have different functions for example glycoprotein IIb-IIIa involves in platelet aggregations and glycoprotein Ib involves in platelet adhesions to subendothelial microfibrills.

The rapid increase in cytoplasm is accommodated by progressive folding, or invagination, of its membrane and it has been proposed, that this process accounts for the appearance of demarcation membranes, which eventually form the boundary of individual platelets. A fully mature megakaryocyte begins to extend pseudopodia, which penetrate through the walls of adjacent individual platelets or larger cytoplasmic fragments. The latter 'proplatelets' are transported through the bloodstream via the heart to the lungs, where final mechanical fragmentation is accomplished in the pulmonary microcirculation. Each megakaryocyte gives rise to as many as 3000 platelets.

Around 20-30% of 'circulating' platelets appear to be sequestered in the spleen, although it is not known if the splenic pool represents the newly released cells. The normal life span of platelets ranges between 8 and 14 days. The platelet count in the peripheral blood is in the range 150-400 x 10^9/l in normal subjects. (Hoffbrand et al.,1999)

1.1.2. Structure and function of the blood platelets.

Platelets that have been fixed immediately after removal from the body appear under the electron microscope as smooth, biconvex discs with a diameter of 2-4μm.

The platelet plasma membrane is vital to the cells function for two reasons. Firstly, it contains a number of specific glycoprotein (GP) receptors, through which platelets interact with aggregating agents, inhibitors, coagulation factors (such as fibrinogen, von Willibrand factor and thrombin) and consequently, with the vessel wall and with each other. Secondly, the platelet membrane also contains phospholipids, which are concerned with prostaglandin synthesis and calcium mobilization within the cell, and with the generation and localization of procoagulant activity on the outer surface of the platelet. (Hoffbrand et al., 1999)

1.2 Blood products

A blood product is defined as any therapeutic substance prepared from human blood. Whole blood is unseparated blood collected into an approved container containing an anticoagulant-preservative solution. Whole blood is obtained from human blood donors by venesection. Whole blood may be suitable for transfusion in many clinical situations, such as red cells replacement in acute blood loss where there is also hypovolaemia. However, the whole blood is also a source of other important components and the separation of whole blood into its constituent component-red cells, platelets and plasma is widely practiced for use when these components only are specifically required.

A blood component is a constituent of blood, separated from whole blood into red cell concentrates, plasma and plasma components such as fresh frozen plasma (FFP) and cryoprecipitate as well as platelet concentrate.

(Emmanuel, 2001)

For platelet concentrates, there are two types which are available for transfusion:

1. platelet from random donor
2. apheresis platelet.

Platelets are prepared from whole blood of apparently healthy donors by differential centrifugation, while apheresis platelets are harvested from individual donors through apheresis with minimal or no contamination by leucocytes or red cells. Although platelets are the predominant cellular component, both products also contain significant amounts of red cells, leucocytes and plasma. All these blood components in platelet concentrates are potentially immunogenic and can lead to various immunological and clinical consequences in transfusion recipients. (Kao et al., 2000)

1.3. Blood transfusion

1.3.1. Indications

Blood transfusion involves the infusion of whole blood or the blood components from one individual (the donor) to another (the recipient).

For the whole blood, it is usually reserved for red cell replacement in acute blood loss with hypovolaemia, for example in traumatic or surgical blood loss, or severe gastrointestinal or uterine haemorrhage. It is also used in exchange transfusion for the case of neonatal jaundice. Sometimes it is given to the patients needing red cell transfusions where red cell concentrates are not available.

Red cell concentrates are the treatment of choice in chronically anaemic patients who require transfusion. In older subjects, a diuretic is often given simultaneously and the infusion should be sufficiently slow to avoid circulatory overload. In the majority of patients with deficiency anaemias appropriate therapy with iron, folate or vitamin B_{12} is sufficient and red cell transfusions are seldom required. In chronic anaemias which do not respond to haematinics, transfusion should be avoided unless the patient is at risk from, or incapacitated by, the anaemia. This concentrates is also used with crystalloid replacement fluids or colloid solution in acute blood loss.

Platelet concentrates as mentioned earlier are harvested by cell separators or from individual donor units of blood. These concentrates are indicated in severely thrombocytopenic patients or patients with platelet dysfunctions in established haemorrhage or prophylactically to prevent bleeding for example during intensive myelotoxic chemotherapy to keep the platelet count above 5-10 X 10^9/l. Their most important use is therefore in the support of patients with severe bone marrow failure for example due to acute leukaemia, aplastic anaemia, myelodysplasia or bone marrow transplantation. If fever, infection or concurrent coagulopathy is present or the platelet count is falling rapidly or potential bleeding sites for example surgical wounds are present, the platelet count should be kept more than 20 X 10^9/l. Platelets may also be needed for patients with platelet functional disorders, following massive blood transfusion during cardiopulmonary by-pass surgery with bleeding not due to surgically correctable disorders and for patients with disseminated intravascular coagulation and bleeding. Platelets are not usually used in immune thrombocytopenias but may be given in auto-immune thrombocytopenia with major haemorrhage and in neonatal alloimmune thrombocytopenia.

For the fresh frozen plasma, it is a rapidly frozen plasma separated from fresh blood and its main use is for the replacement of coagulation factors, for example when specific concentrates are unavailable or after massive transfusions, liver disease, warfarin anticoagulant overdose and disseminated intravascular coagulation (DIC). It is also indicated in thrombotic thrombocytopenic purpura (TTP).

Cryoprecipitate is obtained by thawing fresh frozen plasma at 4°C and contained factor VIII, factor XIII, von Willebrand factors (vWF), fibronectin and fibrinogen. It is used widely as replacement therapy in haemophilia A and von willebrand's disease before more purified preparations of factor VIII became available. It is also used as a source of fibrinogen in acquired coagulopathies eg DIC. (Emmanuel, 2001)

1.3.2. Adverse effects of blood transfusion

Transfusion reactions occur in 7-10% of all recipients of blood or blood components. The majority of them are minor reactions, of which 10% are hemolytic and 90% are non hemolytic reactions. Transfusion reactions may be divided into acute (< 24 hours) and delayed (> 24 hours), as shown in Table 1.0 and Table 1.2.

Table 1.1 Acute Transfusion Reactions.

Immunologic	**Etiology**
Haemolytic transfusion reactions (HTR	ABO incompatibility
Febrile non-haemolytic transfusion reaction (FNHTR)	Cytokines ,antileucocyte antibodies
Allergic	Antibodies to plasma protein
Anaphylaxis	Antibodies to IgA
Transfusion related acute lung injury (TRALI)	Antibodies to leucocytes or to complement activation
Non Immunologic	
Marked fever with shock	Bacterial contamination
Atypical reaction with hypotension	Associated with ACE inhibitors
Congestive cardiac failure	Volume overload
Air embolism	Air infusion via line
Hypocalcaemia	Citrate toxicity

Hypothermia	Rapid infusion of cold blood
Hypokalaemia and hyperkalaemia	Red cell storage

Table 1.2 Delayed adverse reaction to transfusion (>24 hours).

Immunologic	**Etiology**
Alloimmunization to red blood cell (RBC), white blood cell (WBC) and platelets	Exposure to antigen of donor origin-plasma protein, human leucocyte antigen (HLA)
Hemolytic	Anamnestic antibodies to RBC antigens
Transfusion associated graft versus host disease (TAGvHD)	Engraftment of transfused functional lymphocytes Not well understood
Post transfusion purpura	Antiplatelet antibodies
Non Immunologic	
Iron overload	Multiple transfusion
Transfusion transmitted disease	Hepatitis, human immunodeficiency virus (HIV), Cytomegalovirus etc

(Emmanuel, 2001)

1.4. Causes of thrombocytopenia

Thrombocytopenia is defined as a platelet count of less than 150 X 10^9/l. There are many causes of thrombocytopenia which can be divided into non-immune thrombocytopenia and immune thrombocytopenia as shown in Table 1.3.

Table 1.3 Causes of thrombocytopenia.

A. Non immune thrombocytopenia is associated with :
-bone marrow transplant
-sepsis
-disseminated intravascular coagulopathy
-splenomegaly
-fever
-leukaemia/anaemia
-antibiotic therapy
-clinical bleeding
-hepatomegaly
-liver disease
-chemotherapy
-heart valves replacement

B. Immune thrombocytopenia:

1. Autoimmune thrombocytopenia this occurs because of the presence of autoantibodies to the patient's own platelet as in idiopathic thrombocytopenic purpura and acute thrombocytopenic purpura.

2. Alloimmune thrombocytopenic purpura which may be associated with multiple exposures to similar cellular antigens as in post transfusion purpura and neonatal alloimmune thrombocytopenia.

3. Drug induced which may occur following the administration of heparin, quinine, sulpha drugs and others.

1.5. Platelet alloantigens and alloantibodies.

Platelet alloantigens are defined by alloantibodies directed against genetically determined molecular variants of proteins or carbohydrates on the platelet membrane. The alloantibodies are elicited in normal individuals upon exposure to the alloantigen usually during pregnancy and in blood transfusion, or, rarely, by bone marrow transplantation. These alloantibodies bind to the target platelet alloantigens, resulting in immuno-mediated platelet phagocytosis.

Three major group of alloantigens are present on platelets:

1. Blood group antigens
2. Platelet-specific antigens
3. HLA's

There are different types of clinically relevant platelet alloantigens. Alloantigens shared with other blood cells and tissues are referred to as Type I antigens. Among these are the glycoconjugates of the blood group ABH system and the highly polymorphic HLA class I molecules. Type II alloantigens are more or less specific to platelets and are conventionally called platelet-specific alloantigens. It is well documented that platelet-specific antibodies against type II alloantigens play a role in the pathological mechanism of neonatal alloimmune thrombocytopenia (NAIT), post-transfusion purpura (PTP) and post-transfusion refractoriness (PTR). In contrast, platelet antibodies against type I alloantigens seem to be restricted to PTR. (Santoso et al., 2003)

Blood group antigens on platelets include ABH, Lewis, Ii and P antigens. These antigens are present in platelet membrane glycoproteins (Santoso S et al, 1991). Antibodies to ABO, Lewis, and P antigens often develop spontaneously in antigen-negative persons, and antibodies to I antigens are cold reacting autoantibodies (Mollison PL et al, 1993).
It is generally assumed that the expression of ABH antigens on platelets is too weak that anti-A, anti-B isoagglutinins cannot affect considerably the survival time of ABO-incompatible platelets (Santoso et al, 2003) and although the impact of ABO compatibility on post-transfusion platelet recovery, albeit small, has been demonstrated (Lee EJ et al., 1989).

Platelet membrane glycoproteins contain antigenic epitopes that are not found on other types of blood cells. More than 20 such platelet specific alloantigens have been identified (Kunicki et al, 1992), and many have been well characterized at the molecular level (Newman et al, 1995). Many platelet-specific alloantigens have been found on other cells and tissues as well such as the cell adhesion receptor and intergrins. Six diallelic alloantigen systems (HPA-1-5 and HPA-15) and a number of low frequency antigens (HPA-6W-14W, HPA-16W) have been described. Currently, six platelet membrane glycoproteins GPIa, GPIbα , GPIbß , GPIIb, GPIIIa and GPI-linked CD109 have been identified as carriers of platelet alloantigenic determinants (Santoso et al., 2003). These platelet specific alloantigens are present in dimorphic forms, and the allelic frequencies are equally distributed in a high-frequency public form and a low-frequency private form (Kim et al., 1995). They can elicit the production of antibodies through transfusion and pregnancy. The development of such antibodies can result in three clinical conditions:

1. Neonatal alloimmune thrombocytopenia- mother becomes immunized to platelets of fetus and give birth to thrombocytopenic infants;

2. Post-transfusion purpura- patients become immunized to platelet-specific alloantigens after blood transfusion and develop thrombocytopenia;

3. Transfusion-associated alloimmune thrombocytopenia- patients receive multiple platelet transfusions and become refractory to random-donor platelets. (Mcfarland, 1996)

Table 1.4. Human Platelet-Specific Antigen Systems (Adapted from Kim et al, 1995)

Platelet Antigen System	Protein Antigen	Synonyms	Alleles	Antigen Frequency
HPA-1	GPIIIa	PI^A,Zw	HPA-1a = PI^{A1} HPA-1b = PI^{A2}	97% 26%
HPA-2	GPIb	Ko, Sib	HPA-2a HPA-2b	99% 14%
HPA-3	GPIIb	Bak, Lek	HPA-3a HPA-3b	85% 66%
HPA-4	GPIIa	Pen, Yuk	HPA-4a HPA-4b	>99% <1%
HPA-5	GPIa	Br, Hc, Zav	HPA-5a HPA-5b	99% 20%

The platelet membrane glycoproteins (GPs) that carry the human platelet antigen (HPA) epitopes play a fundamental role in platelet function so that platelet alloantibodies may not only cause thrombocytopenia, but may also affect primary haemostasis. (Hoffbrand et al., 1999)

Moreover, HPA antibodies have been found in approximately 20% of alloimmunized patients. (Kiefel et al., 2001)

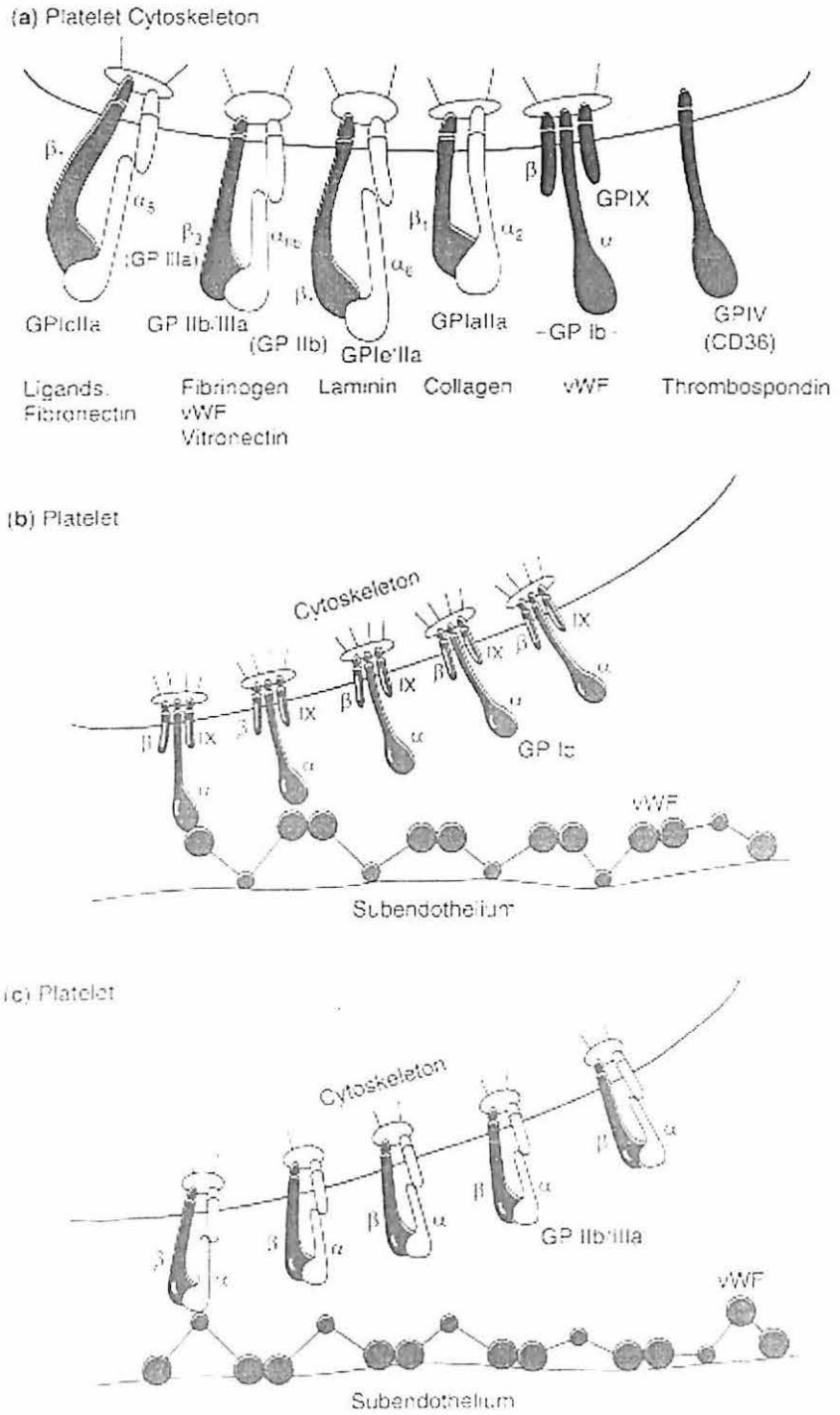

Figure 1.1 Platelet membrane glycoproteins and adhesion to membrane (Adapted from Hoffbrand et al.,1999)

The HLA class 1 molecules constitute the third group of platelet alloantigens. These antigens are heterodimeric membrane glycoproteins that consist of a 44-kD highly polymorphic heavy chain and a 12-kD invariant $ß_2$-microglobulin. These two polypeptides are noncovalently associated with each other and are present in varying quantities on most cells in an individual. The genes encoding HLA heavy chains are located at three different loci (A, B and C) of chromosome 6. On platelets, the expression of HLA-A and B antigens is higher than that of HLA-C antigens. (Santoso et al, 2003)

Functionally, HLAs play a major role in presenting antigenic peptides to cytotoxic T lymphocytes (CTLs) (Zinkernagel et al, 1979) and are essential for the ontogenetic development of CD8+ cytotoxic T cells in the thymus. The expression of HLA antigens on platelets can vary substantially and is influenced by gene dosage and other as yet uncharacterised genetic factors (Liebert et al, 1979) Antibodies to HLAs are responsible for greater than 90% of immune-mediated platelet transfusion refractoriness. (Mcfarland, 1996).

The most common platelet reactive antibodies in transfused patients recognize epitopes on HLA class I molecules which are frequently responsible for febrile nonhemolytic transfusion reactions and immunologically mediated PTR. Although most cases of antibody-mediated PTR related to HLA class I, additional platelet specific antibodies have been identified in sera of transfused patients.

Risk factors for developing HLA antibodies include the presence of more than 1 million donor leucocytes in transfused products, transfusing ABO-mismatched platelets, the presence of an intact immune system (ie absence of cytotoxic or immunosuppressive therapy), female sex (approximately 75% of cases), and a history of multiple transfusions (>20). (Sepulveda et al., 2001)

Refractoriness to platelet transfusion that accompanies alloimmunization is one of the most serious and most difficult to manage among transfusion therapy hazards. It occurs when a patient receives platelet preparation from another person that contains leucocytes. The body reacts to the leucocytes and develops antibodies against them (HLA antibodies). This immune response, called alloimmunization, can cause patients to become refractory (resistant) to subsequent platelet transfusions. This means that they do not respond or benefit from the transfusion, whereby the risk of spontaneous bleeding from the patient. Patients receiving leucocyte-reduced blood products are at much lower risk for refractoriness to platelet transfusion than the recipients of blood that is not leucocyte reduced. (Kruskall, 1997)

There are clinical syndromes associated with platelet alloimmunization:

1. Human leucocyte antigens alloimmunization causes refractoriness to platelet transfusion.This can be reduced by the use of leucocyte-depleted blood components. There are few reported cases of refractoriness due to HPA alloimmunization.

2. Human platelet antigen alloimmunization causes NAIT and PTP. Most HPAs have been implicated in NAIT. Furthermore, HPAs which is associated on GPIIb-IIIa are primarily, if not exclusively, involved in PTP, and the immunochemical properties of this complex may be related to the mechanism of platelet destruction in PTP. (Hoffbrand et al., 1999)

Patients not previously sensitized to develop anti-platelet antibodies approximately 3-4 weeks (10 days to 26 days) after the transfusion and patients previously immunized by transfusion, pregnancy, or organ transplantation, anamnestic responses occur as early as 4 days after transfusion. Macrophages in the liver, spleen, and other tissues of the mononuclear phagocyte system phagocytize and destroy antibody-coated platelets. (Sepulveda et al., 2001)

Other than alloimmunization to platelet antigens, there is also alloimmunization to red cells and granulocytes in multiply transfused patients. (Sepulveda et al., 2001)

Approximately 20-85% of patients who receive multiple transfusions become immunized against platelet antigens (eg HLA, HPA) and approximately 30% of patients who are alloimmunized develop refractoriness to platelet transfusions (Sepulveda et al., 2001). Refractoriness may be caused by immune causes as well whereby the antibodies present in the patient's serum, which can react with antigens present on the platelet membrane and decrease platelet in vivo survival and function. (Rebulla, 2002). In approximately 66% of these patients, non immune factors such as sepsis, antibiotics (for example

amphotericin), hypersplenism, fever, active bleeding and DIC alone are the cause, whereas alloimmunization may be involved in 33% of refractory patients, often in combination with nonimmune causes. (Sepulveda et al., 2001)

Refractoriness to platelet transfusion is most commonly seen in patients with haematologic diseases requiring frequent red blood cell and platelet transfusions. Platelet refractoriness is not frequently seen in surgical patients, who may require less prolonged and intense platelet support than haematology and oncology recipients. (Kaneda et al., 1999)

Frequently, patients with refractoriness to platelet transfusion are asymptomatic and diagnosed by laboratory methods; however, failure to achieve hemostatic levels of platelets may preclude these patients from important procedures, including bone marrow transplantation. Alloimmunization should be avoided at all costs in candidates for bone marrow transplantation. In platelet refractoriness patients, preexisting bleeding resulting from thrombocytopenia may persist after transfusion of an appropriate therapeutic dose of platelets and rarely, spontaneous bleeding may occur after prophylactic transfusion of platelets. (Sepulveda et al., 2001)

But it should be remembered that platelet transfusions in a bleeding patients are usually associated with very low post-transfusion platelet count increments. Nonetheless, such transfusions are considered a success if they are associated with cessation of bleeding. (Rebulla, 2002)

In general, alloimmunization results in the rapid removal of platelets and in lower counts at 10 minutes to 1 hour post-transfusion, whereas nonimmune causes mostly affect the 4- to 24-hour post-transfusion count. Mild alloimmunization, however, can be present with 1-hour increments within the reference range. (Sepulveda et al., 2001)

Strategies to overcome platelet transfusion refractoriness include the selection of platelets from HLA-identical or HLA-compatible donors from HLA-typed donor registries, (Yankee et al, 1969), platelet cross-matching (Murphy et al, 1998), and the antibody specificity prediction method (Petz et al., 2000).

1.6. The immune response

This immune response can be due to human platelet antigens and human leucocyte antigens.

1.6.1. Immune response to Human platelet antigens

An immune response to HPAs is an exception rather than a rule. The most frequently occurring antibody is anti-HPA-Ia which is found in 80-90% of cases. Anti HPA-5b occurs in 10-15% of the cases, and there are only occasional detections of antibodies to the other HPAs. These antibodies almost always occurs in women who have been immunized by pregnancy or in association with PTP. The increased use of platelet transfusion since the early 1980s has not altered this situation; there are few reported cases of refractoriness due to platelet specific alloantibodies, and the incidence and specificity of these alloantibodies in this situation is still uncertain. Although anti-HPA-Ia is the most frequently encountered antibody, less than 10% of HPA-Ia-negative individuals who are exposed to HPA-Ia become immunized. (Hoffbrand et al., 1999)

1.6.2. Immune response to Human leucocyte antigens

Alloimmunization to HLA associated with platelet transfusions is caused by the passenger leucocytes especially the donor dendritic cells, which directly activate the recipients helper T-lymphocytes. Platelets alone seem to be unable to induce primary antibody responses against HLA antigens, due to the absence of HLA class II antigens. (Hoffbrand et al, 1999)

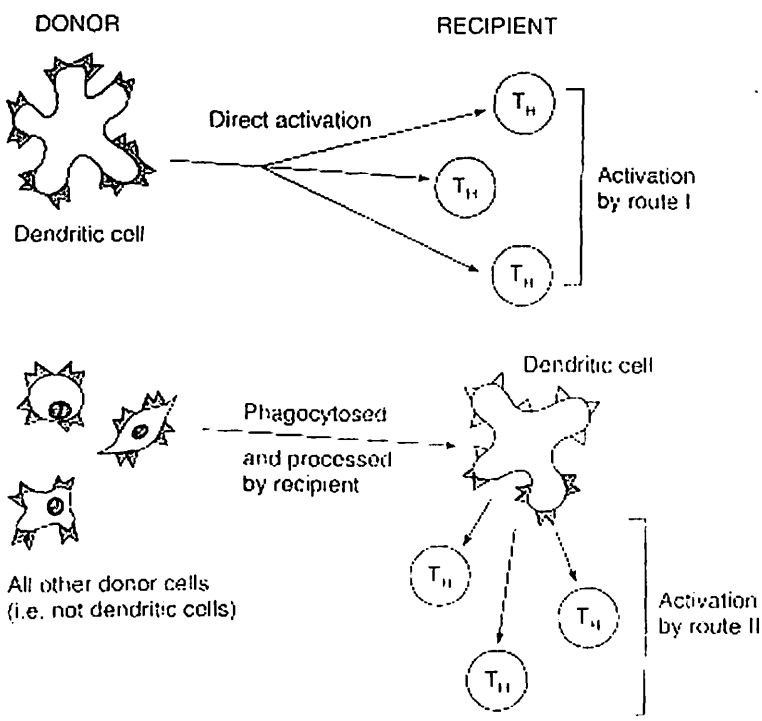

Figure 1.2 Dual route of sensitization by allo-antigen. (Adapted from Hoffbrand et al.,1999)

1.7. Laboratory detection of platelet alloantibody.

1.7.1 Enzyme Linked Immuno-Sorbant Assay (ELISA)

The basic principle of ELISA is to use an enzyme to detect the binding of antigen (Ab) and antibody (Ab). The enzyme converts a colourless substrate (chromogen) to a coloured product, indicating the presence of antigen:antibody binding. An ELISA can be used to detect either the presence of antibodies or antigens in a sample, depending on how the test is designed.

If the test is designed to detect Abs in a sample:

1. Combine Ag and Ab; allow them to bind:

- the antigen is firmly attached to a surface (usually plastic well or filter)
- sample being tested is added , allowed to incubate , then unbound Abs are washed from the surface.

2. Detect Ag:Ab binding:

- an antiglobulin that is covalently attached to an enzyme (ie enzyme-linked) is added, incubated, then unbound antiglobulins are washed from the well.
- a colourless substrate of the enzyme is added
- if the enzyme-linked antiglobulin bound to Abs on the surface, the