Blood transfusions and adverse acute events: a retrospective study from 214 transfusion-dependent pediatric patients comparing transfused blood components by apheresis or by whole blood

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Abstract
Introduction. Blood transfusion is a lifesaving procedure for patients affected by hematological diseases or hemorrhage risk.
Aim. This retrospective study was aimed to evaluate clinical safety of pediatric transfusions by comparing the frequency of adverse events caused by apheresic blood components vs whole blood.
Methods. From 2011 to 2015, 214 patients (blood malignancy patients, n = 144 and thalassemic patients, n = 70) received 12,531 units of blood components. The adverse acute reactions occurred during patient hospitalization were reported to the Hemovigilance system and assessed by fitting a logistic mixed-effect model.
Results. A total of 33 (0.3%) adverse acute events occurred. Odds ratio (OR) of adverse events from apheresis vs whole blood transfusion adjusted by patient classification was not statistically significant (OR [95% CI], 0.75 [0.23-2.47]).
Conclusion. Our findings showed no significant differences in the prevalence of adverse acute events between blood component collected by apheresis vs whole blood in our study center.

Key words
• apheresis
• whole blood
• acute adverse transfusion reactions
• pediatric patients

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INTRODUCTION

Blood transfusion is a lifesaving therapy for patients affected by anemia, coagulation disorders, bone marrow aplasia, as well as many other conditions compromising oxygen transport and hemostatic function [1-3]. Despite the increasing of transfusion safety achieved by both application of good manufacturing practice (GMP) and appropriate therapeutic protocols, blood components can be associated with acute (< 24 h) or delayed (≥ 24 h) adverse effects, especially in transfusion-dependent pediatric patients [2].

Acute or delayed adverse effects are classified as immunological and non immunological reactions. Acute adverse events include hemolytic, febrile non hemolytic, allergic and anaphylactic reactions, as well as lung injury, infection or sepsis, and circulatory overload. Delayed adverse events include erythrocyte and platelet alloimmunization, hemolytic reactions, post-transfusion purpura, immunomodulation, graft versus host disease, as well as iron overload in long-term transfusion [4, 5]. When acute reactions occur, it is necessary to stop the transfusion procedure to establish an appropriate treatment. Besides, the event must be notified to Blood Bank [4, 5]. Laboratory parameters include hemoglobinemia (pink or red serum/plasma), hemoglobinuria, positive direct anti-globulin test (DAT), elevated indirect and direct bilirubin test, and red blood cell (RBC) abnormalities, such as schistocytes in intravascular hemolysis or spherocytes in extravascular hemolysis [4, 5].

According to the Italian Report of Haemovigilance (2016), from January 1st to December 31st 2016, 1958 adverse reactions were notified in recipients of allogeneic blood components (one every 1560 transfused units); taking into account only adverse reactions that are probably or certainly imputable with a high level of severity the frequency is one every 339 543 transfused units. It is estimated a more than double incidence of adverse reactions in pediatric population [9, 13-16]. Transfusion reactions in pediatric populations have not been well explored; however, a study evaluating 126 pediatric patients reported a percentage of 14.4% of acute transfusion reactions (urticarial, cutaneous) and mild respiratory symptoms [16]. In light of this, we report our single-center experience focused on a retrospective analyses aimed to evaluate putative difference between apheresis and whole blood transfusions underlying adverse acute events in 214 pediatric patients.

MATERIALS AND METHODS

Study population

From 2011 to 2015, blood components, collected at the Division of Clinical Immunology, Immunohematology and Transfusion Medicine, were transfused in patients with blood malignancy or thalassemia in the Pediatric Department, at Università degli Studi della Campania “Luigi Vanvitelli”, Naples, Italy. Pediatric patients (n = 214, aged from 8 to 19 years) received randomized blood components obtained by apheresis or by whole blood, according to the availability in the Blood Bank. Exclusion criteria were the presence of pre-existing cardiopulmonary affections and medical history of allergy and anaphylaxis. Detailed forms on the type and rate of transfusion related to adverse events occurred during hospitalization were obtained.
from Pediatric Division and sent mandatorily to our Division for the Hemovigilance Network. The number and the type of blood components transfused under normal clinical practice were obtained from our electronic database. A transfusion reaction was an adverse acute event attributable to a blood product infusion (PLTs, RBCs, plasma). Data on all adverse acute events were registered using authorized and approved procedures. Guidelines on assessing donor suitability for blood donation are reported on DM November 2, 2015 by Italian Minister of Health (www.gazzettaufficiale.it/eli/id/2015/12/28/15A09709/sg) and according to the Society of Transfusion Medicine and Immunohematology (www.centronazionalesangue.it/sites/default/files/it_standards_transf_med.pdf) [17]. Donors were deferred in presence of risk behavior, cardiac pathologies, autoimmune and neoplastic diseases and the use of particular drugs according to Italian legislation.

Blood derivates

Blood donation was performed by apheresis or by whole blood. During the apheresis procedure, the donor is connected through a single venous access to a sterile disposable kit in a closed circuit. The apheresis allows the collection of aliquots of RBCs, PLTs and plasma using Haemonetics MCS® 9000 system blood cell separator (Haemonetics S.A. Signy Centre, Ruedes Fléchères 6, Svizzera). The main advantage of this procedure is the low extracorporeal volume (calculated by volume of the apheresis chamber, the hematocrit (Hct) and total blood volume of the donor), which avoids modifications in donor pressure. Through the centrifugal force, blood cell separators collect blood aliquots during the discontinuous flow procedure throughout the programs for the multicomponent collections of RBCs (erythro-apheresis, EA, 947F) and RBCs-plasma (erythro-plasmapheresis, EPA, 947F) and PLTs-plasma (platelet-plasmapheresis, PLT-A, 994EF) while the remaining part is returned to the donor. The circuit used is sterile and disposable while donor extracorporeal part is anticoagulated by using a citrate solution. For EPA/EA, RBCs target yields were programmed to 230-280 g of absolute RBCs (leukodepleted) and 450 mL of plasma. After collection, 70-80 g saline-adenine-glucose-mannitol preservative solution was automatically added to RBCs and the filtration was performed routinely after the last return cycle by gravity through the integrated filters for leukodepletion. RBCs were stored at 4 °C for 42 days. For PLT-A, platelet target yields were programmed to range 2.5-3.5 x 10^11 of PLTs and 450 mL of plasma. After collection, PLTs were re-suspended in 130-150 mL of SSP solution (Macopharma, Italy) and automatically filtrated according to the current legislation for leukodepletion. PLTs were stored at room temperature on continuous shaker for up to 5 days [18-21]. By apheresis, 400-650 mL of plasma was collected (DM November 2, 2015 by Italian Minister of Health (www.gazzettaufficiale.it/eli/id/2015/12/28/15A09709/sg) and according to the Society of Transfusion Medicine and Immunohematology (www.centronazionalesangue.it/sites/default/files/it_standards_transf_med.pdf) [17].

Blood bags were handled to avoid any bacterial contamination in the blood product collected. Blood products (PLTs and RBCs) were subjected to periodic and randomized checks for microcontaminations [11, 17]. Whole blood was processed within 8 hours after donation to obtained platelet concentrates (PC), fresh frozen plasma (FFP), packed RBCs (EC), and leukodepleted RBCs (EL) by using a closed system collected in citrate-phosphate-double dextrose. RBC target yields were programmed equal to be at least 280 mL of leukodepleted RBCs with a Hct level of 0.50-0.70% and plasma is collected with a volume ranging from 400 mL to 730 mL.

Statistical analysis

Continuous variables were reported as either mean and standard deviation (SD) or median and interquartile range (IQRs) according to their distribution, as assessed by the Shapiro-Wilk test and compared with t-test or Wilcoxon-Mann-Whitney test. Categorical variables were reported as absolute numbers and percentages and compared with Pearson’s chi-square test or Fisher’s exact test as indicated. Rate of adverse events were calculated as number of events divided by number of transfusion and compared between transfusion procedures. The effect of transfusion procedures on adverse event was assessed by fitting a logistic mixed-effects model [22]. An unstructured within-subject covariance matrix was used in the analysis (i.e. variances and covariance were allowed to vary at each observation point). Transfusion procedures according to the classification of patients (blood malignancies and thalassemic pediatric patients) were first tested; if the interaction was statistically significant at 0.05 level (i.e., there was enough evidence that adverse event varied among transfusion procedures), a separate model was performed separately for blood malignancies and thalassemic pediatric patients. All analyses were replicated for patients with both transfusion procedures.

RESULTS

In our single-center experience, 12 531 randomized blood components (n = 2662 blood malignancy and n = 9869 thalassemic patients) were transfused to 214 patients (n = 144 blood malignancy and n = 70 thalassemic patients). As shown in Table 1., the two populations (blood malignancy and thalassemic patients) showed different characteristics and were considered separately. Age, gender, number of transfusion, time of observation, and different blood components transfused were reported in Table 1. Male gender was 60% and 46% in blood malignancies and thalassemic patients, respectively. Mean age was 8.5±5.3 years in blood malignancy and 19.4±12.8 years in thalassemic patients. Median time of observation was 0.50 years (interquartile range (IQR) 0.11-0.83) in blood malignancy patients and 4.9 years (IQR 3.2-4.9) in thalassemic patients.

Adverse acute event rates for total and single transfusion are reported in Table 2. A total of 33/12 531 (0.3%) adverse acute events occurred. Only the adverse reactions observed during hospitalization were reported in our study. They included only mild acute reactions. In particular, 4 for EA (3 minor allergic reactions, and 1
episode of bronchospasm); 4 for EL (2 minor allergic reactions, 1 febrile episode, and 1 episode of vomiting); and 6 for EC (3 minor allergic reactions, 1 febrile episode, and 2 episodes of vomiting). Regarding PTL transfusion, we registered 19 adverse acute events for PC (9 minor allergic reactions, 2 febrile episodes, 1 episode of vomiting, and 7 episodes of bronchospasm). No adverse acute events for PLT-A, FFP, and plasma apheresis were reported (P = NS). No significant difference in rate of adverse acute events between blood components obtained by apheresis or whole blood was observed. Data have been confirmed by considering also patients who received both types of blood components (n = 78 blood malignancies patients and n = 66 thalassemic patients for a total n = 144 pediatric patients).

**DISCUSSION**

Adverse events in transfusion medicine are largely determined by the clinical conditions and the patient state of immune reactivity, in which the choice of the transfused product can be useful in limiting subsequent adverse reactions. Whereas the blood components collected both by AF and by SI are valid in the same way, the availability of both allows us to allocate the best product to a certain type of patient, reducing adverse events in a personalized dimension. Pediatric patients are more vulnerable than adults showing a higher frequency of transfusion related side effects [7]. To our knowledge, this is

<table>
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<tr>
<th>Table 1</th>
<th>Characteristics of transfusion-dependent pediatric patients</th>
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<tr>
<td></td>
<td>Blood malignancy patients n = 44</td>
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<tr>
<td>Male gender n (%)</td>
<td>87 (60.4)</td>
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<tr>
<td>Age mean (sd)</td>
<td>8.5 (5.3)</td>
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<tr>
<td>Number of transfusion median (IQR range)</td>
<td>10.5 (4-25.3)</td>
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<tr>
<td>Period of observation, years (median (IQR range))</td>
<td>0.50 (0.11-0.83)</td>
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<tr>
<td>Type of blood components n (%)</td>
<td></td>
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<tr>
<td>Packed Red Blood Cells without Buffy Coat (EC)</td>
<td>88 (61.1)</td>
</tr>
<tr>
<td>Leukodepleted Red Blood Cells (EL)</td>
<td>84 (58.3)</td>
</tr>
<tr>
<td>Erithro-Apheresis (EA)</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>Erithro-Plasma-Apheresis (EPA)</td>
<td>65 (45.1)</td>
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<tr>
<td>Platelet Concentrates (PC)</td>
<td>45 (31.3)</td>
</tr>
<tr>
<td>Pool Platelet from Buffy Coat (Pool-PLT)</td>
<td>50 (34.7)</td>
</tr>
<tr>
<td>Platelet Apheresis (PLT-A)</td>
<td>45 (31.3)</td>
</tr>
<tr>
<td>Plasma Fresh Frozen (FFP)</td>
<td>16 (11.1)</td>
</tr>
<tr>
<td>Plasma from Erithro-Plasma-Apheresis (P-EPA)</td>
<td>8 (5.6)</td>
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<tr>
<td>Plasma from Platelet-Plasma-Apheresis (P-PPA)</td>
<td>3 (2.1)</td>
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*Fisher’s exact test

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<th>Table 2</th>
<th>Distribution of adverse events by each transfusion in patients subgroups</th>
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<tr>
<td></td>
<td>Total</td>
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<tr>
<td>Only blood malignancy patients (n = 144)</td>
<td>15/2662 (0.56%)</td>
</tr>
<tr>
<td>Only thalassemic patients (n = 70)</td>
<td>18/9869 (0.18%)</td>
</tr>
<tr>
<td>Blood malignancy patients with both transfusion procedures (n = 78)</td>
<td>11/2248 (0.49%)</td>
</tr>
<tr>
<td>Thalassemic patients with both transfusion procedures (n = 66)</td>
<td>17/9860 (0.17%)</td>
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Data are reported as number of events/number of transfusion (percentage).
the first study investigating a putative different rate of collateral effects between apheresis and whole blood collection in pediatric patients. By results, no difference in adverse acute events occurred from blood components (RBCs, PLTs, plasma) obtained by apheresis or by whole blood in blood malignancy and thalassemic pediatric patients. Interestingly, adverse acute reactions observed (n = 33) were mild and did not cause further hospitalization. This may be related to the benefits of the increased use of leukodepleted whole blood components characterized by helpful pre-storage and bedside filters, as required by Italian legislation [17]. Since it is well known that cytokines released from residual leukocytes contaminating blood components are actively involved in a number of blood transfusion complications, most authors recommended the leukodepletion, especially for selected categories of patients, such as recipients of “long-term” transfusion regimes [23]. Leukodepleted RBCs and PLTs produced both from whole blood and apheresis reduced the risk of HLA immunization in recipients, as well as transmission of new variant Creutzfeldt-Jakob or cytomegalovirus avoiding febrile reactions [24]. The introduction of apheresis changed the final blood products with modification of storage solution and a lower volumes of residual plasma, thus producing RBCs with controlled volumes and increased Hct value [25]. In addition, apheresis provided PLTs with 4 fold lower exposure to donor antigens as compared to a single pool of PCs (with a consequent lower risk of infection, development of alloimmunity, refractoriness, and transfusion-related acute lung injury in recipients).

Consistently to our data, a recent preliminary report indicated that PLT transfusion reactions did not occur more often in recipients transfused with apheresis vs buffy coat platelet concentrates [25]. Although apheresis RBCs present a citrate concentration 2.5-3.0-fold higher than standard RBCs (5.72±3.01 vs 1.88±0.31), no adverse acute events correlated to lower plasma calcium (muscle tremor, paresthesia, cardiac arrhythmia) caused by citrate toxicity were registered [25].

CONCLUSIONS
Globally, our study data confirm a low incidence of pediatric transfusion reactions (0.33%). Regarding the RBCs, our data showed that the pre-filtered RBCs collected with the AF are absolutely superimposable to those collected from SI. Indeed, the new systems of decomposition of the whole blood allow a separation of all the components of the blood in an automatic way reducing the risk of leaving a part of plasma adhered to the red cells. Consequently the use of apheresis for red blood donors, in most CTs only concerns rare phenotypes in which it is possible to collect a double unit of red blood cells tailored on donor Hb. However hematocrit of our whole red blood sacs is around 60% while apheresis has 70% and, obviously, this must be evaluated in clinical practice as it should affect transfusion intervals. As regards platelets, apheresis have shown a greater transfusion safety and they are preferred in our Center through a massive and constant policy of increasing donations.

However, our single-center experience presents many limitations due to the heterogeneity of population examined, to the possible underestimation of symptoms, due to the inability of children to report correctly pathological signs, as well as the lack of data in the population from 0 to 8 years and the patient condition of immunosuppression. In addition, reported adverse events included only reactions occurred when patients was under medical supervision. Transfusion reactions after hospital discharge was not monitored. Despite the low reported adverse events in pediatric patients, we cannot exclude the infectious risk of non-removable blood components even with the latest methods of analysis. These considerations according with the latest directives and strategies of the PBM impose an ever lower recourse to allogeneic blood components and a greater use of alternative anesthetic, pharmacological and surgical strategies to blood transfusion. Particularly, in pediatric patients we advocate large-scale planning and application of restrictive PBMs models.

In our opinion, a randomized study involving a larger number of patients should be performed to suggest additional strategies to establish more accurate criteria to prevent adverse acute reactions in transfusion-dependent pediatric patients.

Authorship contributions
MRDP, AB, LS, AS, MV, CF performed this study. MRDP, AB, LS, SS, AS, MV, GB wrote the manuscript. CN reviewed and edited the manuscript. MRDP, AB, LS, SS, MV, AS, CF, GD, MC, SP, FC, RA, GB, GFN, and CN approved the final version of the manuscript. CN is the guarantor of this work.

Conflict of interest statement
The Authors declare no conflicts of interest.

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<th>Table 3</th>
<th>Statistical significance of transfusion adverse events: logistic mixed-effect model</th>
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<tr>
<td></td>
<td>All pediatric patients</td>
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<td></td>
<td>Odds Ratio of adverse events (95% CI)</td>
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<tr>
<td>Apheresis vs whole blood</td>
<td>0.75 (0.23-2.47)</td>
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<tr>
<td>Thalassemic vs blood malignancy patients</td>
<td>0.30 (0.04-2.10)</td>
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REFERENCES


