Hemovigilance: is it making a difference to safety in the transfusion chain?

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CHAPTER 5

Clinical predictors of alloimmunization after red blood cell transfusion

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ABSTRACT

Background

Development of new red blood cell (RBC) alloantibodies (alloimmunization) is one of the most frequent adverse reactions after an RBC transfusion. Few studies have investigated clinical risk factors for alloimmunization.

Study design and methods

In this case-control study, the characteristics of all patients in whom alloimmunization occurred for the first time after an RBC transfusion in two hospitals between January 1, 2003, and May 5, 2005, were examined and compared to a randomly selected control group who received RBC transfusions in the same hospitals during the same period without alloimmunization. Odds ratios (ORs) for the association between these characteristics and alloimmunization were calculated and analyzed with a logistic regression model.

Results

Eighty-seven cases were found, and 101 controls were selected. Female sex (OR, 1.89; 95% confidence interval [CI], 1.05-3.38), diabetes mellitus (OR, 2.15; 95% CI, 0.91-5.05), solid malignancy (OR, 2.07; 95% CI, 1.00-4.30), and previous allogeneic hematopoietic peripheral blood progenitor cell (PBPC) transplantation (OR, 2.24; 95% CI, 0.64-7.81) were associated most strongly with alloimmunization, whereas lymphoproliferative disorders (OR, 0.33; 95% CI, 0.13-0.81) and symptomatic atherosclerosis (OR, 0.52; 95% CI, 0.25-1.08) were associated with the absence of alloimmunization. All of these associations except for female sex became stronger after adjustment for possible confounders.

Conclusion

Female sex, diabetes mellitus, solid malignancy, and previous allogeneic PBPC transplantation seem to be risk factors for alloimmunization, whereas lymphoproliferative disorders and symptomatic atherosclerosis seem to protect against it. Further studies are needed to confirm these associations and investigate underlying mechanisms.

INTRODUCTION

Although blood transfusion is generally very safe, adverse reactions to blood transfusions remain an important clinical problem. Since 2002, transfusion reactions in The Netherlands have been reported to the TRIP (Transfusion Reactions in Patients) Dutch National Hemovigilance Office. TRIP captures not only severe transfusion reactions, like Serious Hazards of Transfusions (SHOT) in the United Kingdom, but also nonsevere transfusion reactions. In 2004 and 2005, the most frequent adverse reaction reported to TRIP was the development of new red cell (RBC) antibodies (alloimmunization).[1] Few studies have investigated clinical risk factors for alloimmunization, such as characteristics of the recipient, and previous findings have been inconsistent. Knowledge of clinical conditions that predispose to alloimmunization is important in two ways. First, it may influence the management of a patient. If a certain category of patients has a high risk of alloimmunization, the consequence could be more extensive antigen typing and matching. Second, more knowledge of associations between clinical conditions and alloimmunization may lead to a better understanding of the etiology of this transfusion reaction.

In this case-control study, we examined the case records of patients who developed alloimmunization after a blood transfusion and compared them to patients who never developed such a reaction after a blood transfusion to identify risk factors for alloimmunization. As a secondary objective, we wanted to evaluate whether the TRIP database facilitates the study of such risk factors.

MATERIALS AND METHODS

Cases and controls

We examined the case records of all patients in whom alloimmunization was reported in the Leiden University Medical Center and Haga Teaching Hospital in The Hague from January 1, 2003, to May 5, 2005. We chose these two hospitals from the 82 hospitals that reported transfusion reactions to the TRIP organization in 2003 because they are large affiliated hospitals that cooperate on transfusion policy, and both are closely associated with the TRIP foundation. Alloimmunization was defined as the finding of a new antibody against RBC antigens other than Rhesus D and the ABO system. Only first-ever alloimmunizations were taken into account. We were looking for alloimmunization as a result of transfusion, not childbirth, so the patient must have had at least one earlier RBC transfusion to which the alloimmunization could be ascribed. Alloimmunization was ascribed to the last RBC transfusion given before the finding of the new alloantibody. All in-hospital patient records concerning the period around the time of the transfusion event were checked and analyzed by two of us (MB and JW).

The control group for the alloimmunization cases was created by randomly selecting a RBC transfusion administered to a patient in the same hospital on the day after the alloimmunization was reported. Control patients had to fulfill the same criteria as case patients except for the fact that they did not develop an alloantibody. Therefore, the selected RBC transfusion should have been preceded by at least one "type-and-screen" procedure during which no new alloantibody was found. Furthermore, the patient should have received at least one RBC transfusion previously. If a control patient did not meet these criteria, another RBC transfusion was randomly selected from the following day's list. A flow chart of the study design is shown in Fig. 1.

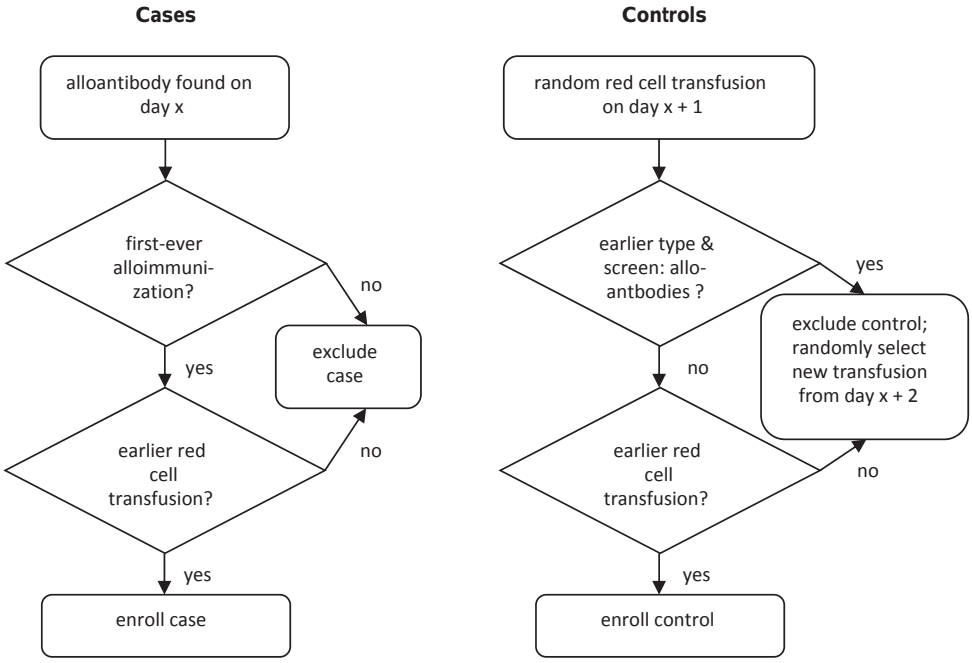

Figure 1. Flow chart of study design

Patient characteristics

Demographic patient characteristics that we recorded were sex, age, and a non-European surname, as a measure of a potential genetic background difference from the main blood donor population, which is known to consist predominantly of non-immigrants in The Netherlands. The clinical characteristics we studied were the indication for the blood transfusion, the reason for the hospital admission during which the blood transfusion took place, previous transfusion reactions, the number of previous transfusions given in the same hospital (including the transfusion to which the reaction was ascribed; only RBC transfusions were counted), the

number of children in the case of women, a history of autoimmunity (defined as any disease in which autoantibodies play a clear etiologic role or any disease of unknown etiology associated with autoantibodies [see Table 1 for list of conditions]), systemic inflammatory diseases without a clear association with autoantibodies, myelogenous marrow disorders (myelodysplasia, myeloproliferative disorders and myelogenous leukemia), lymphoproliferative diseases, a history of an allogeneic hematopoietic peripheral blood progenitor cell (PBPC) transplantation, a history of a solid organ transplantation, chemotherapy before the transfusion, and allergies.

Table 1. List of diseases defined as autoimmune

Graves' disease
Hashimoto's thyroiditis
Type 1 diabetes mellitus
Autoimmune adrenalitis
Autoimmune hemolytic anemia
Aplastic anemia
Idiopathic thrombocytopenic purpura
Thrombotic thrombocytopenic purpura associated with antibodies against ADAMTS13
Acquired hemophilia
Antiphospholipid antibody syndrome
Pernicious anemia
Celiac disease
Rheumatoid arthritis
Systemic lupus erythematosus
Still's disease
Felty's syndrome
Sjögren's syndrome
Systemic sclerosis
Polymyositis dermatomyositis
Mixed connective tissue disease
Autoimmune hepatitis
Primary biliary cirrhosis
Primary sclerosing cholangitis
ANCA-associated vasculitis syndromes
Goodpasture's syndrome
Relapsing polychondritis
Myasthenia gravis
Lambert-Eaton syndrome
Anti-Hu-associated diseases
Multiple sclerosis
Vitiligo dermatitis
Herpetiformis pemphigus
Bullous pemphigoid
Lichen sclerosus
Vogt-Koyanagi-Harada syndrome

Abbreviations: ADAMTS13 = circulating protease of the ADAMTS family; ANCA = anti-neutrophil cytoplasmic antibodies; Anti-Hu = antibody directed against neuronal Hu antigen

These are all conditions that could have a plausible relationship with a patient's immune status. Other major disease categories apart from immune disorders that we investigated were a solid malignancy, diabetes mellitus, renal failure (defined as a repeatedly measured serum creatinine concentration of more than 150 mmol/L for at least 1 week; this cutoff level was chosen because it is elevated even for muscular young men and newborns), liver cirrhosis, and symptomatic atherosclerosis. All conditions were only taken into account if they were present at the time of the transfusion. Finally, we examined whether the patient had died since his blood transfusion.

Blood products

We examined the following characteristics of the blood product to which the transfusion reaction was ascribed: leukoreduction, washing, subtyping (for C, c, E, e, Kell, Duffy (a), Duffy (b), Kidd (a), Kidd (b), M, N, S, and P1 antigens in addition to ABO and Rhesus D) and/or irradiation.

Analysis

For categorical variables, we calculated Mantel-Haenszel common odds ratio (OR) estimates for the correlation between transfusion reactions and patient characteristics with computer software (SPSS 11.0 for Windows, SPSS, Inc., Chicago, IL). We calculated adjusted ORs with a logistic regression model with the same program. We compared continuous variables with a t test.

RESULTS

We identified 70 cases of alloimmunization in the Leiden University Medical Center and 31 in Haga Teaching Hospital. Aiming for an equal number of control patients, we selected 101 control patients in both hospitals according to the method described above. Patients who had an earlier alloimmunization episode were excluded from the case group, so that 67 cases from the Leiden University Medical Center and 20 from Haga Teaching Hospital remained. Information on patient and clinical characteristics was obtained from hospital computer files or patient charts. Information on previous childbirths and allergies could not be obtained for all patients. ORs for the association between patient characteristics and alloimmunization are listed in Table 2. A solid malignancy, female sex, and diabetes mellitus seemed risk factors for alloimmunization. A previous allogeneic PBPC transplantation might be a comparable risk factor, although the confidence interval of the OR was wide. Lymphoproliferative disorders and to a lesser extent symptomatic atherosclerosis seemed to protect against alloimmunization. Thirty-six patients in the control group had died since their transfusion versus 23 in the case group; this difference is not significant.

Table 2. Mantel-Haenszel adjusted odds ratios (95% confidence intervals) for association between patient characteristics and alloimmunization, stratified by hospital.

Patient characteristic	Nr in case group (87)	Nr in control group (101)	Odds ratio (95% CI)
Demographics			
Female sex	51	44	1.89 (1.05 to 3.38)
Non-European surname	9	12	0.88 (0.35 to 2.20)
Previous childbirth	25 (n=32)*	21 (n=23)*	0.34 (0.06 to 1.82)
Transfusion history			
Previous transfusion reaction other than alloimmunization	7	4	1.96 (0.56 to 6.93)
Number of previous transfusions over 12.5	28	35	0.83 (0.45 to 1.55)
Immunologically mediated diseases			
Autoimmune disease	13	11	1.38 (0.59 to 3.23)
Other systemic inflammatory non-infectious diseases	7	10	0.76 (0.28 to 2.11)
Allergies	14 (n=79)*	18 (n=95)*	0.92 (0.42 to 1.98)
Haematological disorders			
Aplastic anaemia	3	1	3.23 (0.33 to 31.89)
Myelodysplasia, myeloproliferative disorders, myelogenous leukaemia	10	14	0.85 (0.35 to 2.02)
Acute myelogenous leukaemia	5	10	0.54 (0.18 to 1.64)
Myelodysplasia	2	2	1.40 (0.20 to 9.80)
Lymphoproliferative disorders	7	22	0.33 (0.13 to 0.81)
Previous allogeneic haematopoietic stem cell transplantation	8	4	2.24 (0.64 to 7.81)
Other			
Solid malignancy	23	15	2.07 (1.00 to 4.30)
Chemotherapy within one month prior to alloimmunization	18	24	0.84 (0.42 to 1.67)
Chemotherapy within six months prior to alloimmunization	15 (n=67)*	23 (n=70)*	0.59 (0.28 to 1.26)
Previous solid organ transplantation	3	3	1.05 (0.20 to 5.38)
Renal failure	10	13	0.86 (0.35 to 2.08)
Liver cirrhosis	2	2	1.16 (0.15 to 8.81)
Symptomatic atherosclerosis	14	28	0.52 (0.25 to 1.08)
Diabetes mellitus	16	10	2.15 (0.91 to 5.05)

Descriptive statistics for the continuous variables in the case group and the corresponding control group are shown in Table 3. The patients in the control group had received slightly more RBC transfusions than those in the case group, although the difference was not significant.

The means of the age and the number of childbirths for the case and control group were not significantly different either.

Table 3. Means and 5th and 95th percentiles (p5 and p95) for continuous variables for the alloimmunization cases and corresponding controls

		Median	p5	p95
Age (years)	Cases	56.5	12.8	84.2
	Controls	56.0	8.1	84.3
Nr of previous red blood cell transfusions	Cases	6.0	1.0	40.0
	Controls	9.0	1.1	65.2
Number of childbirths*	Cases	2.0	0.0	4.0
	Controls	2.0	0.0	4.0

* women for whom information on childbirths was available

To correct the ORs for potential confounders, we performed logistic regression analysis. Given the relatively small numbers of cases, we only entered three or four variables together in a single model. We selected confounders that have an obvious relationship with certain risk factors. For example, allogeneic PBPC transplantation is usually used to treat lymphoproliferative or myelogenous marrow disorders and is obviously associated with previous chemotherapy. The results are listed in Table 4.

Table 4. Crude and adjusted odds ratios (OR; 95% confidence intervals) for association between patient characteristics and alloimmunization

Patient characteristic	Crude OR	Confounder	Adjusted OR
Female sex	1.89 (1.05 to 3.38)	Diabetes mellitus; symptomatic atherosclerosis	1,74 (0.96 to 3.16)
Lymphoproliferative disorders	0.33 (0.13 to 0.81)	Chemotherapy within one month prior to alloimmunization; previous allogeneic haematopoietic stem cell transplantation	0.26 (0.09 to 0.71)
Previous allogeneic haematopoietic stem cell transplantation	2.24 (0.64 to 7.81)	Lymphoproliferative disorders; myelogenous marrow disorders; chemotherapy within one month prior to alloimmunization	3.70 (0.94 to 14.63)
Solid malignancy	2.07 (1.00 to 4.30)	Chemotherapy within one month before alloimmunization	2.13 (1.02 to 4.44)
Symptomatic atherosclerosis	0.52 (0.25 to 1.08)	Diabetes mellitus; female sex	0.46 (0.21 to 0.99)
Diabetes mellitus	2.15 (0.91 to 5.05)	Female sex; symptomatic atherosclerosis	2.66 (1.07 to 6.63)

The associations between alloimmunization and solid malignancy and diabetes mellitus became stronger after correction. Female sex was slightly less strongly associated with alloimmunization after correction. Strikingly, previous allogeneic PBPC transplantation seemed a much stronger risk factor after correction for lymphoproliferative disorders, myelogenous marrow disorders, and previous chemotherapy. The protective effects of lymphoproliferative disorders and atherosclerosis seemed stronger after correction. The number of patients with female sex was not significantly different between patients with and without a lymphoproliferative disease. There were slightly more women among the patients with a solid malignancy than without a malignancy, but regression analysis did not influence the ORs much. The number of previous RBC transfusions was not a confounder.

DISCUSSION

In this exploratory study, we found a number of associations between patient characteristics and alloimmunization. Our data suggest that solid malignancy, previous allogeneic PBPC transplantation, diabetes mellitus, and female sex are risk factors for alloimmunization against RBC antigens, whereas lymphoproliferative disorders and symptomatic atherosclerosis protect against it. All these associations except for female sex were stronger after correction for possible confounders.

The main weakness of this study is the relatively small number of patients, which limits the power to detect small differences. This project, however, has demonstrated the usefulness of identifying side effects in databases like TRIP's for etiologic studies and future studies can be undertaken with a larger number of participating hospitals.

The way in which the control patients were selected resulted in an overrepresentation of patients who had received many RBC transfusions. For that reason, the number of previous transfusions could not be evaluated as a risk factor. This ensures, however, that controls had enough exposure to develop antibodies. Certain RBC antibodies become undetectable within months after their development.[2] Therefore, alloantibodies that have developed may be missed if a type and screen procedure is performed a long time after the RBC transfusion that caused their development. Owing to the fact that control patients received more RBC transfusions, the transfusion intervals in the control group, that is, the intervals between the last RBC transfusion and the transfusion for which a new type-and-screen procedure was performed, were usually shorter than the transfusion intervals in the case group, so that short-lived antibodies had a larger chance to be detected in the control group than in the case group. Therefore, differences found between cases and controls cannot be caused by confounding by the duration of the transfusion interval.

In contrast, it is possible that patients with slowly forming alloantibodies are still in the control group because their antibodies are not yet apparent. If the risk factors for slow-forming alloantibodies are the same as those for early alloantibodies, this means that any associations found between alloimmunization and possible risk factors would be weakened because cases with an excess of risk factors are hidden in the control group. Therefore, if an association is found, it can only be stronger than suggested by the present data, a phenomenon called nondifferential misclassification. Furthermore, we judge the chances of slowly forming antibodies being missed in the control group to be small due to the large number of transfusions the control group received and the inherently high frequency of screening for alloantibodies.

The case-control design is a powerful tool for the detection of several risk factors at the same time. This design has rarely been applied in studies investigating risk factors for transfusion reactions.

Part of our findings are consistent with earlier studies. Female sex has been indicated as a risk factor.[3-6] This is biologically plausible, because women are exposed to alloantigens during pregnancy and childbirth. Earlier studies suggested that chronic lymphocytic leukemia, a lymphoproliferative disorder, protects against alloimmunization.[3,7] Our study found lymphoproliferative disorders as a group to be protective. This is also biologically plausible, because the malignant clone may displace functional T and B cells. Moreover, most of the patients with lymphoproliferative disorders receive intensive chemotherapy, which suppresses immunity. A protective effect of intensive chemotherapy has been suggested in an earlier study.[8] Our study suggests a slightly protective effect of chemotherapy. Aplastic anemia has been suggested to be a risk factor.[3] Our data are consistent with this, although the numbers are very small.

Some of our findings are inconsistent with earlier studies. In a number of studies, alloimmunization seemed to be associated mainly with racial differences between donor and recipient populations and the number of previous blood transfusions.[10-13] We found no indications for this with our rather crude method of comparing surnames. Furthermore, an association between alloimmunization and autoimmune diseases has been reported,[14] which we did not find either. One study found liver cirrhosis and myelodysplastic syndromes to be risk factors,[3] which we could not confirm, possibly due to the very low frequency of these conditions in our study population. Finally, splenectomy was found to be a risk factor in one study.[12] In our study population, only one control patient had had a splenectomy.

Finally, we found associations that have not been reported earlier. A new finding is the increased risk of alloimmunization in patients with solid malignancy, in spite of the fact that

many of them were receiving chemotherapy. A possible mechanism for this is a state of increased immune activation. A recent animal model suggests that alloantibodies are formed more easily in the context of an inflammatory state.[15] Another finding that has not been published earlier to our knowledge is the increased risk of alloimmunization after an allogeneic PBPC transplantation. There are several reports on hemolysis due to alloantibodies after an allogeneic PBPC transplantation, however. The development of these antibodies might have a relationship with major and minor incompatibility between donor and recipient and the persistence of mixed chimerism. This might play a direct role because part of the recipient's immune system might recognize the transfused antigens as foreign, or an indirect, role — again in the context of an inflammatory state. Also, a passenger lymphocyte mechanism has been implicated.[9] Surprisingly, we found diabetes mellitus to be a risk factor. For this finding we have no pathogenetic explanation. We also found symptomatic atherosclerosis to protect against alloimmunization, although we have no theory for a possible underlying biologic mechanism. Obviously, we do not know whether these findings are the result of an unknown pathophysiologic mechanism or the association is caused by random variation in the small numbers.

In conclusion, female sex, diabetes mellitus, solid malignancy, and previous allogeneic PBPC transplantation seem to be risk factors for alloimmunization against RBC antigens, whereas lymphoproliferative disorders and symptomatic atherosclerosis seem to protect against it. Further studies are needed to confirm these associations and investigate their possible underlying mechanisms. For this goal, databases such as TRIP can be very useful.

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1 Reference incorrect in the published version. The reference should be to TRIP Report 2005, The Hague 2006, ISBN 978-90-78631-01-9.