CHAPTER 3

RED BLOOD CELL ALLOIMMUNIZATION AND NUMBER OF RED BLOOD CELL TRANSFUSIONS

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ABSTRACT

Background

Patients receiving red blood cells may form antibodies against the alloantigens expressed by red blood cells, with the risk of serious morbidity and the need for extensive phenotype-matching in subsequent transfusions. The incidence of alloimmunization is considered variable for specific patient groups and for first time antibody formation. We therefore studied the cumulative incidence of the first formed alloantibody as a function of red blood cells exposure.

Methods

We performed a new user cohort among all previously non-transfused non-alloimmunized patients that received non-extended matched (ABO and RhD) red blood cells transfusions from January 2005 to December 2009 in our university medical centre. Alloimmunization incidences were estimated by Kaplan-Meier survival-analysis.

Results

A total of 3002 previously non- transfused patients received 31,103 red blood cell units. A first time alloantibody forming event was experienced by 54 (1.8%) patients. The cumulative incidence of alloimmunization was 1.0% at 5 units, 2.4% at 10 units, 3.4% at 20 units, and 6.5% at 40 units of red blood cells transfused.

Conclusion

The risk to develop a first red blood cells alloantibody increases up to the 40th transfusion and is similar for men and women. More data is needed to examine the risk after 40th transfusion.

INTRODUCTION

Patients exposed to red blood cell alloantigens by transfusion, pregnancy, or transplantation may produce antibodies against alloantigens expressed by red blood cells. These can cause acute and delayed hemolytic transfusion reactions and potentially serious morbidity and even mortality. Additionally, alloimmunization makes more extensive matching of subsequent transfusions necessary.

Transfusion mediated alloimmunization could in theory be prevented by exact matching of all donor blood to the recipient's phenotype. Implementation of such a strategy, however, will be laborious and cost- consuming and its logistical burden may hamper the timely availability of matched blood for patients.

For some transfusion populations for e.g. sickle cell anemia and thalassemia, matching for Rh and K antigens to reduce the high immunization incidence associated with these diseases is often performed in most high income countries. We could maximally prevent alloimmunization by administrating matched blood to patients that are particularly at risk for alloimmunization, based on their clinical and transfusion risk factors. To estimate the feasibility of such an approach, an accurate antibody incidence measurement is an essential starting point. The reported frequency of red blood cell alloimmunization, however, varies considerably from 2 to 21%.[1-6] Besides, differences in study design and populations studied, the exact number of received transfusions before antibody formation is often unknown or poorly documented in many studies. Increased exposure to red blood cell antigens i.e. more red blood cell transfusions are likely to cause a higher risk for alloimmunization. Also, other studies included patients with already formed antibodies [7], or patients receiving large amounts of transfusions due to their predisposing indications [6, 8, 9].

Considering that red blood cell transfusions likely determine exposure to alloantigens and the risk for subsequent antibody formation and that preventative matching will alter the observed alloimmunization risk, we aimed to study the incidence of first alloantibody formation due to only ABO and D matched red blood transfusions in a general patient population from their first transfusion onwards. We also studied the incidence among females in the reproductive age for whom red blood cell transfusions are additionally matched for K antigen.

METHODS

Study Design and study population

We performed a retrospective incident new user cohort study among consecutive transfused patients at the Leiden University Medical Center (LUMC), the Netherlands from January 2005 to December 2009, to study adverse events (alloimmunization to red blood cell transfusions) among first time red blood cell transfused patients in an electronic patient follow up database. [10, 11] Eligible were all patients who received their first ever red blood cell transfusion within the study period in our centre. Transfusions were included only if they were preceded by a negative antibody screen and followed by a post transfusion antibody screen.

Patients who routinely received Rh phenotype (C, c, E and e) and K red blood cell transfusion, e.g. haemoglobinopathies and women who received intra uterine transfusions (IUT) were excluded. We also excluded infants under 6 months of age because they are presumed to have a reduced capacity to form red blood cell antibodies as their immune system is not fully developed [12-14]. According to Dutch transfusion guidelines (24), apart from ABO and RhD, K antigen blood group matching is mandatory since 2004 for female patients in the reproductive age (≤45 years). We did not exclude women who received ABO, RhD and K matched blood but analyzed them separately from the main cohort.

First time alloantibody

The endpoint of our study was the first time post-transfusion formation of clinically significant red blood cell alloantibodies as screened by a 3 cell serology panel at 37 degree Celsius, in patients with prior negative red blood cell antibody screens. All patients were routinely screened for alloantibodies before transfusion which is repeated at least every 72 hours, if further transfusions are required. The antibodies are screened for by a 3 cell panel including an indirect antiglobulin test (LISS Diamed ID gel system, Murten, Switzerland) and subsequently identified by standard 11 cell panels in the same gel test system. Additional techniques such as Poly-ethylene glycol, Bovine serum albumin and enzyme method were used when required. Non-red blood cell transfusion induced antibodies (5 cases of anti-D by passive acquisition), antibodies against low-frequency antigens that are not routinely present on antibody screenings panels (12 cases of anti Wr^a) and cold-reacting (12 cases) antibodies were not classified as endpoints.

Data acquisition

All data on antibody screening, antibody identification and transfusions were routinely recorded in the laboratory electronic data system General *Laboratory* Information *Management System* (GLIMS). We gathered transfusion dates for every transfused red blood cell unit, dates and results of antibody screens, antibody specificity, dates of birth and gender of all transfused patients.

Statistical analyses

The incidence of alloimmunization was estimated using Kaplan-Meier survival tables.

The cumulative transfusion exposure expressed as cumulative units of red blood cells transfused was used as the time axis.

We calculated incidences of new antibody development among all males and females older than 45 years. In addition, women under 45 years of age who had received K-matched red blood cells were analyzed separately. The association between sex and age of the patients and incidence of alloimmunization was also assessed and presented with log rank test p values.(significance level $p<0.05$)

RESULTS

After careful exclusion of patients that had received transfusions that had extended antigen matching, infants and patients without post-transfusion antibody follow-up (Table 1), the study cohort comprised of 3002 patients who had received a total of 31,103 red blood cell transfusions in the 5 year study period with a median of 6 units per patient (range 1-133). The main cohort comprised of almost twice more men than women, since the women with child bearing potential (under 45 years) were analyzed as a separate cohort on account of receiving transfusions that had limited antigen matching. After the first transfusion the median antibody follow-up period was 60 days and comparable for men (55 days) and women (69 days); (log rank p=0.12) (Table 2).

Table 1. The study population with the excluded number of patients

	Patients excluded	Patients in study
Transfused patients		8629
Haemoglobinopathies	100	8529
Intrauterine transfusions	49	8480
Infants <6 months of age	1089	7391
Patients with pre-transfusion positive screens	127	7264
Patients without antibody follow-up after a single transfusion event	3752	3512
Women ≤45 years of age (analyzed separately)	510	3002

Table 2. Transfused patient characteristics

Patient characteristics	Men	Women >45 yrs	Total	Women ≤45 yrs
Number of patients (%)	1929 (64)	1073 (36)	3002	510
Age in years (median, IQR)	60 (44, 70)	65 (56, 74)	62 (51, 71)	30 (15, 37)
Cumulative units	20,960	10,143	31,103	5093
Units per patient (median, IQR)	7 (4, 13)	6 (4, 11)	6 (4, 12)	6 (3, 12)
Follow-up period (median, IQR)[1]	55 (13, 256)	69 (14, 287)	60 (13, 266)	147 (22, 527)
Patients forming first antibodies (%)	36 (65)	18 (35)	54	7
Sensitization frequency (%)	1.9	1.7	1.8	1.4

[1] Follow-up period in days from first transfusion to last antibody screen in non-immunized patients and to first positive antibody screen in immunized patients.
IQR – Inter quartile range

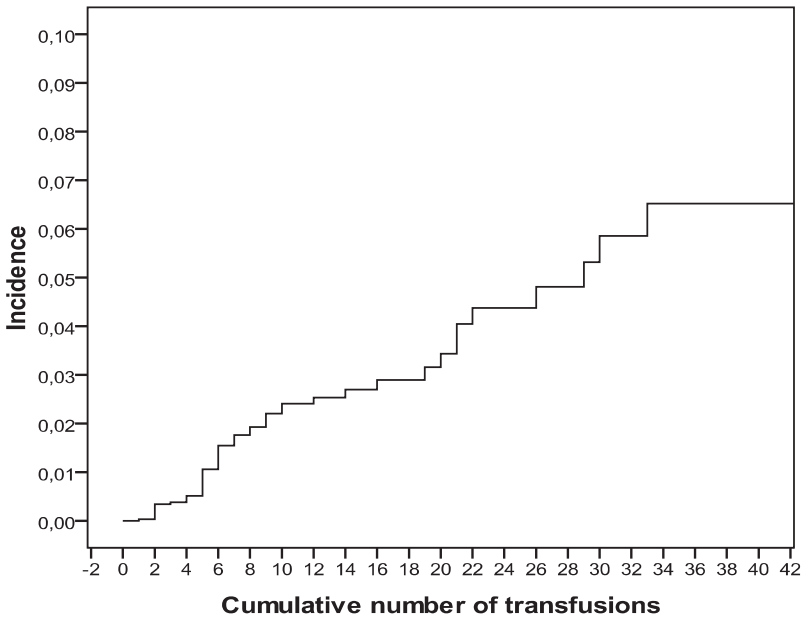

Figure 1. Red blood cell alloimmunization incidence in general red blood cell transfused population according to transfused units

Addendum to figure 1
Number of patients at risk at various cumulative transfusion units:

Number of units	Patients at risk
5	1830
10	953
20	370
40	94

In 54 patients (1.8%) 69 clinically significant antibodies were detected (Table 3). Single antibody specificities were found in 40 patients and multiple specificities in 14 patients. Multiple antibody combinations occurred with Anti E in 13 of 14 cases. Patients who developed antibodies had received a median of 6 red blood cell units (IQR 4, 10) and non-antibody formers had had a median of 6 red blood cell units (IQR 3, 11) units. Among women aged less than 45 years 7 women (1.4%) formed 9 alloantibodies (Table 3). We recorded 6 men and 2 women with a first time alloantibody formed in less than 2 weeks of their first transfusion at our center.(3x anti-Jk^a, 2x anti- E, 2x anti- Le^a and 1x anti-M). one male patient formed an anti-C^w after receiving 66 red blood cell units in 8 transfusion episodes.

The eventual cumulative incidence of alloimmunization as a function of exposure to the number of units, amongst 3002 red blood cell recipients was 1.0% at 5 units, 2.4% at

Table 3. Specificity and frequencies of antibodies among routine (ABO-D) and extended (ABO-D, K) matched patients between 2005-2009 at LUMC, Leiden

Alloantibody specificity	ABO, D matched			ABO, D, K-matched
	Men	Women	Total	Women ≤45
E	17	10	27	4
K	10	5	15	0
Jk^a	4	2	6	1
C^w	4	1	5	0
M	3	1	4	0
C	1	1	2	0
c	1	1	2	3
S	0	1	1	1
Fy^a	1	0	1	0
Le^a	2	0	2	0
e	2	0	2	0
Lu^a	1	0	1	0
Kp^a	1	0	1	0
Total	47	22	69	9

10 units, 3.4% at 20 units and 6.5% at 40 units of red blood cells transfused and was comparable for men and women (Fig. 2 and Table 4). In women aged less than 45 years (n=510), who had received K-matched transfusions, the incidence was 0.8% at 5 units and 2.5% after 10 units of red blood cells transfused (Fig. 2, Table 4). No association of age, categorized as young (<= 30 years), middle (> 31-60 years) and old (> 61 years) was found in the total study population with incidence of alloimmunization (log rank p= 0.18). Comparing men under 45 and over 45 years of age, again no association between age and alloimmunization was noticed. (Log rank p= 0.5)

Table 4. Antibody incidence according to number of transfused units in different transfusion recipient populations

Number of transfused units	Incidence of alloimmunization (95% CI)			
	Men (n=1929)	Women >45 years (n=1073)	All patients (n= 3002)	Women ≤45 years (n=510)
5	1.0 (0.9-5.6)	1.2 (1.1-8.4)	1.0 (0.9-3.8)	0.8 (0.7-20)
10	2.4 (2.1-5.7)	2.4 (2.3-9.7)	2.4 (1.8-4.2)	2.5 (2.4-16)
20	3.4 (3.1-8.0)	3.5 (3.3-15)	3.4 (2.6-5.9)	2.5 (2.4-16)
30	6.5 (4.9-11)	5.0 (4.9-21)	5.8 (4.4-9.1)	-
40	6.5 (4.9-11)	7.4 (7.1-24)	6.5 (4.9-9.9)	-

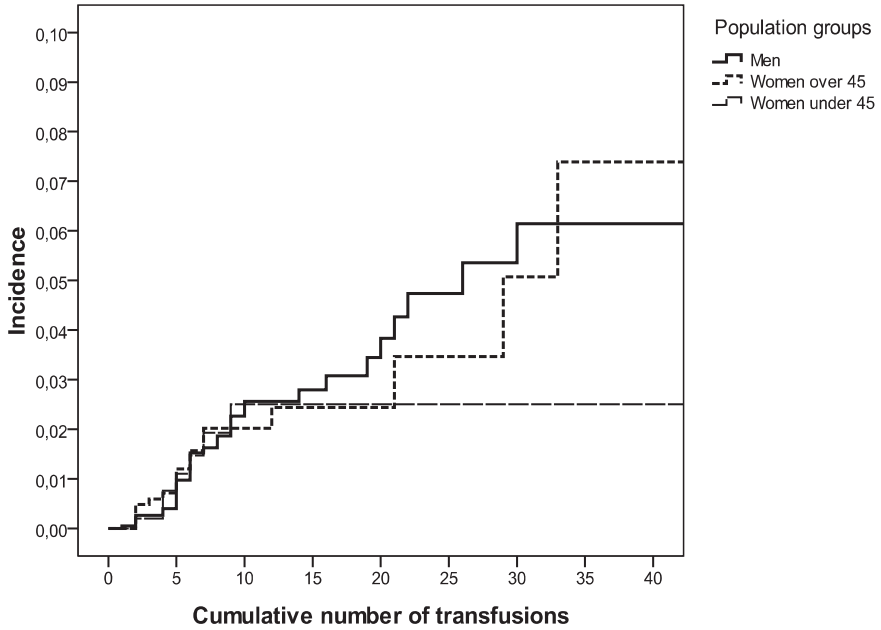

Figure 2. Red blood cell alloimmunization incidence in men, women >45 years (ABO, D matched) and women ≤45 years (ABO, D and K matched) according to transfused units

DISCUSSION

Our study, in general non-transfused and non-immunized transfusion recipients, demonstrated that the risk of first time alloimmunization to red blood cell antigens increases to 6.5 percent with the number of red blood cell transfusions up until 40th unit. This immunization rate was comparable for men and women over the age of 45 years as well as among different age groups. Young women who additionally received K- matched transfusions showed an immunization risk of 2.5% at the 10th transfusion, which was comparable to men and older women who received equal amounts of only ABO-D matched transfusions.

The alloimmunization risk to red blood cell antigens is debated and is suggested to be dependent on other factors such as the population studied, gender and genetic background. The number of transfusions however, seems most likely to have a major influence [7, 9, 15, 16]. Indeed, the incidences reported in several studies on transfused patients seem to indicate that the risk of alloantibody formation rises with an increasing number of transfusions. In sickle cell disease patients, Sarnaik et al. reported alloimmunization frequencies up to 11.5 percent, with increasing number of red blood cell transfusions [15], Reisner et al reported a 10 percent immunization rate in less than 50 units transfused [17] and in another study the rate of alloimmunization increased exponentially with higher numbers of transfused units [9]. In addition, Hoeltge et al showed, in a general transfused population, that the number of antibodies in a patient was positively correlated with the mean number

of red blood cell transfusions [16]. In these studies either the antibody specificities were not reported or clinically not- significant antibodies were included. Fluit et al, studying antibody specificities comparable to our study, reported an increase in first immunization frequencies from 4 percent before the 10th unit to 14 percent before the 40th unit.[7]. In lesser transfused populations (medical, obstetrics, trauma, elective surgery patients), as compared to the heavily transfused groups mentioned above, prospectively studied alloimmunization was described between 3 percent after a maximum of 24 units [18] and 8 percent after a maximum of 10 units [19]. In these two studies, however, more than 30 percent of patients had a pre-study transfusion history. The fact that our study comprised of a non-transfused population, likely explains our overall lower immunization rate. Seemingly in contrast with our findings, a recent study addressing this issue proposed that the number of transfusions was only a weak determinant of alloantibody formation [5]. In the latter study however, it is unclear how the patients with hemoglobinopathies and infants were dealt with. Such patients, along with patients that have previously formed antibodies – who were documented in the study- are likely to be receiving broader matched blood. Matching on more blood groups will decrease alloimmunization risk. Compared to other studies, we specifically excluded patients who had received extended matched blood for various chronic conditions. In addition, patients with previous alloimmunization were excluded, because of their enhanced immune response against donor red blood cell alloantigens compared to first antibody responders [20, 21].

In our data, we show that the association of the immunization rate and the number of transfusions is not weak, and increases up to 40th transfusion.

Finally, our study also showed a lower alloimmunization frequency among women aged less than 45 years of age, compared to the general female population (1.4 percent vs.1.7 percent), and an immunization rate of 2.5 percent after 10 units. The effectiveness of the current matching policies can be seen in these women where no K antibodies were observed.

A strength of our study was using the incident new user cohort study design, that reduces the investigator error as well as avoids selection bias, without compromising on study validity. [10, 11] Our data collection approach allowed a prospective follow up of previously non-transfused and non-immunized patients during subsequent transfusions up to the appearance of a first alloantibody. We thus feel that this cohort represents the general transfused population and is appropriate to study the incidence of first time alloimmunization. Another strength of the study is that the incidence of alloimmunization increased with the increased number of units despite the median and range of red blood cell units being relatively similar among men, women > 45 years and women < 45 years of age.

Although we did exclude all patients who had received transfusions before 2005, there could be a possibility that the patients entering the cohort have had transfusions prior to the start of study period in other hospitals.

In addition, the incidence of alloimmunization, in the antibody forming patients as well as in the patients without observed alloimmunization in our study, may be under-estimated, because antibodies can stay undetected due to variable follow up intervals after transfusion, e.g. between 1 and 1825 days in our study. Moreover, the time that is needed before

antibodies can be detected differs and once formed they can disappear again, depending on antigen types [22]. This inherently will lead to a number of undetected alloimmunizations and thus, our presented results could be an underestimate of actual incidence.

Our data being retrospectively acquired unfortunately did not allow for a comprehensive check on previous pregnancies. But we did exclude women receiving Intra Uterine transfusions and pre-transfusion antibodies. Additionally, we also analyzed women under 45 cohort separately and it did not show different results. Pregnancy induced antibodies can lead to an overestimation of transfusion induced first time alloantibody respondents. We, however, feel that this potential problem plays a minor part in our study.

Alloimmunization to red blood cell transfusions is the single largest transfusion adverse reaction category reported in the Netherlands every year, since 2002 (23). Accurate incidence figures of alloimmunization provide a good platform to further study clinical implications and environmental risk factors of all immunization.

We conclude that the risk to develop alloantibodies increases with the cumulative number of red blood cell transfused units. This risk increases at least up to 6.5% at 40 transfusions and does not differ for men and women. We were not able to find a plateau of sensitization and even beyond 40 units, there maybe be more alloimmunization taking place. More data is required to examine the risk of alloimmunization after more than 40th transfused unit. (addendum to figure 1)

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