R-fact study: risk factors for alloimmunization after red blood cell transfusions : methodology, risk factors and challenges in transfusion medicine research

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CHAPTER 1

INTRODUCTION

Red blood cells are transfused with the intent to improve the oxygen carrying capacity of blood during and after a clinical event which has led to severe bleeding, or in case of other types of anemia leading to cardiovascular symptoms. Over half a million red cell concentrates are transfused annually in the Netherlands (source: Sanquin Blood Supply Annual Report 2011). With improvements in blood donation, storage and transfusion procedures, red cell transfusions have come a long way in terms of safety. Yet, donated transfused blood is a foreign, non-self tissue and therefore carries an intrinsic hazard for the recipient. One of these hazards is antibody formation. The aim of the studies described in this thesis was to examine the effect of transfusion related and patient specific risk factors on the occurrence red cell alloantibody formation (or alloimmunization). Because the design, analysis and interpretation of observational studies in clinical transfusion medicine present specific methodologic challenges, the methods of the studies are extensively discussed in several chapters of this thesis.

RED BLOOD CELL ANTIGENS AND RISK OF ALLOIMMUNIZATION

Red blood cells membranes have embedded carbohydrate, protein and lipid structures whose presence or absence is genetically determined. These so called blood group antigens, are mostly determined by single nucleotide differences in the encoding genes and, which as the name implies, define a person's blood group type. Including the commonly known *ABO* and rhesus *D* blood group systems (or, major blood group antigens), there are 33 recognized blood groups systems (or, minor blood group antigens- for example other rhesus antigens like C, E, c, e; Kell; Duffy; Kidd groups) carrying around 600 antigens (*Blood Transfusion in Clinical Medicine, 11th edition by P.L. Mollison*).

The distribution of minor blood group antigens (the focus of this thesis) varies in different populations: for example rhesus C- 68% of Caucasians, 27% blacks and 93% Asian are positive for this blood group. On the other hand, 29% of Caucasian, 22% of blacks and 39% of Asians are positive for rhesus E blood group type. Kell (K) is found in only 9% of Caucasians, 2% in blacks and up to 25% in Arab population (Reid ME and Lomas-Francis C. The Blood Group Antigen Facts Book. Second ed. 2004, New York: Elsevier Academic Press). Thus, quite a few of these antigens are rare and or have a skewed presence in different ethnic groups.

A non-self (or *allogenic*) blood group antigen comes in contact with the transfusion recipient's immune system mostly via a blood transfusion and pregnancy; the recipient's immune system may react leading to formation of antibodies against these foreign red cell antigens. These antibodies are called *alloantibodies* and the phenomenon- *alloimmunization*. Alloantibodies, which could cause such hemolytic reactions, are also called clinically relevant alloantibodies. Besides alloantibodies against incompatible transfusions and pregnancies, naturally occurring antibodies like IgM (immunoglobin M type) against the A and/ or B antigen occur in the absence of a previous red cell transfusion, previous pregnancy or an organ transplant (likely sources of red cell antigen exposure). Contact with such antigens in the environment that resemble non self red blood cell antigens (antigens located outside the

red cell membrane, or from other foreign substances such as bacteria) are held responsible for the formation of such naturally occurring antibodies.

Alloimmunization has various clinical consequences. Alloimmunization may present itself as an acute (within 24 hours of transfusion) hemolytic reaction or a delayed (more than 24 hours after transfusion) hemolytic transfusion reaction. Symptoms may include fever, rigors, nausea, hypotension, tachycardia, skin flushing, hemoglobinemia, hemoglobinuria and bleeding (*Blood Transfusion in Clinical Medicine, 11th edition by P.L. Mollison*).

In the Netherlands, close to 800 cases of alloimmunization against red cell transfusions are reported yearly, according to the Transfusion Reactions in Patients (TRIP) national hemovigilance office. This reported number could well be an underestimation since the reporting from hospitals is voluntary.

Each antigen differs in its immunogenicity, with the *ABO*, *D* and Kell (*K*) highly immunogenic whereas the majority are not. The ability to detect transfusion related (or from previous pregnancy, previous organ transplant) as well as naturally occurring antibodies leads us to avoid subsequent hemolytic transfusion reactions by selecting donors with red cells lacking the antibody targeted antigen. Determining both the red blood cell type of patient and donor in principle also allows matching of transfused donor blood to patient's blood type, and thus preventing alloimmunization in the first place. For example, the severely immunogenic antigen groups- ABO and rhesus D with significant percentages of the population that differ in their presence are as a standard tested for, both in patients that need blood and their donors. Transfusions are matched for these antigens; in other words patients thus receive red blood cell units that are compatible with their own ABO and D type. This is done not only to avoid a severe intravascular hemolysis mediated by the naturally occurring IgM antibodies, but also to avoid (further) (IgG) immunization against these antigens.

Such preventive measures before an allogeneic red cell transfusion hence consist of routinely matching the recipient's blood group type to the donor red cell unit. While A*BO* and D matching is practiced in all patients, additional matching is more sensible- a) for common, highly immunogenic antigens other than ABO and D like *K* and *e* antigens and b) in special patient populations with chronic requirement of blood transfusions (sickle cell anemia, thalassemia, auto-immune hemolytic anemia, myelodysplastic syndrome- *CBO richtlijn- Bloedtransfusie 2011*) as well as women in the reproductive age. The *Danger Model* theory of immune response[1,2] suggests that the extent of the immune response depends not only on the exposure to a foreign antigen itself, but also on the immune modulating conditions surrounding that exposure- transfusion related and co-existing patient clinical profile. Identification of patients or clinical conditions that are associated with high risks for alloimmunization and subsequently transfusing these high risk patients with more extensive matched blood could be the third most likely and most cost-effective strategy to prevent alloimmunization. A first step for this strategy would be to define the transfusion and clinical risk factors and in this way identify the patients with the highest risk for alloimmunization. In this respect, female sex, diabetes, solid malignancies and progenitor cell transplants

were shown to be associated with a higher risk of clinical alloimmunization[3]; and lympho-proliferative disorders and atherosclerosis with a lower risk of alloimmunization[3].

In studying clinical risk factors for an adverse event, (alloimmunization in this instance) case- control epidemiological study design (with its variants) and cohort design are logical and feasible study designs. A major pitfall of such case control study designs though is improper selection of a control patient population for comparisons with the case patients. It is essential that the control patients are a good representative sample of the population at risk for alloimmunization, or the source population. This less optimal sampling of control patients was a limitation of a study[3] which examined clinical risk factors (or predictors) of alloimmunization.

OUTLINE OF THIS THESIS

The scope of the work described in this thesis is to examine whether potential transfusion related and clinical risk factors modulate the risk of alloimmunization in a general, previously not transfused, non alloimmunized population of transfusion recipients. In these studies we emphasize the methodological aspects of observational research in clinical transfusion medicine.

Briefly, in chapter 2, we presented the design of our case- referent study as a benchmark for the rest of the thesis. We then aimed to study the risk of alloimmunization and the number of transfusions, using a new user cohort study design in chapter 3. The aim of the case- referent study conducted in chapter 4 was to examine storage time of red cells as a risk factor of alloimmunization. In chapter 5, we examined the intensity (or the dose) of red cell transfusions and the risk of alloimmunization. The effect of concomitant immunosuppressive medications as a clinical risk factor for alloimmunization was studied in chapter 6. Finally, in chapter 7, we aimed to highlight the distinction between etiologic and prediction observational research in clinical transfusion medicine.

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