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HPA antibodies in Algerian multitransfused patients: Prevalence and involvement in platelet refractoriness

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ABSTRACT

Background: Patients receiving cellular blood components may form HLA or HPA antibodies. The frequency and the specificity of HPA antibodies after a series of blood transfusions have never been reported in the Algerian population which is ethnically diverse and runs a higher risk of platelet alloimmunization due to high b allelic frequencies observed for the HPA systems.
Methods: 117 polytransfused patients were included in this study; the detection of HPA antibodies was performed by the Monoclonal Antibody-specific Immobilization of Platelet Antigens method (MAIPA). Post-transfusion platelet effectiveness was evaluated by the calculation of corrected count increment (CCI).
Results: The antibodies against platelets were detected in 10.26% of the patients. In this study, the platelet systems concerned by the alloimmunizations were specifically HPA-1, -3 and -5 with particular predominance of HPA-1. Twenty two patients were refractory to platelet transfusion, as assessed by a CCI; in which 64% have factors associated with increased platelet consumption. Platelet Immunization was found in 14% of platelet refractoriness (PTR) cases. 03 Anti-platelet antibodies were directed against GPIb-IX (n = 1), anti-HPA-1b (n = 1) and anti HPA-5b (n = 1) associated with anti-HLA antibodies in two cases.
Conclusion: HLA and HPA alloimmunization is common among chronically transfused patients. PTR detection, identification of the underlying causes, and selection of the appropriate product for transfusion are fundamental to reduce the risk of major bleedings.

1. Introduction

Platelet-specific alloimmunization results from exposure of normal subjects to allotypic determinants. This is mainly encountered during pregnancy or after transfusion with platelet (PLT) concentrates or red blood cell concentrates that contain platelets. Patients with HPA antibodies (that could be a result of previous pregnancy or transfusion) are at risk of developing post-transfusion purpura (PTP) following subsequent exposure to cellular blood products. Alloimmunization may cause platelet destruction [1]. In contrast with the abundant literature on anti-HLA post-transfusion alloimmunization, the frequency of specific platelet antibodies in multitransfused patients and their role in platelet refractoriness (PTR) remain a subject of debate and controversy [1]. The main reason is the difficulty of detecting specific antibodies. Some data have reported the

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involvement of specific antibodies in 8–20% [1,2]. However, The Trial to Reduce Alloimmunization to Platelets (TRAP) Study Group showed that PTR due to antibodies against platelet glycoproteins is ≤2% of immune PTR [3], and higher in individuals who also have HLA antibodies, with rates estimated to be between 9% and 25% among HLA alloimmunized recipients [4]. Platelet allo-immunization after a series of blood transfusions has never been studied in the Algerian population. The high risk of allo-immunization in this population due to high frequencies of allele b in some HPA systems [5] may lead to different data on platelet antibody formation from those observed in previously published studies on this topic.

Platelet refractoriness is an important problem in multitransfused patients [6]. Its incidence is estimated to reach 30–70% of patients transfused with non-leukoreduced components. Several causes have been reported, such as alloimmunization against HLA and/or specific-platelet antigens and non-immune causes such as fever, infection, disseminated intravascular coagulation (DIC), splenomegaly (SPM) [7,8]. Identification of these factors and their correction allow prevention of serious hemorrhagic events and reduce utilization of platelets that represent a scarce and expensive product [9]. HLA/HPA alloimmunized patients are dependent on platelets derived from selected individual donors for platelet support therapy [10] hence the difficulty of finding compatible donors. Platelet refractoriness due to alloimmunization can be treated with compatible platelet concentrates [11]. A number of clinical trials have confirmed that leukocyte reduction of cellular components to a residual leukocyte level of $<5 \times 10^6$ is highly effective at reducing HLA alloimmunization rates in multitransfused patients [12]. Leukocyte depletion does not appear to reduce the frequency of HPA alloantibodies [13].

Objective: The objective of our study is to review the frequency and specificity of platelet alloimmunization in multitransfused patients, and to study its implication in the platelet refractoriness cohort.

2. Materials and methods

2.1. Patient selection

Over a period of six months, 117 multitransfused patients seen at the hematology department of University Hospital Center in Annaba are included in this study, of which 58 (49.6%) with no alloantibodies at base line, have hemoglobinopathies (β thalassemia, sickle cell disease), receiving only non-leukoreduced red blood cell ABO compatible (RBC; Group 1 = Anemic patients), and 59 (50.4%) leukemic patients who received non-leukoreduced platelet concentrate ABO identical (PC; Group 2 = Leukemic patients) produced by the method of PRP. The study was approved by the institutional review board.

2.2. Antibody testing

The search for anti-platelet antibodies was performed by the Monoclonal Antibody-specific Immobilization Platelet Antigens (MAIPA) using 10 μL of monoclonal antibodies (anti-GPIaIIa clone P16; anti-GPIIbIIIa clones PL1-64 and PL2-46; anti-GPIbIX clone GRP; anti-HLA class I clone B1G6 anti B2-microglobuline 'Beckman'), 30 μL of donor platelets typed in the HPA-1,-3 and -5 systems and Goat anti mouse 'Interchim' [14]. The MAIPA is the gold standard reference technique in platelet immunology and has been the main topic for standardization in the 2006 International Society of Blood Transfusion (ISBT) – International Platelet Workshop [15,16].

2.3. HPA genotyping

Genomic DNA was isolated using the 'QIAamp DNA blood Mini Kit' (Qiagen) according to the manufacturer's instructions. HPA-1, -2, -3 and -5 genotypings were determined by polymerase chain reaction–sequence specific primer (PCR-SSP) [17].

2.4. Calculation of the corrected count increment

The corrected count increment (CCI) used to evaluate effectiveness of platelet transfusion in group 2 was calculated as the difference between the platelet count within an hour and 24 hours after transfusion and the platelet count before transfusion, multiplied by the body surface area (in square meters) and divided by the number of platelets transfused [18].

Platelet content was available for the majority of PC transfused. The majority of PC transfused is aged from 2 to 3 days.

A CCI of >7.5 at 1 hour after transfusion or >4.5 at 20–24 hours after transfusion is considered as acceptable. Platelet refractoriness is defined as the failure to achieve an expected CCI after two consecutive transfusion episodes [19].

2.5. Statistical analysis

The frequency of alloimmunization in patients transfused by PC (Leukemic patients) and those receiving only RBC (Anemic patients) was compared by Fisher's exact test.

2.6. Protocol

The cohort of patients receiving only RBC transfusions (Anemic patients) was included in the study because residual platelets in RBC can be the source of platelet alloimmunization and may even develop a PTP. Platelet refractoriness cases are studied separately in the cohort of patients receiving platelet concentrates (Leukemic patients).

3. Results

The multitransfused population is composed of 76 men (65%) and 41 women (35%), the sex ratio is 1.85. The patients were aged 10–82 years; the average age is 37.6 ± 19.8 and the median is 47 (Table 1).

The frequency of platelet-specific immunization was 10.3% (12/117 cases). Despite the fact that both groups contain almost the same number of samples (Table 2), the number of cases of platelet-specific immunization for Leukemic patients (multitransfused patients with PC) is less than the number found for Anemic patients (multitransfused patients with RBC) respectively, 5 (8.5%) *versus* 7 (12.1%)