

Storage Duration and Cell Density as Predictors of Red Blood Cell-Related Febrile Non-Hemolytic Transfusion Reactions: A Matched Case-Control Study

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ABSTRACT

Background and objectives: Blood component storage lesion has been already associated with undesirable clinical endpoints. We have designed a case-control study evaluating the effect of storage time and supernatant content in the incidence of febrile non-hemolytic transfusion reaction (FNHTR).

Material and methods: Matched case-control study at a tertiary complexity hospital. cases were FNHTR experiencing patients from September 1st, 2015 to September 1st, 2016. Controls were selected from patients transfused in the same day, matched over gender, age, transfusion adverse events (TAE) and transfusion history and use of premedication. Only PRBC transfusions were enrolled. Parameters evaluated on PRBC were irradiation, leukoreduction, [Na⁺], [K⁺], hematocrit (Ht), hemoglobin (Hb), microbiological culture and storage time.

Results: We have found an annual FNHTR incidence of 0.38%. A total of 117 transfusions were accrued (FNHTR-39/ControlA-39/ControlB-39). Groups were balanced over matching variables. On univariable analysis, irradiation, leukoreduction and storage time did not associate with FNHTR. [Na⁺] decreased (Rho=-0.49, p<0.001) and [K⁺] increased (Rho=0.52, p<0.001) significantly over storage time, but concentrations did not differ between study groups. Microbiological culture was thoroughly negative for all groups. Ht (72.4[68.8-75.7] × 68.1[62.75-72]) and Hb (23.7[22.1-24.4] × 22.3[20.5-24]) were significantly higher in FNHTR group. Final multivariable regression model rendered two significantly associated variables: Hb and storage time. For each increase in 1 day of storage time, FNHTR chance increased a mean of 6.7% (95%CI:0.4%-13.4%); while for each elevation of 1 g/dL of hemoglobin, a mean increase of 49.1% (95%CI:17.5%-89.3%) in the chance of FNHTR was observed.

Conclusion: Cell density (estimated by hemoglobin and hematocrit concentration) and storage time seems to be associated to FNHTR incidence. Causal relation should be further evaluated.

Keywords: Transfusion reaction; Storage lesion; Packed red blood cell

INTRODUCTION

Blood transfusion is a main part of medical therapy and one of the most frequently performed procedures in public and private hospitals [1]. Unfortunately, despite of an undeniable development of laboratory techniques over the last decades, transfusion adverse reactions still pose a difficult challenge for transfusion medicine. Recent research [2,3], while analyzing 125 datasets representing 25 different countries included in the International Haemovigilance Network Database, found an estimate of 660 transfusion adverse events (TAE) per 100,000 individuals; almost 3% of these were

classified as severe. The mortality rate was 0.26 deaths per 100,000 and more than half of these events were secondary to transfusion-associated circulatory overload (TACO), transfusion-related lung injury (TRALI), and transfusion-associated dyspnea (TAD).

A growing knowledge on pathophysiology of TAE has allowed for preventive measures in some situations [4]. Among different mechanisms postulated, blood component storage time (BCST) association with negative clinical endpoints in diverse scenarios has been gaining increasing attention from scientific community [5-12]. BCST can exert a main influence on complement activation,

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extracellular potassium and sodium, supernatant lactate, red cell deformability, mean corpuscular hemoglobin concentration and blood component (BC) pH [6,11,13-16]. Irradiation of BC seems to potentiate this effect [5]. Whether through these pathways or others still unknown, BCST has been linked to increased rates of TAE [8,9], and even mortality [10] (although this latter association has been still a matter of controversy [12,17]).

This association is still far from being thoroughly explored. A better understanding on the effect of BCST effect on TAE, along with other clinically important endpoints, would be pivotal in precipitating modifications in methods of BC preservation/rejuvenation and shelf-life limits consensus. Among TAE, febrile non-hemolytic transfusion reaction (FNHTR) may be a peculiarly important marker of blood component cellular lesion due to its etiological connection to BCST-induced cell apoptosis [18]. In this research, we have designed a matched case-control study to assess which laboratory and clinical parameters would differentiate patients developing FNHTR from the remaining population, focusing on BC variables, such as storage time and other supernatant biomarkers, while controlling for patient intrinsic characteristics.

METHODS

Study design

This study was developed at a high complexity referral hospital (Hospital de Clínicas de Porto Alegre, Porto Alegre/Brazil) which delivers over 2,000 transfusions monthly to patients under various conditions, but mainly onco-hematological diseases and bone marrow and solid organ transplants. The research aimed at assessing varied packed red blood cell (PRBC) laboratory parameters and storage time between BC transfused to patients exhibiting signs and symptoms of FNHTR compared to those experiencing no adverse event. It had a case-control design, with a proportion of two control patients for each case. The study was performed over the period of one year, from September 1st/2015 to September 1st/2016.

Our main hypothesis was that red blood cell injury secondary to progressively longer storage times would result in an increased rate of FNHTR, that could be demonstrated by the release of biochemical markers into the PRBC supernatant. Thus, the main outcome was PRBC storage time (compared between cases and controls) and secondary outcomes were hematological and biochemical parameters estimated on cases and controls transfused BC.

Selection criteria

FNHTR was defined as the increase of 1 degree Celsius from basal axillary patient temperature. For the purpose of this study, we have only considered FNHTR secondary to PRBC transfusions. Controls were selected randomly from patients undergoing transfusion of PRBC at our institution on the same day of FNHTR cases, but not experiencing TAE (of any type). Two groups of controls (A and B) were recruited in a 1:2 proportion, due to anticipated low incidence of TAE and statistical power concerns. The sample was made up of inpatients that were monitored during the whole transfusion within the blood bank facility. No other therapies were administered during transfusion. Due to the low incidence of the outcome studied and logistic difficulties of a long-lasting enrollment process, accrual of cases and controls was based on

convenience sampling (all events occurring during study period). Leukoreduction at our institution is performed pre-storage, but not universally, due to financial restrictions.

Data sources

From each patient enrolled, clinical information was obtained from medical files (gender, age, main disease, previous history of TAE, frequency of previous transfusions and premedication use). Except for the main disease, all other characteristics were evaluated and matching performed between case and control groups, within a reasonable age spread tolerance (e.g., maximum of 5 years of difference in age). Hierarchically, matching was pursued for previous history of TAE, frequency of previous transfusions, use of premedication, age and gender, in this order.

PRBC transfused in both FNHTR or control groups had samples extracted from BC residual volume immediately after transfusion interruption or completion. These samples were analyzed for different laboratory parameters (sodium [Na⁺] in mEq/L, potassium [K⁺] in mEq/L, complete blood count [CBC] and aerobic microbiological culture [AMC]), storage time and blood type. Biochemical parameters were estimated in Cobas C702 Chemistry Analyzer (Roche Diagnostics, USA) and CBC in XE-5000 Hematology Analyzer (Sysmex, Japan). Increased hemoglobin/hematocrit parameter was considered a surrogate marker for cell density in this study.

Statistical analysis

Continuous variables were described as mean and standard deviation or median and interquartile range, pending on normality of distributions evaluated by Shapiro-Wilk test. Categorical variables were described as absolute and relative frequencies. Evaluation of continuous variables differences between FNHTR and control groups were performed using Repeated Measures ANOVA. Sphericity assumption was tested with Mauchly's test and if sphericity assumption was not fulfilled, correction of estimates was performed via Greenhouse-Geisser method. Proportion differences were evaluated with McNemar-Bowker test in pairs of measures (case-control 1 and case-control 2). A logistic regression model was built to assess concomitantly the association between study parameters and FNHTR endpoint. It has been assembled in a backward stepwise method designed to excluded predictors showing significance probabilities of 0.2 and over. Final model was evaluated for goodness-of-fit with Hosmer and Lemeshow test and collinearity of continuous predictors with variance inflation factor and tolerance parameters. Analyses were performed with IBM SPSS Statistics 20.0 (Armonk, NY: IBM Corporation).

Ethical considerations

This research project was approved by the institutional Research Ethics Committee (CEP/HCPA-UFRGS) under Plataforma Brasil protocol identification number (CAAE) 53339316.5.000.5327. It has followed all ethical guidance recommended in National Health Council Resolution (number 466/2012). Informed consent from patients was considered unnecessary as biologic samples analyzed in this study would be only originated from formerly discarded material. Identification concealment of participants was secured.

RESULTS

During the study period 10,283 PRBC were transfused and 39 FNHTR were identified resulting in an incidence of 0.38% (for PRBC transfusions only). A total of 117 PRBC transfusions (39 in FNHTR group, 39 in control group A and 39 in control group B) was included, in a proportion of 1 case: 2 controls. In 7 opportunities during enrolment, availability of more than 2 suitable controls made it possible for us to collect one additional control subject (for whom all parameters were obtained). We did this in order to replace one control subject if its parameters could not be determined due to handling errors or resulted in inadequate material for measurement (a situation that eventually was not observed in any of the subjects studied). Univariate paired sample analyses were performed with the 39 FNHTR group and two groups of 39 controls (Control A and Control B). Baseline characteristics from FNHTR and control groups are described in Table 1. Groups were similar considering gender, age, previous history of TAE, main disease and number of previous transfusions. Control group A had a slightly higher frequency of premedication use and control group B had a higher frequency of O negative PRBC.

Concerning PRBC characteristics, there was an overall higher frequency of leukoreduction and irradiation in control groups, although none of these differences reached statistical significance ($p>0.05$). Control groups PRBC showed a lower mean storage time when compared to the FNHTR group, but also did not reach statistical significance ($p=0.15$). Details are available in (Table 2).

Characteristics of fifty Japanese patients are shown in Table 1.

Table 1: Baseline characteristics from FNHTR and control groups.

Variable	FNHTR	Control A	Control B	p-value
Gender (Male)	16 (41.02%)	17 (43.6%)	17 (43.6%)	1.00/1.00
Age	64 (42-71)	69 (56-77)	66 (56-71)	0.18
Main Disease	-	-	-	-
Hematological				
Malignancy	5 (12.8%)	7 (17.9%)	5 (12.8%)	-
Solid Tumor	18 (46.2%)	20 (51.3%)	23 (59%)	0.26/0.2
Myelodysplastic Syndrome	3 (7.7%)	7 (17.9%)	6 (15.4%)	-
Solid Organ Transplant	2 (5.1%)	0	0	-
Puerperium	2 (5.1%)	0	0	-
Chronic Renal Failure	3 (7.7%)	1 (2.6%)	0	-
Other conditions	6 (15.4%)	4 (10.3%)	5 (12.8%)	-
Previous TAE				
FNHTR	2 (5.1%)	2 (5.1%)	2 (5.1%)	NC
Allergic	2 (5.1%)	2 (5.1%)	2 (5.1%)	-
None	35 (89.7%)	35 (89.7%)	35 (89.7%)	-
Number of Previous Transfusions	5 (2-11)	5 (2-7)	6 (2-8)	0.064
No Use of Premedication	28 (71.8%)	32 (82.1%)	30 (76.9%)	0.005a/0.78
PRBC Blood Typing				
O+	19 (48.7%)	18 (46.2%)	14 (35.9%)	-
A+	11 (28.2%)	14 (35.9%)	13 (33.3%)	-
B+	3 (7.7%)	2 (5.1%)	3 (7.7%)	0.19/0.03b

AB+	0	1 (2.6%)	2 (5.1%)	-
O-	1 (2.6%)	3 (7.7%)	5 (12.8%)	-
A-	5 (12.8%)	1 (2.6%)	2 (5.1%)	-

Table 2: Included PRBC characteristics.

Characteristic	FNHTR	Control A	Control B	p-value
Irradiation	14 (35.9%)	19 (48.7%)	19 (48.7%)	0.19/0.51
Leukoreduction	20 (51.3%)	24 (61.5%)	19 (48.7%)	0.33/1.0
Storage Time (Days)	12.13 (8.16)	9.69 (5.65)	9.95 (5.96)	0.19

Biochemical, microbiological and hematological analyses are shown in (Table 3). Hematocrit and hemoglobin concentration from PRBC transfused in the FNHTR group were significantly higher than those transfused in control groups ($P<0.05$). Other parameters did not differ between groups.

We have also analyzed laboratory parameters correlation with storage time, to identify potential modifications occurring in PRBC as a consequence of storage injury. Correlation analyses can be seen in (Table 4). Supernatant Na^+ concentrations decreased and K^+ increased significantly along storage time. Correlation between those variables was considered high.

Table 3: Biochemical, microbiological and hematological analyses in FNHTR and control groups.

Parameter	FNHTR	Control A	Control B	p-value
Na^+ (mEq/L)	128 (112-147)	127 (109-148)	133 (113-149)	0.91
K^+ (mEq/L)	54.8 (31.5-75.7)	55.8 (34.3-73)	47.5 (32.5-72.5)	0.92
Total Leukocytes ($\times 10^3/\text{mL}$)*	8.17 (5.14-9.61)	7.38 (5.61-11.8)	7.69 (5.6-11.01)	0.51
Hematocrit (%)	72.4 (68.8-75.7)	68.5 (61.2-71.6)	66.9 (62.4-73.1)	<0.001
Hemoglobin (g/dL)	23.7 (22.1-24.4)	22.4 (19.6-24)	22.2 (20.5-23.6)	<0.001
Microbiologic Culture (Negative)	39 (100%)	39 (100%)	39 (100%)	NC

*Non-leukoreduced PRBC. NA, Not computed (proportions are identical).

Table 4: Correlation between biochemical and hematological parameters and storage time.

Parameter	Spearman Correlation Coefficient (Rho)	p-Value
Na^+	-0.49	<0.001
K^+	0.52	<0.001
Hematocrit	0.16	0.86
Hemoglobin	-0.12	0.19

A logistic regression model was built to evaluate main study variables (PRBC irradiation and leukoreduction status, storage time, hemoglobin concentration [hematocrit was not considered due to collinearity issues], and patient previous TAE history and use of premedication) association with FNHTR. This final multivariate model incorporated all collected data (124 observations – 117 subjects from previously mentioned groups plus 7 additional control subjects). Variables were selected in a backward stepwise approach and maintained in the final model only if they presented statistical significance. Final model is detailed in (Table 5). Eventually, two

PRBC factors remained in the multivariable model: storage time and hemoglobin concentration. For each increase in 1 day of storage time, FNHTR chance increased a mean of 6.7% (95% CI 0.4%-13.4%); while for each elevation of 1 g/dL of hemoglobin, a mean increase of 49.1% (95% CI 17.5%-89.3%) in the chance of FNHTR was observed.

Table 5: Final logistic regression model concerning possible predictors of FNHTR*.

Variable	OR	95% CI	p-value
Storage Time (Days)	1.067	1.004-1.134	0.037
Hemoglobin Concentration (g/dL)	1.491	1.175-1.893	0.001

*In this logistic regression model, study parameters (storage time, biochemical and hematological parameters and patient characteristics) association with main outcome (FNHTR) were tested altogether. Non-significant factors were excluded from model one by one, and significance of model parameters were estimated again at each step. Final model illustrates the two parameters with statistically significant association with main outcome. For each day of further storage, the probability of FNHTR increased by 6.7% (95%CI 0.4-13.4%) and for each increase of 1 g/dL in PRBC hemoglobin concentration, the same probability increased by 49.1% (95%CI 17.5-89.3%).

DISCUSSION

TAE are yet a relevant challenge to transfusion medicine specialists worldwide. Limited understanding on etiopathogenic mechanisms limits available alternatives of management and prophylaxis. Accordingly, population awareness on transfusion risks gradually mounts. Graw et al. [19] reported that 10.9% of patients and 14.6% of healthcare professionals considered blood transfusion as a potentially harmful procedure. These undesired damages not only compromise patient well-being but also burden healthcare resources. A recent study by Janssen et al. [20] estimated an annual in-hospital cost of transfusion reactions in the Netherlands of €933,356 per year, two-thirds of which are incurred by non-serious transfusions reactions, as FNHTR. This type of TAE leads not only to treatment-related costs, but it also leads to inherent diagnostic procedures that also charges healthcare system. A recent Canadian research [21] found that one-quarter of patients experiencing FNHTR underwent chest imaging, and 79% had blood cultures. Also, for transfusions performed at an outpatient setting, 15% of patients had to be admitted to the hospital to exclude other causes of fever [21].

We have found an incidence of FNHTR comparable to other centers [21-23]. Control group seemed to show a higher frequency of “younger”, leukoreduced and irradiated PRBC, which seems intuitive, but did not reach statistical significance on univariable analysis. Supernatant analysis ratified previous findings [13,24] in regard to PRBC storage-induced modifications, with a rising K⁺ and declining Na⁺ concentration, due to Na⁺/K⁺ ATPase gradual compromise. Both Na⁺ and K⁺ did not show any association with FNHTR. Furthermore, most interestingly, logistic regression analysis, while improving precision on estimates by concomitantly evaluating the isolated effects of different variables on outcome, found a significant association of storage time and hemoglobin concentration (which could already be seen in univariable analysis) with FNHTR incidence.

Cell injury during storage has been a major concern for a long time. Efforts to improve our understanding over storage lesion have

evolved from measuring supernatant hemolysis to metabolomics analysis. But still little could be done in order to effectively control it, or even fathom its influence on TAE. Long-standing knowledge [15,25] has found that hemoglobin/hematocrit concentration in PRBC (i.e., cell density) could influence viability of red blood cells decreasing their deformability, possibly due to availability of substrate and/or storage conditions. Storage time exerted similar effects [14]. Release of different chemical mediators [6] under this continuous pernicious phenomenon could be etiological cornerstone for many FNHTR events. In the present study, we have found a positive association between storage time and FNHTR, and also identified an increased probability of this type of TAE among PRBC exhibiting higher Ht/Hb estimates, which corroborates previous findings on the importance of cell density while in storage. Both factors possibly interact and potentiate each other during shelf life.

CONCLUSION

However, these results should be evaluated carefully. Some limitations are inherent to its case-control design, such as the lack of randomization. Also, the low incidence of FNHTR (and resulting statistical power limitations) has prevented stratified analysis for some parameters of importance (e.g., use of premedication). Although we have attempted to match groups for established risk factors for TAE, other confounding factors could have influenced results. Study was also susceptible to recall bias due to errors in medical file filling of some key information, such as number of transfusions and previous history of TAE. Measurement of laboratory parameters is also prone to variations due to sample handling and storage inadequacies. Also, convenience sampling implied potential restrictions in statistical power over some comparisons (e.g., leukoreduction rate).

Despite of these potential drawbacks, this study raises important issues over processing and storage of BC, linking these processes to clinical outcomes, as TAE (specifically FNHTR). Causal relation between parameters studied, as well as the effects of cell density over storage duration, should be subject to further research.

CONFLICT OF INTEREST

There are no conflicts of interest to disclose from author.

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