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Transfusion of older red blood cell units, cytokine burst and alloimmunization: a case–control study

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ABSTRACT

Background: Experimental data have shown that the transfusion of older red blood cell units causes alloimmunization, but the clinical applicability of this statement has never been properly assessed in non-sickle cell patients. It has been hypothesized that older units have higher numbers of cytokines, increasing the risk of alloimmunization related to antigen-presenting events. The goal of this study was to evaluate the association between the transfusion of older red blood cell units subjected to bedside leukodepletion and alloimmunization.

Methods: All patients submitted to transfusions of bedside leukodepletion red blood cell units proven to have become alloimmunized in one oncologic service between 2009 and 2013 were enrolled in this study. A control group was formed by matching patients without alloimmunization in terms of number of transfusions and medical specialty. The median age of transfused units, the percentage of transfused red blood cell units >14 days of storage in relation to fresher red cell units (≤14 days of storage) and the mean age of transfused units older than 14 days were compared between the groups.

Results: Alloimmunized and control groups were homogeneous regarding the most relevant clinical variables (age, gender, type of oncological disease) and inflammatory background (C-reactive protein and Karnofsky scale). The median age of transfused red blood cell units, the ratio of older units transfused compared to fresher units and the mean age of transfused units older than 14 days did not differ between alloimmunized and control patients (17 vs. 17; 68/32 vs. 63.2/36.8 and 21.8 ± 7.0 vs. 21.04 ± 7.9; respectively).

Conclusion: The transfusion of older red blood cell units subjected to bedside leukodepletion is not a key risk factor for alloimmunization. Strategies of providing fresh red cell units aiming to avoid alloimmunization are thus not justified.

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Introduction

Alloimmunization against red blood cell (RBC) antigens is a late transfusion complication, the clinical predictors of which in non-sickle cell patients are unclear.[1,2] Alloimmunization stems from the antigen-presentation process, which is positively influenced by pro-inflammatory cytokines and negatively by T regulatory cells.[3] The genetics of the patient, especially their human leukocyte antigen (HLA) type, plays a role in the alloimmunization process,[4] but other environmental factors also seem to contribute and have been explored little in the medical literature.[5]

The most important environmental risk factor for alloimmunization is the recipient's inflammatory background,[6] which even causes antibody development in sickle cell patients.[7] Recently it was observed that the presence of acute chest syndrome and vaso-occlusive crises are associated with a higher risk of alloimmunization.[8] Experimental data have demonstrated that the transfusion of older RBC units is also a risk factor,[5] but the mechanisms for this have not been elucidated yet. It has been hypothesized that older RBC units may contain higher levels of pro-inflammatory cytokines and products of RBC degeneration, which may cause a burst in the antigen-presentation process and lead to alloimmunization.[5]

The beneficial effect of the transfusion of pre-storage leukodepleted RBC units to reduce alloimmunization is well-known.[9] However, whether the transfusion of older RBC units subjected to bedside leukodepletion is a risk factor for alloimmunization has never been evaluated. As the accumulation of pro-inflammatory interleukins increases during the storage, the infusion of older RBC units may possibly lead to a more intense cytokine burst and, according to experimental results, to a higher risk of alloimmunization. The exact role played by all leukocyte-derived cytokines in the development of alloantibodies, however, has not been defined even in the experimental scenario.[9]

The goal of this case–control study was to evaluate the association between the transfusion of older RBC units subjected to bedside leukodepletion and alloimmunization, hence justifying the prescription of fresher units as prophylaxis.

Methods

This study was approved by the Local Ethics Committee and exempted from the application of informed consent form. All solid cancer patients proven to have become alloimmunized in this service (2009–2013) were enrolled. The choice to select only patients with non-hematological cancer was an attempt to maximize the homogeneity of the study population. A control group was formed in parallel to the alloimmunized group matched both in terms of the number of transfusions and medical specialty. In this regard, every time an alloimmunized patient was included in the study, the number of transfused RBC units until the development of the first alloantibody was calculated and, then, one or two non-alloimmunized control patients were added to the control group with the same number of transfusions and a similar diagnosis. Antibody identification was performed using the gel methodology (Bio-Rad laboratories) following the manufacturer's instructions.

All RBC units were collected in citrate-phosphate-dextrose with adenine (CPDA-1) bags (Fresenius-Kabi) and were submitted to bedside-leukodepletion (BioR filters, Fresenius-Kabi) before transfusion. None of the patients received phenotyped units as a primary alloimmunization prophylaxis or received RBC units outside this service. The patients' inflammatory background was assessed using cancer prognostic scales capable of evaluating systemic inflammation (Karnofsky)[1] and C-reactive protein levels (measured at inclusion in the study).

Alloimmunized and control groups were compared in terms of the median age of transfused units, the percentage of older RBC units (>14 days of storage)/fresher RBC units (≤14 days of storage) transfused and the mean age of the older (>14 days of storage) transfused units. The data considered for this comparison involved all transfused RBC units until the appearance of the first alloantibody. Considering the known risks associated with the transfusion of older RBC units, this study design was approved by the local Ethics Committee.

The design of the study allowed a three-day storage difference between the groups with the inclusion of 50 patients in each arm (80% of power). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 18). The Mann–Whitney test was used to compare variables with unequal distribution and the Student *t*-test, to compare variables with normal distribution. A *p*-value less than 0.05 was considered significant.

Results

Fifty alloimmunized and 59 non-alloimmunized (control) patients were enrolled in the study. The mean age of patients was 56.74 ± 15.42 and the mean number of transfused RBC units per patient was 4.76 ± 3.85. Groups were initially compared in terms of the most relevant clinical variables (age, gender, body mass index, diagnosis) and proved to be homogeneous (Table 1). The patients' inflammation background, evaluated using the Karnofsky cancer prognostic scale and C-reactive protein levels, was also similar between the groups (Table 1).

Median age of transfused RBC units was equal for both groups (17 days; p-value = 0.15). The percentage of transfused units older than 14 days was 68.0% in the alloimmunized group and 63.3% in control group (p-value = 0.28). The mean age of the transfused units older than 14 days was 21.8 ± 7.0 in the alloimmunized group and 21.04 ± 7.9 in the control group (p-value = 0.6). Table 1 compares the groups in terms of the most important demographical and transfusion variables.

Discussion

This study demonstrates that the transfusion of older RBC units is not a key risk factor for alloimmunization and, consequently, it does not support previous experimental data. The hypothesis of a cytokine burst contributing to

Table 1 – Comparison between alloimmunized and non-alloimmunized patients regarding clinical variables, inflammatory background and age of red blood cell units transfused.

	Alloimmunized group ($n = 50$)	Control group ($n = 59$)	p-Value
Age – years (mean ± SD)	56.2 ± 15	57.3 ± 16	0.7
Gender – female/male (%)	59.3/40.7	64/36	0.69
Diagnosis (%)			
Gastrointestinal system cancer	33.9	46.9	0.21
Genitourinary system cancer	42.4	26.6	
Other solid cancer	23.7	26.5	
C-reactive protein – mg/L (mean)	128.8	162.4	0.36
Karnofsky Scale (median)	80	85	0.42
Age of transfused RBC units – days (median ± IQR)	17 ± 17.8	17 ± 16.7	0.15
RBC units >14 days transfused (%)	68.0	63.2	0.28
Mean age of RBC units >14 days – days (mean ± SD)	21.8 ± 7.0	21.04 ± 7.9	0.6

RBC: red blood cell; SD: standard deviation; IQR: interquartile range.

RBC alloimmunization was not suggested by these results, since alloimmunized and non-alloimmunized patients were homogenous in terms of length of storage of transfused units and the number of RBC units subjected to bedside leukodepletion, which have a higher level of pro-inflammatory cytokines. To the best of our knowledge, this is the first case–control study to evaluate the risk of alloimmunization associated with the transfusion of older RBC units.

One previous clinical study also found no association between older RBC units and alloimmunization, but it had two major limitations: the use of units submitted only to pre-storage leukodepletion, which precluded the evaluation of leukocyte-derived cytokines, and it did not evaluate the recipient inflammation status, an extremely important factor for alloantibody formation.[10] The present study did not find any association between age of the transfused unit and alloimmunization, and ruled out any possible bias regarding differences in the inflammatory background of participants. In contrast to these results, there is consistent experimental data associating the accumulation of leukocyte-derived transforming growth factor-β (TGF-β) in transfused units and lower rates of alloimmunization.[9] However, the age of storage of the RBC units in this specific study was significantly shorter (three days vs. fourteen days) and only one leukocyte-derived cytokine was evaluated, while the effect of many others known to cause pro-inflammatory stimulation were not evaluated.

It is important to highlight that this study did not exclude the hypothesis of antibody recrudescence after transfusions of RBC units. However, as none of the patients was transfused outside the current service, this event would only be due to pregnancy-related alloimmunization. As no antibody with anti-D specificity was identified within the studied population, this bias probably had no significant impact on the results.

Conclusions

The transfusion of older RBC units is not a key risk factor for the development of RBC alloantibodies in non-sickle cell patients. Strategies of providing fresher units as prophylaxis for alloimmunization are therefore not justified.

Conflicts of interest

The authors declare no conflicts of interest.

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