Clinical determinants of red cell alloimmunizatiom, implications for preventative antigen matching strategies

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IMMUNOSUPPRESSANTS AND ALLOIMMUNIZATION AGAINST RED BLOOD CELL TRANSFUSIONS

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Abstract

Introduction

Patients receiving red blood cell transfusions are at risk of developing alloantibodies against donor red cell antigens. The risk of alloimmunization is dependent on the number of units administered and the patient's genetic predispostion, but has also been suggested to be modulated by a patient's clinical profile. Our aim was to examine whether immunosuppressants suppress the development of clinically relevant red cell alloantibodies.

Methods

A two-center case- referent study was performed where case patients and control patients were sampled from all consecutive patients (N=17,750) who had received their first and subsequent red cell transfusions in a five year period in the study centers. Cases were all patients with a first detected red cell alloantibody preceded by negative antibody screens. Control patients were two-to-one matched to the case patients based on the number of red cell transfusions. Logistic regression analysis was used to examine the association between immunosuppressant exposure and the subsequent occurrence of red cell alloimmunization.

Results

A total of 156 case patients and 312 control patients in the study received a median of 6 transfusions (interquartile ranges 3-11). Among the total study population, 207 patients received immunosuppressive therapy, with 142 patients receiving only corticosteroids, 4 receiving only other immunosuppressants and 61 receiving both. The incidence of alloimmunization among patients using immunosuppressants was lower than among other patients receiving red blood cells, adjusted relative rate (RR) 0.55 (95% confidence interval (CI) 0.34- 0.91).

Interpretation

Our findings support a considerably lower risk of alloimmunization with the use of immunosuppressive medications.

Introduction

Patients receiving red blood cell transfusions are at risk of developing alloantibodies against donor red blood cell antigens.[1] Alloimmunization against clinically relevant red cell antigens can cause serious complications like acute and delayed hemolytic transfusion reactions. In light of this, it becomes important to study the risk factors associated with alloimmunization in detail, in order to predict which patients are most vulnerable to alloimmunization and may be considered for more extended matched red blood cell transfusions. On the other hand, identifying clinical factors protecting patients against alloimmunization would be equally important.

The risk of alloimmunization is dependent on the number of red cell units administered.[1] The extent of alloimmunization has been studied in various populations with the incidence of alloimmunization increasing with the number of units, ranging from 7% to 13% in a general transfused population.[1-2] The risk of alloimmunization is also determined by a patient's genetic predisposition to form an immune response to these non-self antigens.[3] In addition, it has been suggested that a patient's clinical condition is associated with modulation of the alloimmunization risk.[4] Immunosuppressive therapy could be of particular importance in this respect, because red blood cell transfusions and immunosuppressive therapy often coincide in intensive care, trauma, active autoimmune disorder, cancer, and organ transplant patients.

The use of immunosuppressants among a general transfused population and its effect on the risk of clinically relevant red cell alloimmunization, however, has not been reported and was the purpose of this study.

Methods

Design and study population

A matched case-referent study was performed at two Dutch university hospitals (Leiden University Medical Center, Leiden and University Medical Center Utrecht, Utrecht, the Netherlands). Details of our case-referent study design have been previously published[5] and are presented in chapter 3 of this thesis. In short, the source population comprised of all previously non-transfused, non-alloimmunized patients who received their first red cell transfusion at one of the study centers. The study period was January 2005 to December 2010 at Leiden University Medical Center and January 2006 to December 2011 at University Medical Center Utrecht, Utrecht.

Case patients were patients with first-time detected clinically relevant red cell alloantibodies and control patients were patients who did not have formed any clinically relevant red cell alloantibody after the same number of transfusions as the matched case. The control sampling was conducted on the principles of a risk-set sampling strategy,[6-7]

i.e. for any given case (with *N* red cell units received up until alloantibody formation), two control patients with at least the same number of units were randomly selected from the source population (figure 1). Control patients were then matched to case patients based on the *N* number of units received (figure 1). Case and control patients were also matched on the study center.

The transfusion policy in the study centers was as follows: 1. routinely transfused red cell concentrates were in SAGM and pre-storage leukoreduced and 2. all patients were routinely screened for alloantibodies before transfusion, which was repeated at least every 72 hour, if further transfusions were required.

Alloimmunization risk period

We first set out to define an 'alloimmunization risk period' preceding the antibody detection in order to identify the concurrent clinical conditions that in combination with an antigen mismatched transfused unit (implicated unit) could have led to alloimmunization.[5,8]
We measured all the study variables within this alloimmunization risk period.

This risk period stretched from 30 days before up to seven days after the implicated unit. We chose the risk period not to include the week just before the positive screen to permit at least one week to allow appropriate time for the development of alloantibodies (lag period). The risk period definition is illustrated in figure 1. A similar clinical risk period surrounding the *Nth* transfusion was defined for the matched control patients with the *Nth* transfusion corresponding to the implicated unit received by the case (figure 1).

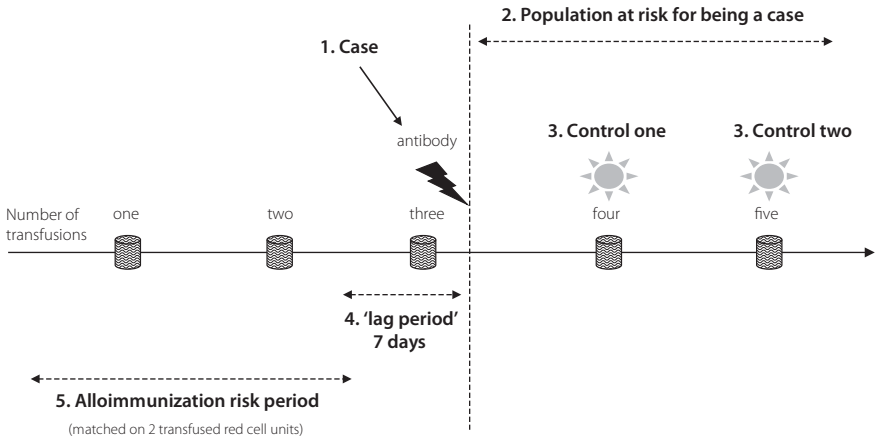
Using the above defined method to establish an alloimmunization risk period, we found in the majority (88%) of our case patients at least one transfusion with the mismatched antigen in the risk period immediately preceding the antibody identification. For the remainder of case patients, we looked further back into their transfusion history to identify the transfused unit with a mismatched antigen and re-defined the alloimmunization risk period as per the above mentioned definition around that particular mismatched transfusion.

Identification of initial (first time formed) clinically relevant red cell alloantibodies

Red cell alloantibodies were defined as warm reacting clinically significant antibodies (against: C, E, c, e, C^w, K, Fy^a, Fy^b, Jk^a, Jk^b, Le^a, Le^b, Lu^a, Lu^b, M, N, S and s), and were screened for using a three cell panel including an indirect antiglobulin test (LISS Diamed ID gel system) throughout the study period. Positive screening in the three cell panel led to subsequent identification of the antibody or antibodies by a standard 11 cell panel using the same technique.

Alloantibodies of other specificities than those mentioned, as well as cold reacting alloantibodies are not routinely detected by the three cell panel screening method and were thus not considered to be included as cases of clinical alloimmunization.

Figure 1 Control patient selection and the alloimmunization risk period.



The chronological order from case patient identification to alloimmunization risk period definition is marked from step 1 to 5.

1. A case is detected after three units of red cells received.
2. All transfusion recipients who received at least three units of red cells and developed no antibodies up until three transfusions are considered as the referent population.
3. From this referent population, two controls are selected at random.
4. A 'lag period' of seven days is introduced between the day of antibody detection to prevent the inclusion of patients demonstrating possible recall events. As such, the second but not the third unit transfused here might have mounted an alloimmune response and is defined as the implicated transfusion.
5. For both the case and the two matched controls, an alloimmunization risk period stretching from 30 days before up to 7 days after the second unit transfused is established as the alloimmunization risk period.

Medication classification

To classify the immunosuppressive therapy into corticosteroids and other immunosuppressants categories (table 1), the World Health Organization's ATC (Anatomical Therapeutic Chemical) classification index was used (source: http://www.whocc.no/atc_ddd_index). Medications classified under category H, subcategory H02 were included as corticosteroids; medications classified under category L, subcategory L04 were included as (other) immunosuppressants (table 1).

Data collection and definitions

Transfusion dates, results of the antibody investigations, patients' date of birth, gender, and clinical data on the presence of chronic obstructive pulmonary disease (COPD), infections (bacterial, viral, or fungal; infections diagnosed by laboratory serological techniques

including blood and tissue cultures), fever (temperature above 38.5 °C), transplants (organ and hematopoietic stem cell), allergies (food, dust, animal and chemical), autoimmune diseases, leukemia (acute lymphoblastic, acute myeloid, chronic lymphocytic, juvenile myelomonocytic), mature lymphoma, chemotherapy, surgeries (thoracic, abdominal, cranial and facial, upper and lower limbs excluding transluminal angiography), traumas (high impact traumas including cars, motorbikes and bicycles; falls) and diabetes (type 1 and type 2) were collected from clinical files within the defined alloimmunization risk period of alloimmunization. Use of immunosuppressive medications within this risk period was verified by consulting the hospitals' electronic patient dossiers and information management systems.

At the time of this analysis, we had not yet reached the target number of hospitals stated in the R-FACT protocol (500 case patients) due a general delay in initiating the R-FACT study protocol in other hospitals.

Data analyses

The association between the use of immunosuppressive medications and alloimmunization was modeled using a logistic regression model. Odds ratios were interpreted as relative rates throughout the manuscript. All relative rates (RR) were corrected for the matching factors (i.e. total number of transfusions and study center) and presented with a 95% confidence interval (CI).

We compared patients receiving 1. any immunosuppressive medication, 2. exclusively corticosteroids, 3. exclusively other immunosuppressants, and 4. both of these in combination, to patients not exposed to any of these medications, within the alloimmunization risk period.

The adjusted relative rates were adjusted for the above mentioned potential clinical confounders with age categorized as ≤25, 26-50, 50-75, and >75 years of age.

Results

Characteristics of the study population

Out of a total of 17,750 transfused patients, 468 patients were studied (156 case patients, 312 control patients). Fifty-six percent (N=261) of patients were from Utrecht and 44% (N=207) were from the Leiden study center. The study population had a median age of 59 years, (interquartile range (IQR) 38-70) and comprised of 56% males. Case patients had received a median of 6 units of red cells (IQR 3-11) before alloantibody formation. Antibodies were detected for the first time after a median of 123 days (IQR 25-333) following the first transfusion.

Use of immunosuppressive therapy in the alloimmunization risk period

A total of 207 patients used any immunosuppressant medications during the alloimmunization risk period including 54 cases (34.6%) and 153 controls (49.0%). Prednisone/ prednisolone (50.2%), dexamethasone (46.9%), hydrocortisone (24.1%), mycophenolate mofetil (17.9%) and cyclosporine (16.4%) were the most used immunosuppressants (Table 1). Information on medications and immunosuppressive therapy could not be traced for 18 patients (9 controls and 9 cases) and these patients were omitted from the analysis.

Table 1 Types of Immunosuppressive medication used by 207 out of 468 patients (44.2%) of the total study population.

Class and type	Number (%)
Corticosteroids	
Prednisolone/prednisone	104 (50.2)
Dexamethasone	97 (46.9)
Hydrocortisone	50 (24.1)
Methylprednisolone	34 (16.4)
Other	1 (0.5)
Other immunosuppressive medications	
Cyclosporine	34 (16.4)
Mycophenolate mofetil	37 (17.9)
Azathioprine	5 (2.4)
Antithymocyte globulin	9 (4.3)
Basiliximab	16 (7.7)
Tacrolimus	22 (10.6)
Thalidomide	3 (1.4)
Other	1 (0.5)

Among the source population, (i.e. represented by the control patients), patients using immunosuppressive medications were more often males, and younger as compared to patients not using immunosuppressive medications. Patients using immunosuppressive medications more often had (any type of) infection, allergies, leukemia, and mature lymphoma, more often underwent transplants, and more often used chemotherapy. They less frequently underwent surgeries and traumas as compared to patients not using immunosuppressive medications (Table 2). The distribution of auto-immune diseases, diabetes type 1 and type 2 was similar in both patient populations.

Table 2 Characteristics of 312 non-alloimmunized sampled controls during the alloimmunization period according to their exposure to immunosuppressive medications.

Characteristics, N (%)	None	Corticosteroids or other immuno-suppressant	Only corticosteroids	Only other immuno-suppressants	Corticosteroids and other immuno-suppressants
	(N=150)	(N=153)	(N=99)	(N=3)	(N=51)
Men	88 (58.7)	79 (51.6)	52 (52.5)	0 (0)	27 (52.9)
Age in years (median, IQR)	63 (53-75)	49 (31-65)	53 (37-70)	44 (26-44)	43 (28-59)
COPD	3 (2.0)	7 (4.6)	4 (4.0)	0 (0)	3 (5.8)
Infection*	36 (24.0)	56 (36.6)	31 (31.3)	1 (33.3)	24 (47.1)
Fever†	36 (24.0)	39 (25.5)	22 (22.2)	2 (66.7)	15 (29.4)
Transplants (organ and hematopoietic stem cell)	2 (1.3)	31 (20.3)	4 (4.0)	1 (33.3)	26 (51.0)
Allergies	7 (4.7)	12 (7.8)	3 (3.0)	1 (33.3)	8 (15.7)
Auto-immune diseases	4 (2.7)	4 (2.7)	4 (4.0)	0 (0)	0 (0)
Acute or chronic leukemia	11 (7.3)	25 (16.3)	14 (14.1)	2 (66.7)	9 (17.6)
Mature lymphoma	2 (1.3)	12 (7.8)	9 (9.1)	1 (33.3)	2 (3.9)
Chemotherapy	11 (7.3)	33 (21.6)	29 (29.3)	1 (33.3)	3 (5.9)
Surgeries	90 (60.0)	73 (47.7)	50 (50.5)	0 (0)	23 (45.1)
Trauma	16 (10.7)	4 (2.6)	4 (4.0)	0 (0)	0 (0)
Diabetes type 1	2 (1.3)	3 (2.0)	2 (2.0)	0 (0.)	1 (2.0)
Diabetes type 2	13 (8.7)	13 (8.5)	7 (7.1)	0 (0)	6 (11.8)

Values are n (%), unless otherwise stated. IQR = interquartile range. Values are n (%), unless otherwise stated. IQR = interquartile range. Information on immunosuppressive therapy could not be traced for 9 controls.

* includes bacterial, viral and fungal infections. † defined as temperature ≥ 38 °C at least once measured during the alloimmunization risk period.

Immunosuppressives and risk of alloimmunization

Table 3 presents relative rates for patients using any type of immunosuppressants, only corticosteroids, only other immunosuppressants or both, as compared to patients using none of these. Compared with patients not using any immunosuppressive medications, patients using only corticosteroids, only other immunosuppressants, or both all had a lower alloimmunization rate with an adjusted RR of 0.70 (CI 0.42-1.16), 0.51 (CI 0.04-7.10), and 0.19 (CI 0.07-0.53), respectively.

Table 3 Relative rate of alloimmunization in patients using only corticosteroids, only other immunosuppressants and both as compared to using none.

Type of immunosuppressant	Case patients	Control patients	Crude RR (CI) *	Adjusted RR (CI) †
None	96	150	*ref*	*ref*
Corticosteroids and/or immuno-suppressants	23	75	0.53 (0.34-0.81)	0.55 (0.34-0.91)
Only corticosteroids	43	99	0.68 (0.43-1.08)	0.70 (0.42-1.16)
Only immunosuppressant	1	3	0.45 (0.04-5.00)	0.51 (0.04-7.10)
Corticosteroids and immuno-suppressants	10	51	0.28 (0.13-0.59)	0.19 (0.07-0.53)

RR = relative risk. CI = 95% confidence interval.
* adjusted for the matching variables (number of matched transfusions and hospital).
† adjusted for matching variables, sex, age, COPD, infection, fever, transplants, allergies, auto-immune diseases, leukemia, mature lymphoma, chemotherapy, surgeries, trauma, diabetes type 1, and diabetes type 2.

Discussion

In our case-referent study among previously non-transfused, non-alloimmunized patients, exposure to immunosuppressives was associated with a lower incidence of clinically relevant red cell alloantibodies against donor red blood cells.

The number of patients using *only* other immunosuppressants was very low and hence, RRs presented with wide CIs. These low patient numbers reflect the standard clinical practice where immunosuppressive therapy frequently encompasses prednisone or other corticosteroids.

To appreciate our findings, several aspects need to be discussed. Strength of our study is the control sampling strategy. By using a risk-set sampling strategy, our control patients formed a representative sample of the source population.[7] In this study we

examined the combined immune modulating effects of transfusion exposure and that of immunosuppressives administered in the defined alloimmunization risk period. For this purpose, we carefully defined this risk period aiming to be able to study clinical concurrent events with possible immune modulating effects. While the observed protective association between immunosuppressive therapy and alloimmunization may in part be the result of other risk factors for alloimmunization that are also associated with the use of immunosuppressants (confounding factors), we carefully measured other risk factors and adjusted for them in our analyses.

Although the possibility of unknown transfusions at a different hospital cannot be entirely ruled out by our strategy due to absence of such information in the transfusion records of the study centers, all selected patients needed to have a negative antibody screen preceding the first transfusion and at least followed by one post transfusion antibody screen. This strategy is not entirely excluding recall immune responses to earlier primary immunizations. We, however, do not expect this to have affected our study findings as there is no reason to believe that patients with unknown previous transfusions or with unknown previous antibodies are more likely to be exposed (or unexposed) to any of the potential confounding variables.

To our knowledge, this is the first study in humans that shows the presence and extent of the protective effect of immune suppressive medications on alloimmunization against clinically relevant red cell antigens. A causal nature of the observed association with use of immunosuppressants is biologically plausible. Their role in suppressing transplant rejection in patients undergoing solid organ transplants has been well documented.[9] In addition, immunosuppressive therapy has been shown to impair humoral immune responses to vaccines and antigens.[10-11] With respect to corticosteroids, hydrocortisone has been shown to diminish *in vitro* responses to streptokinase-streptodornase and tetanus toxoid vaccinations as indication of a suppressed immune response.[12] This diminished immune response in the presence of corticosteroids has been attributed to transient lymphocytopenia by the redistribution of circulating T cells to other body compartments.[13] It has been also demonstrated that proliferation of T cells can be inhibited by corticosteroids.[14-19] For example, glucocorticoids inhibit production of T cell growth factor and block the clonal expansion necessary to amplify a primary response.[17,20,21]

Other immunosuppressive drugs also suppress T cell responses.[22] Proliferation of B and T lymphocytes is inhibited by immunosuppressants like mycophenolate and rituximab,[11,23]while agents like cyclosporine and tacrolimus inhibit the activation and differentiation of T cells by inhibiting calcineurin. In addition, a lower influenza vaccine antibody response and diminished T cell proliferation responses have been shown with these drugs in immunosuppressed liver transplant patients.[24]

Considering the mechanisms of alloimmunization against red cell antigens, this process is both B cell and T helper cell dependent. Although the short lived formation of non-naturally occurring IgM antibodies by IgM B cell memory cells is mainly T cell independent,

the subsequent memory B cell response and the formation of more high affinity IgG is T cell helper dependent. It is therefore likely that in the presence of corticosteroids and other immunosuppressive drugs, the T cell mediated responses to donor red cell antigens are impaired. Of course, the observed mediated risk reduction of alloimmunization need not be entirely caused by immunosuppressive agents, however, a direct attributive effect is strongly plausible.

As such, when aiming for an eventual alloimmunization risk prediction on the basis of clinical factors, immunosuppressives might be added to such a prediction score. This may enable to distinguish high risk patients for alloimmunization who might benefit from cost effective, extended donor blood phenotype matching strategies.

In summary, corticosteroids and other immunosuppressant medications appear to have a considerable protective effect on alloimmunization in patients transfused with donor red blood cells. While immune activating conditions are often the reason to start these drugs and coincide with their use, the inhibiting effect that was observed in our studies might be even an underestimation of the true effect of these drugs on the alloimmunization response.

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