Out for blood: causal inference in clinical transfusion research

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Chapter 1

General introduction and outline of the thesis

Part 1. Introduction

Blood transfusions have been performed since the early 1800's in order to reduce complications due to bleeding and anemia. Their life-saving potential, ultimately improving tissue oxygenation, is well-established in the acutely bleeding patient seen in the trauma and surgery setting. For the non-acute anemic patient, benefits of transfusions are less clear.[1] Importantly, these benefits need to be weighed against increasing healthcare expenses, the limited availability of blood, and both avoidable and unavoidable risks. These risks – admittedly – have become relatively small but can still lead to severe, life-threatening side effects, e.g. anaphylaxis, transfusion associated circulatory overload and transfusion-associated Graft-Vs-Host disease.[2] In this context, it is understandable that a restrictive transfusion strategy is the default in many settings[3], and an increased interest in potentially avoidable risks has developed. When referring to avoidable risks associated with transfusions, an example which comes to mind is plasma from female donors being associated with increased risk of transfusion-related acute lung injury (TRALI).[4] Using observational data, the 'culprit' was found to be the transfer of antibodies against human leukocyte antigens (HLA), which are more abundant in parous women. This observation prompted a policy change – in the Netherlands – where use of male-only plasma or pooled plasma was made the standard, which resulted in a 33% reduction in TRALI cases.[5, 6] There are still more uncertainties about avoidable harms from transfusions, e.g. other risks relating to sex of the donor[7, 8] and the impact of storage of blood products[9]. The role of clinical transfusion research using observational data to investigate such potential risk factors is evident: at first sight, observational data seems exceptionally suited to investigate a large number of patients exposed to a specific blood product characteristic, and to follow these patients for the occurrence of relatively rare outcomes (such as mortality) within a large time frame. There are, however, a number of caveats when analysing data originating from such uncontrolled settings. In this thesis, characteristics of red blood cell products and their connection with patient outcomes are carefully described, in order to achieve an understanding of the intricate relationship between product, and patient. Ultimately, the goal of the research described here is to contribute to the further development of clinical transfusion practice, by performing epidemiological studies that can estimate causal effects, and thereby discerning ways to mitigate avoidable transfusion risks.

First, to better define the field of clinical transfusion research, the different types of blood products and their uses need to be introduced (*Figure 1*). When the first transfusions were performed, these consisted of whole blood, a product which

is used today but has limited applications.[10] The development of centrifugation and filtration made the individualized transfusion of leukocyte-reduced blood components possible.[11] Red blood cell units, or erythrocyte transfusions, are used to treat anemia and bleeding, and are indicated for symptomatic anemia with pre-transfusion hemoglobin thresholds ranging from 4 to 6 mmol/L.[11] Fresh frozen plasma is used to treat patients with massive bleeding-associated co-

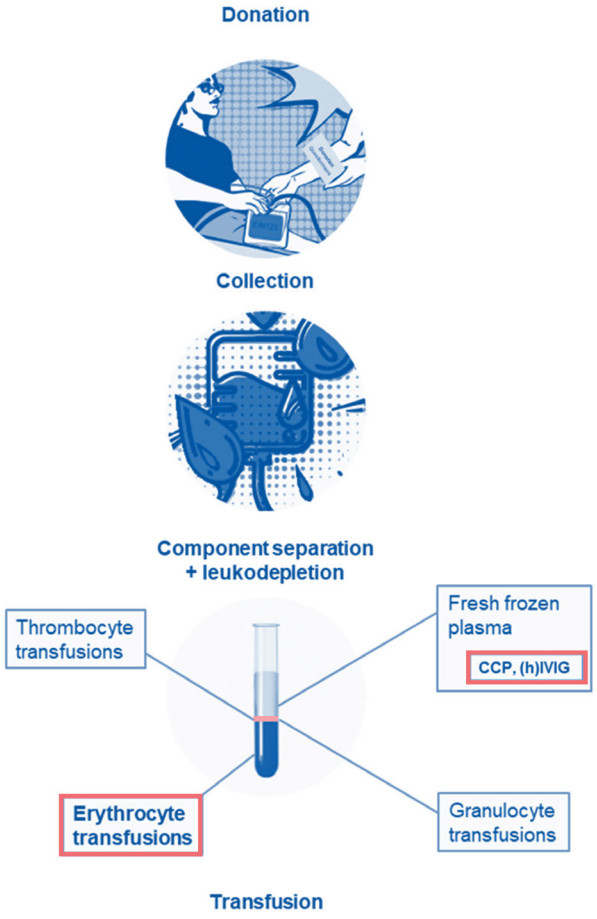

Figure 1

The process from donation to transfusion in the Netherlands consists of multiple steps. First, the donation is performed in one of the blood collection centers from a voluntary non-remunerated donor, and a self-reported questionnaire on risk behaviour and health status is taken. Each donation is assigned a unique number (unit identification number, or "EIN"). The central collection of whole blood is performed in two locations, and component separation by centrifugation is performed, including a leukodepletion filtration step. Whole blood separates into erythrocytes (45%, in dark blue), buffy coat (<1%, in pink) and plasma (55%, in light blue) during the centrifugation process. The separation of whole blood into the blood components for transfusion is further facilitated by the addition of different types of storage media and the use of advanced separation techniques. At multiple timepoints, donors and products are tested for infectious risks to ensure safety of blood products. In this thesis, the safety and efficacy of erythrocyte transfusions, or red blood cell units, and convalescent plasma for COVID-19 and hyperimmune immunoglobulin (hIVIG) are the main focus.

agulopathy and for therapeutic plasma exchange. From plasma, other products can be prepared, such as clotting factor concentrates, albumin and intravenous immunoglobulin (IVIG). Thrombocyte transfusions (platelets) are prepared from the buffy coat of the centrifugated whole blood, by pooling platelet-rich plasma, or by single-donor apheresis and used to treat and prevent thrombocytopenia-associated bleeding. Other blood products have limited indications. One of them is the granulocyte transfusion, derived from granulocyte colony-stimulating factor' treated donors by apheresis, or from pooled buffy coats. These granulocytes are sometimes prescribed for severely neutropenic patients with active infections or prophylactically for patients entering a neutropenic period. Other less common products are convalescent plasma and hyperimmune immunoglobulin (hIVIG) obtained from plasma of individuals who have recovered from a specific infection or that are recently vaccinated, and have been primarily studied in the context of H1N1 influenza and Coronavirus Disease 2019 (COVID-19). These products contain antibodies developed by the donor immune system as a response to infections, and can be used to treat other individuals who are currently infected with the same pathogen.

Clinical transfusion research of blood product characteristics: two perspectives

Blood product characteristics research borders on various fields in the scientific spectrum, and the considerations for researchers in this field can be viewed from two perspectives: the biological perspective and the epidemiological perspective. From the biological perspective, understanding of the different blood products and their uses is essential. It is important to understand the pathophysiology of the various diseases in which transfusions are indicated. As opposed to pharmaceutical products, blood products are intrinsically heterogeneous: they derive from various donors, and can be produced in varying ways across the world. Whereas in pharmaceutical interventions, we intend to give the patient a single treatment, blood products can be considered to contain a multitude of active components, ranging from plasma, red blood cells, leukocytes, cytokines, and various other compounds, that can also change throughout the products' timeline from donation to transfusion. These factors all contribute to the challenging nature of blood product characteristics research from a biological perspective.[12]

From an epidemiological perspective, there are also challenges which contribute to the difficulty of blood product characteristics research.[13] Rather than comparing a single intervention in a randomized controlled clinical trial (RCT), blood product characteristics research is mostly based on observational data from

patients receiving – what can be perceived as – a range of different interventions, which commonly also includes the transfusion of multiple units, over time.

As opposed to creating a division, we want to emphasize that these two perspectives are inherently complementary. The epidemiologists' toolbox will naturally be supplied with a large dose of fundamental scientific information and, in turn, clinical research provides the rationale for laboratory investigations. These perspectives together should be kept in mind by clinical transfusion researchers when investigating blood product characteristics, as they enhance each other and thereby allow the study of complex research questions.

Donor sex and clinical transfusion research

Donor characteristics and transfusion recipient outcomes have been frequently studied. Previously, an association was observed between transfusions of red blood cells from female donors with increased mortality in male recipients under 50 years of age.[14] This association of red blood cell transfusions from female donors with decreased survival of male recipients was later confirmed in an independent cohort.[7] The association was furthermore shown to be limited to female donors with a history of pregnancy, and it was estimated that this association could be responsible for one potentially preventable death per day in the Netherlands.[7] Several studies, although performed in different populations and with a different methodology, have since been published which were not able to confirm these findings.[8, 15] Hence, there still remain a number of unanswered questions about the significance of donor sex and parity in clinical transfusion research, e.g. whether this finding is replicable, and if it is, how it can be explained (i.e. "biological plausibility"). The epidemiological perspective and the biological perspective, that were introduced earlier, coincide here.

As already suggested, for the investigation of the association between donor sex and recipient outcomes several methodological challenges should be addressed. First, due to the nature of observational data, the existence of time-varying exposures, mixed exposures, and incomplete confounder information (e.g. underlying disease severity) make analyses complex. Second, the possibility of bias due to 'treatment-confounder feedback' that will be explained in detail in this thesis, poses a newly recognized challenge. This phenomenon can occur when the exposure of interest is a time-varying product characteristic which has a relation with both the outcome, and with the ability of the product to increase the hemoglobin level of the patient (e.g. through a different Hb content of the product, or if the post-transfusion yield is affected by a changed clearance of donor cells). In this setting, treatment-confounder feedback results in differen-

tial transfusion needs for the exposure of interest, and thereby makes standard confounding adjustment prone to bias.[8] Lastly, the presence of country-specific production methods and practices, population differences of both donor and patient, and differences in analysis methods could account for disparities between studies in detecting an association between donor sex and recipient outcomes. The interpretation of the available evidence requires careful consideration of all these challenges.

The red blood cell storage lesion: lessons from the past

Whether young or old red blood cells are better for clinical outcome is still the subject of debate, with studies showing both positive and negative associations between the storage time of red blood cells and clinical outcomes, including mortality.[16-27] Earlier, our research group showed that very fresh red cells were associated with increased mortality in an observational cohort study.[28] Several randomized trials on this subject have been since published.[19, 22-27] These trials were designed expecting the transfusion of older red cells to be associated with increased mortality. Therefore it was considered unethical to transfuse very old red cells, like those during the last two weeks of the maximum allowed storage time. This resulted in less pronounced storage time contrasts between the treatment arms. Moreover, these trials were not designed and sufficiently powered to accommodate an unexpected inverse association, i.e. fresh red cells potentially being harmful. Still, blood products stored for short storage duration were not found to be superior to older units; although not statistically significant in any individual trial, harm from fresh units was more likely.[19, 22-28]

When comparing these trials to observational research, they are not in agreement.[29, 30] Although results from observational studies and RCTs need not coincide[31], in this specific situation the expectation is that they should, if the observational studies sufficiently eliminate bias due to confounding and selection. To explain this, we need to look at the causal question the observational studies were attempting to answer. In general, the observational studies answered the general question "what is the effect of receiving only 'fresher' transfusions compared to receiving only 'older' transfusions", but without taking into account the time-varying aspect of receiving additional transfusions. Because patients receive multiple transfusions over time, and their probability to receive only one of these categories consequently diminishes, analysing restricted subgroups of patients receiving "only-fresh" and "only-old" units throughout their follow-up would be subject to bias. These inconsistencies have made the scientific community aware of limitations of past studies, but still the conclusions of these studies – namely that stored blood is inferior to fresher units – persist. More

attention to methods in clinical transfusion research can shed light on the causal relations that give rise to the observed data.

Evidence sources: observational research and rapid reviews

Although observational research has a number of limitations, especially in the field of transfusion medicine there is a large role for observational research in providing answers to causal questions. Indeed, for longer term outcomes that are not determined by blood transfusion alone, the size of RCTs needed would not be feasible, and answers should be sought, and can be found, elsewhere. We argue this is especially true when investigating blood product characteristics. This is because the setting in which transfusions are administered can be considered to be similar to a "natural experiment".[8] In this setting, there is no risk of traditional confounding or confounding by indication being introduced, because particular exposure characteristics:

- can typically not be chosen or avoided,
- are not known at the time of transfusion,
- cannot influence how outcomes are reported.

This is why it is puzzling to see that implying causality in an observational study publication can lead to a swift rejection from some scientific journals. Rather than avoiding the "c-word", causality, and only describing associations, observational studies can play an important role in assessing the effects of different interventions, especially when patients are randomly exposed with respect to their prognosis.[32] However, this does not mean clinical transfusion research with observational data in this setting is similar to analysing a true randomized experiment. Rather, clinical transfusion research is complicated by patients receiving multiple transfusions over time, exponentially increasing the probability of mixed exposure. At the same time, transfusion efficacy and safety is likely varying per product, and patient disease severity and outcome effects will additionally vary throughout transfusion episodes. With proper epidemiological study designs and appropriate statistical analyses, however, these difficulties can be overcome. Hence, causality can be inferred from observational research, and importantly, observational studies can serve to inform clinical practice.

In the context of public health emergencies, the use of rapid reviews has become increasingly popular as a means of synthesizing evidence in a timely and efficient manner.[33] The accelerated nature of the review process can reduce costs and increase efficiency, but this approach can also be a limitation, as the review may not be as comprehensive or rigorous as a traditional systematic review. Evidence sources may also be limited to non-randomized studies, such as case reports and

case series. There are also advantages of performing early rapid reviews. Systematic reviews that include non-randomized studies allow researchers to identify and evaluate the full range of evidence available at that time. Indeed, valuable insights can be obtained by carefully selecting and evaluating the studies to be included in the review and assessing the risk of bias in every study. Gaps in the existing literature can be identified to guide future research efforts. Finally, RCTs, as opposed to observational research, often include a strongly selected patient group and are not representative of clinical practice. On the other hand, it is important to note that especially smaller, non-randomized studies that have not been carefully designed can be subject to biases. We hence should always assess carefully where the validity and reliability of findings of both observational studies and RCTs could be compromised.

Part 2. Aim and outline of the thesis

The research questions in this thesis relate to two themes: investigating donor- and blood product characteristics, and investigating safety and efficacy of convalescent plasma for people with Coronavirus disease 2019 (COVID-19). The research in this thesis uses observational data and thorough epidemiological methods to answer several research questions in these two areas.

In **Chapter 2**, the different biological mechanisms potentially underlying associations of donor sex and pregnancy history with mortality in the clinical transfusion field are discussed in the form of a narrative review. Past studies have drawn attention to the potential adverse effects of donor and blood product characteristics. In particular, donor sex and parity have been implicated in increased mortality among transfusion recipients.[7] Interestingly, sex mismatch has long been recognized as a risk factor in solid organ and stem cell transplantation, with the strongest association observed for multiparous female donors and male recipients of hematopoietic stem cell transplantations.[34] In this chapter, the available evidence from transfusion, solid organ transplantation, and stem cell transplantation medicine is summarized and possible biological mechanisms underlying the association between donor parity and red blood cell unit recipient mortality are discussed. A key aspect of this chapter is the possible role of cellular microchimerism in immune modulation of transfusion recipients, and how this may contribute to adverse outcomes.

At the same time, longer blood product storage is associated with mortality in randomized clinical trials, with 'fresh' blood associated with a non-significant

mortality risk.[30] We postulated that both donor pregnancy history and blood product storage may impact the safety of red blood cell transfusions, with 'fresh' units from ever-pregnant donors providing the highest risk after transfusion. This is investigated in **Chapter 3,** where we examined the association between donor pregnancy history and storage with mortality of recipients in a cohort study of first-ever transfusion recipients in the Netherlands. We proposed that both associations were mediated through residual leukocytes which decay during storage, and studied this using Cox proportional hazards models, with the number of units with each product characteristic as exposure.

In **Chapter 4**, a rapid review investigating convalescent plasma and hyperimmune immunoglobulin for treating individuals with COVID-19 is described. This review constitutes the first instalment of the systematic review series investigating these therapies using Cochrane systematic review methodology. Convalescent plasma and hyperimmune immunoglobulin are therapies that have been studied in the context of several respiratory viral infections, but it was not clear whether they were safe and effective for people with COVID-19. A thorough understanding of the most current evidence regarding their benefits and risks at the time was required. Therefore, the objective of this study was as follows: to assess the efficacy and safety of convalescent plasma and hyperimmune immunoglobulin transfusion for treating people with COVID-19 at the start of the COVID-19 pandemic. We conducted a systematic review of the literature, searching multiple databases for completed and ongoing studies as of April 2020.

Chapter 5 continues on the conflicting evidence on the effect of donor sex on outcomes after red blood cell transfusion. Some studies have suggested that transfusion of blood from female donors may increase the risk of adverse outcomes, while others found no difference between male and female donor blood.[7, 8, 15, 35] Donor pregnancy may partly explain this association, with a study from our research group showing increased risk of mortality, predominantly in younger adult male patients.[7] Whether sex of the donor's offspring (i.e., whether the donor had sons or daughters) has any impact on transfusion outcomes is unknown, but because earlier studies showed an association with mortality in especially male patients, we postulated offspring male sex could potentially mediate this association. On that account, we performed a large observational cohort study with information on donor characteristics and their offspring, and studied the association of these characteristics with mortality using inverse probability weighting (IPW). The use of IPW or other advanced statistical modelling techniques is required here, because of the presence of time-varying treatment-confounder feedback. This is due to the probability of additional transfusions

depending on donor sex, with female donors providing a red blood cell unit with lower hemoglobin concentration compared to male donors; and the relation between the number of transfusions and mortality, with patients who are more ill requiring more transfusions on average.

As shown in chapter 5, advanced statistical modelling techniques, like IPW, allow for sophisticated analysis of complex data, and these methods may be required to estimate causal effects when time-varying treatment and confounder information is of interest. There are several reasons why IPW and other similar analytical approaches have not been widely adopted in clinical transfusion research. First, these methods require an understanding of advanced statistical concepts and techniques, and close collaboration with methodological experts. Second, there may be a lack of awareness of the benefits or necessity of these techniques among researchers. Third, some of these methods can be computationally complex and may require large sample sizes, which may be a challenge in the setting of clinical transfusion research. Finally, their results are more challenging to interpret and hence appreciate by the general scientific community. In **Chapter 6,** we provide a detailed tutorial of the use of IPW in clinical transfusion research, including a sample dataset. The situations where the use of IPW is necessary are discussed in depth, and guidance for future research is provided.

In **Chapter 7** the main findings of the thesis are summarized and the implications for future research are discussed. Here, a balance is sought between the epidemiological and the biological perspectives, by combining insights from clinical and fundamental research in the interpretation of the evidence on blood product characteristics and their influence on patient health.

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