Out for blood: causal inference in clinical transfusion research

Valk, S.J.

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Chapter 7

General discussion and summary

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Blood products, by many measures, have evolved to be an extremely safe and fundamental part of hospital care. Moreover, they are a valuable resource that should be respected and safeguarded. In this thesis, we studied the relation of donor and product characteristics with patient outcomes in detail. By using thorough epidemiological methods, we found that there are still causes for concern pertaining to donor characteristics and transfusion recipient outcomes. In all chapters, in addition to describing the results and the most relevant aspects for clinical transfusion practice, we extensively described study limitations, and in a number of chapters we acknowledge that methodological limitations preclude causal claims. It should be noted that the goal of the research included in this thesis is not to criticize the use of blood products as a whole. Rather, the continuous improvement of a therapy's safety and effectivity for those in need of it is always justified, and is the ultimate target of the research described here.

Donor parity and storage of blood products: where do we stand?

In **Chapter 2,** the different potential biological mechanisms that could underlie the association between donor sex and donor pregnancy history with mortality are discussed. We propose the most likely mechanism is the unintentional transfer of donor-derived white blood cells of ever-pregnant female donors, potentially specific to male-targeted minor histocompatibility antigens (HY-antigens), and provide the biological rationale to study this in a later chapter. In brief, exposure to alloantigens during pregnancy, transfusion, or transplantation can induce human neutrophil antigen or human leukocyte antigen (HLA)-specific leukocytes, which we theorize are transferred to recipients by means of a transfusion. We further elaborate on this potential biological mechanism in **Chapter 3**, where we hypothesize that blood product storage, and with it, the decay of residual donor leukocytes in the blood product, might explain the observations pertaining to storage of blood products. In this chapter, we investigate the role of storage in the association between exposure to ever-pregnant donor red blood cell units with mortality. Although we are unable to conclusively show that storage modifies the association between donor characteristics and patient mortality, the most likely direction of the association seems to be harm from fresh products. Therefore, storage remains a factor of interest in the study of blood product safety and effectiveness.

Storage of blood products and its association with patient outcomes indeed have been frequently studied, and researchers remain divided on its relevance.[1] Observational studies were conducted, and an association between prolonged storage and mortality was found, with storage hypothesized to affect red blood

cell transfusion efficacy and safety due to the so-called "storage lesion". However, the more early studies on this matter were conducted while not taking into account the decreasing probability of patients to receive all their transfusions from a single storage category, and are therefore not estimating a causal effect of storage. Moreover, we now understand that even when carefully designing these studies and incorporating the time-varying aspects of transfusion exposures, their conclusions can still be subject to biases. This is due to the possibility of treatment-confounder feedback by smaller hemoglobin-increments observed with stored products, as described in this thesis and in the publication by Zhao et al.[2]. Products that are stored, indeed have a lower capacity to increase the patient's hemoglobin concentration, with a dose-response relationship between storage and hemoglobin increment.[3] Randomized controlled trials have also not given a decisive answer, with the results from a large meta-analysis showing potential for 1-2% benefit and up to 9% harm (HR 1.04, 95% confidence interval 0.98-1.09).[4] The difficulty with these randomized controlled trials is that they investigated overlapping categories of storage and did not restrict themselves to the very fresh and very old groups, which led to a lack of power to determine the effect of the very furthest ends of the storage spectrum. It is understandable that physicians might not want to subject their patients to perceived inferior blood products, being very fresh or very old, respectively. However, as the evidence base for the impact of storage on mortality is not yet fully established, we do consider such trials to be justified, in particular, to find out if a minimum storage threshold might be an easy to implement measure to decrease transfusion-associated risks.

In relation to the aforementioned treatment-confounder feedback by differing levels of product hemoglobin, we would like to draw attention to a relatively recent development in clinical transfusion: patient blood management (PBM). PBM is a strategy that aims to reduce the amount of blood products and thereby make the restrictive transfusion policy more common, while also reducing the patient's own blood loss and, if possible, salvaging the blood that a patient loses and returning it to them. Together, these actions have been shown to lead to a safer and more cost-effective blood supply.[5] We propose that a better understanding of the effects of product characteristics (i.e. storage or donor sex) could contribute to the evolution of PBM. As transfusion researchers, we need to consider the ultimate target of transfusion: to improve tissue oxygenation by providing oxygen-carrying capacity in the form of hemoglobin on the red blood cell. So, why then are donor and product characteristics associated with hemoglobin increment not taken into account here? It would be interesting to see whether patient outcomes might be improved by using information known

to be associated with lower hemoglobin-raising capacity of products (again, as can be caused by donor sex and storage, but also by novel concepts like 'poor storing donors'[6]) to tailor transfusions to patients with certain characteristics (e.g. different strategies for transfusion indications and/or relevant patient characteristics such as sex). The undertaking of a pragmatic randomized controlled trial comparing such a tailored transfusion strategy to a standard one could help further understanding of associations in observational research, and subsequently improve both patient outcomes and cost-effectiveness.

Convalescent plasma for COVID-19

The objective of **Chapter 4** was to quantify the available evidence on the efficacy and safety of convalescent plasma or hyperimmune immunoglobulin transfusion as a treatment for individuals with COVID-19. The results of the review indicated that the evidence on the effectiveness of convalescent plasma therapy for individuals hospitalized with COVID-19 is highly uncertain due to inconsistent reporting of results, which made it difficult to draw definitive conclusions. We found very low-certainty evidence on the effectiveness and safety of convalescent plasma therapy for individuals with COVID-19, as all studies included in the review had a high risk of bias and low reporting quality. Furthermore, at the time of publication there were no completed randomized controlled trials or controlled non-randomized studies that evaluated the benefits and harms of convalescent plasma therapy. Since then, a plethora of studies of varying quality have become public and it has become clear that convalescent plasma does not reduce mortality and has little to no impact on clinical improvement for hospitalized patients with moderate to severe disease (*Figure 1*).[7] For outpatient with mild disease, evidence from five randomized trials suggests that early and high-titer convalescent plasma is safe and effective against hospitalization.[8]

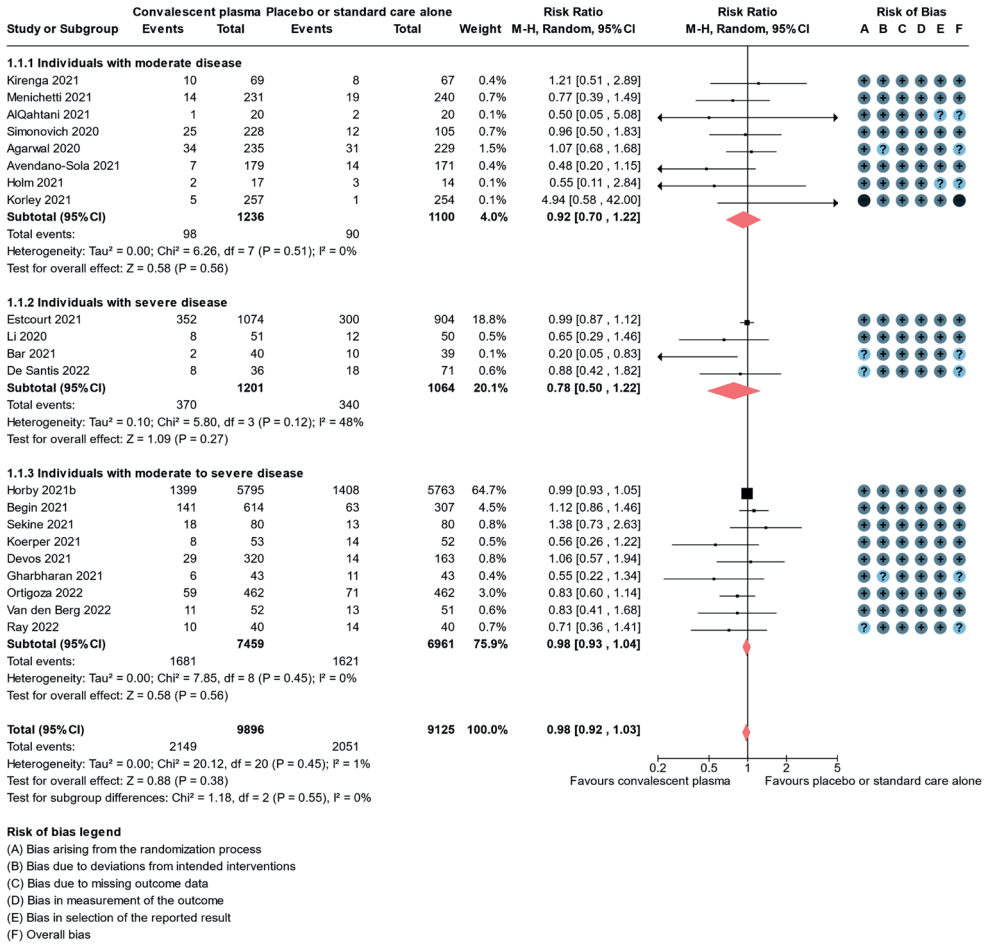

Figure 1

The most recent update of the systematic review investigating the safety and effectiveness of convalescent plasma transfusion as a treatment for individuals with COVID-19 showed there was no benefit in the primary outcome of 28-day mortality when transfusing convalescent plasma compared to placebo or standard care in hospitalized patients with moderate to severe disease (HR 0.98, 95% CI 0.92-1.03, adapted from Iannizzi et al.[7]).

The continued threat of the emergence of variants, like the Omicron variant of concern of SARS-CoV-2, has posed a significant challenge to the treatment of COVID-19 with plasma or antibodies. Omicron indeed has shown increased resistance to the anti-Spike monoclonal antibodies (mAbs) that have been authorized for emergency use.[9] Although plasma obtained following vaccination designed against earlier strains might also be less effective against new variants, cross-reactivity is more likely, while plasma of patients having cleared the new variant might also be available rapidly. As a result, there is ongoing interest in the use of COVID-19 convalescent plasma, particularly for immunocompromised patients.[10]

In the context of the COVID-19 pandemic, notwithstanding effectivity given early and with high titers for also immunocompetent patients, immunosuppressed patients could especially benefit. They are more vulnerable to severe illness and death, due to their decreased ability to mount an effective immune response to the virus, and an estimated 2% of patients without a functional B-cell response present with persistent COVID-19 and progressive respiratory failure.[11] On top of this, immunosuppressed patients are viewed as a potential public health threat because of the development of new variants under these conditions.[12] There are indications that a proportion of patients without antibody responses to SARS-CoV-2 vaccines are able develop T cell responses, but evidence is inconsistent.[13] Better treatment and prevention strategies are needed to protect this vulnerable patient population, both now and in the future.

We recognize there are ample grounds to consider the use of convalescent plasma in immunocompromised patients as a treatment option.[10] First, immunocompetent individuals have responded well to early antibody-based therapy of sufficient dosage, with some studies indicating benefit in patients without antibodies at baseline.[7, 14] Second, convalescent plasma contains a broader spectrum of also non-IgG type immunoglobulins than other formulations, such as hyperimmune immunoglobulin[15] and monoclonal antibodies[9]. Third, preliminary evidence suggests that even late administration of convalescent plasma may have potential benefits in immunocompromised patients.[10] While the definitive benefits of convalescent plasma in immunocompromised patients remain to be demonstrated, the available evidence supports its efficacy as a treatment option and the therapy is available on compassionate need basis in the Netherlands. A trial is in preparation on this subject.

The role of donor sex and pregnancy history of the donor

In 2017, our research group was the first to show that exposure to ever-pregnant donor red blood cell products was associated with mortality, with a hazard ratio (HR) of 1.43 (95% confidence interval (CI) 1.13-1.82) in male patients between 18 and 50 years of age.[16] This research was rooted in multiple studies consistently indicating an association between female donor sex and mortality of male patients.[16-26] However, the association between donor parity and mortality was not confirmed in a large observational cohort study in Sweden, Denmark, and the USA[27], while another study applying different statistical methodology also was unable to detect an association[2]. What's more, the recent publication of the iTADS trial showed that there was no difference in mortality rates at 30 days, 3 months, 6 months, 1 year, and 2 years when comparing assignment to male donor units to assignment to female donor units in a large pragmatic random-

ized controlled trial in Canada.[28] There was, however, a noteworthy association between exposure to female donor units and mortality in the combined group of male and female patients aged between 20-29 years, which was not explained. The question remains: why did these studies not find the same effect? The answer could lie in differences in country-specific production methods, population differences in both donors and patients, and differences in the applied statistical analyses. This last point pertaining to methodology, as we are able to show in **Chapter 5**, does not alter our initial observation on the potential for harm from exposure to ever-pregnant female donors for male patients.

We investigated whether offspring sex explains the association between donor pregnancy history and mortality using newly collected data from the municipality registration in the Netherlands on over 130,000 female donors, and we relate this to patient mortality using marginal structural models. Offspring sex was not shown to play a role in the earlier observed association, and thereby the hypothesis that blood products from female donors with sons was driving the previously observed associations in clinical transfusion research could not be confirmed. One noteworthy aspect that could have influenced the interpretation of the study's findings, is that female donors who have been pregnant are on average older compared to never-pregnant women, meaning the association could also (partly) be driven by this factor. Notwithstanding any donor age-mediated effects, the methodology used here was able to account for treatment-confounder feedback due to donor hemoglobin concentration differences, and thereby this research question has been thoroughly investigated.

Interestingly, the study was able to independently replicate that exposure to ever-pregnant donors is associated with male mortality in the age group between 18 and 50 years. Residual leukocytes from blood products persisting in the patient, with some products containing up to $5x10^6$ residual white blood cells following leukodepletion, might cause this consistent observation. We can only speculate, however, why male patients of the age group between 18 and 50 could be more sensitive to these female products. First of all, concerning the higher sensitivity of males between 18 and 50, we hypothesize that this is not because of their Y-chromosome, but might be due to the different transfusion indications and medical needs in that male age group. Young male patients indeed more often receive their transfusions for trauma indications[29], in which the induced raise in Hb (or lack of it, e.g. by female donor units) could be more critical. Additionally, trauma is known to lead to a rapid and, at times, sustained depression in cellular and humoral immunity (*Figure 2*).[30] It is important to point out the exclusion of a proportion of the trauma patients from the study by design, because these

patients often receive multiple transfusions, leading to limited generalizability to this subgroup. Still, these patients are present in the study population, albeit in lower numbers

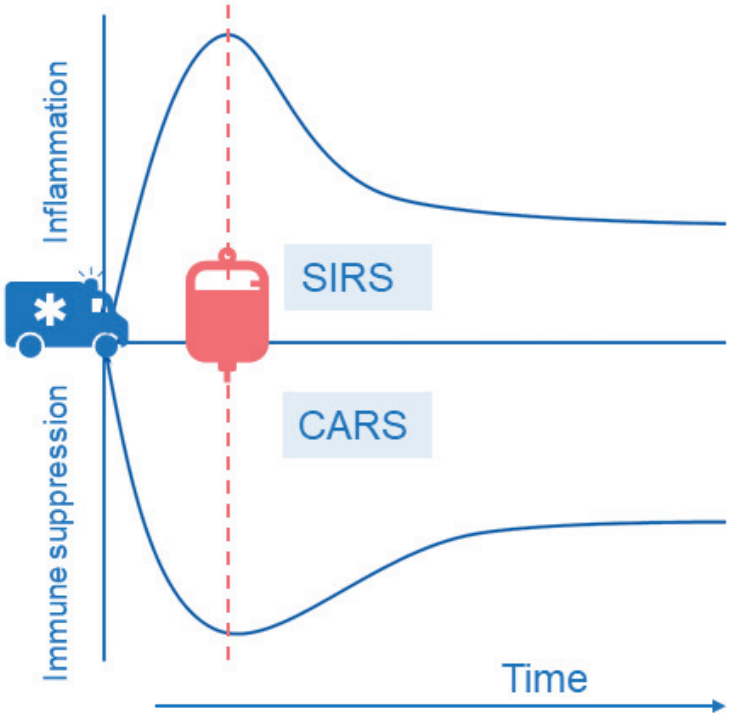

Figure 2

The immune response to trauma forms a complex interplay of both pro-inflammatory compounds and mechanisms (i.e. cytokines, fever, capillary leak, and early organ dysfunction) and anti-inflammatory responses (i.e. anti-inflammatory mediators, suppressed cytokine production, antigen presentation capacity, and cellular immunity) known as the systemic inflammatory response syndrome (SIRS) and the compensatory anti-inflammatory response syndrome (CARS). Transfusions are given at a time when these two responses are active, and the delayed return to homeostasis over time is hypothesized to be a facilitator of microchimerism development. (adapted from Shah et al.[31])

Acting in tandem with the latter, is that HLA alloimmunization of women is more common after pregnancy, and this immunization also having been shown to be a strong predictor of Graft-vs-Host disease after stem cell transplantation.[32] While it would be interesting to study the association between exposure to multiparity of the donor, and patient mortality, this is complicated by the small sample size of patients exposed only to women with multiple children, and a dose-response relationship will be difficult to establish. Nevertheless, there is still a need to further clarify 1. whether male patients of a certain age group are really at risk, 2. if the underlying transfusion indication further drives the epidemiological associations, and 3. what the causative agent(s) in the transfusion product might

be, inducing that risk. Moreover, these speculations should include storage time if the interest lies in studying its combined effects, with two different potential risk modulating mechanisms: leukocytes are less present in older products with hence less expected immunomodulating effects, and older products induce a lower Hb increase and hence less efficiency. Because of the many expected opposing risk modulations of blood product characteristics, the combined effect of these mechanisms is hard to predict. Data on cause-specific mortality have an important role here and should have a high priority, as these could give valuable insight into the potential biological mechanisms underlying the observed epidemiological associations.

Methodological implications for the field of transfusion medicine

Following up on the previous chapter, in Chapter 6 the importance of a thorough understanding of clinical epidemiology in the clinical transfusion research field is further emphasized. We present a structured approach to investigate any exposure that is associated with both the subsequent probability of receiving additional transfusions, and the outcome. This approach can be widely adopted by researchers studying transfusion exposures, which are generally sustained over time (e.g. trauma patients, receiving multiple transfusions in a short time frame, or chronically transfused cancer patients receiving multiple transfusions divided over a long period). Before this can be attempted, however, we recommend the use of directed acyclic graphs (DAGs) to visualize the researcher's assumptions about the data before embarking on the analytical task.

DAGs are a visual tool for causal inference, and can be used in conjunction with the target trial framework, aiding variable selection in statistical models and thereby avoiding self-inflicted bias by the researcher.[33] By definition, DAGs are acyclic, meaning variables cannot have effects on their own parent variables occurring before them in time. While this has led to the misconception that DAGs cannot display bidirectional effects, bidirectional effects can, in fact, be incorporated into DAGs by specifying how causal relationships evolve over time.[34] Incorporating bidirectional effects can improve the validity of statistical models and provide a more accurate representation of complex biological relationships. Not properly doing so can lead to mistakes that make a causal interpretation of estimates from studies inappropriate. Therefore, clinical transfusion researchers are encouraged to embrace incorporating bidirectional effects into DAGs, as we demonstrated in this thesis.

When to stop? An epidemiological perspective on innovation vs. replication

In the academic world, there is a lack of decisiveness regarding the need for pursuing the same research topic, or on the other hand, replicating and validating previous findings. This is reflected in the overrepresentation of new prediction models and the difficulty in publishing validation studies.[35] The sentiment can be summarized in the following quote, which was studied in psychological science but can be extrapolated to science in general: 'Innovation points out paths that are possible; replication points out paths that are likely; progress relies on both.'[36] Therefore, it is crucial for clinical transfusion researchers to strike a balance between innovation and replication to ensure that progress is made towards improving transfusion practices and patient outcomes. Both can coexist even within the same paper, as we demonstrated, but the decision to study either one of them or both should be made after careful consideration.

The goal of clinical transfusion research is to further the knowledge and understanding about transfusion products and their effects, and thereby help improve health. It is sometimes not clear when this goal is reached, and what a new study would add to the available body of evidence on an intervention. There are always additional research questions, study limitations and new research ideas, often indicated by the phrase 'more research is needed' at the end of a manuscript. Rather than paving the road for promising new research questions, this phrase has become an empty one without meaning. There comes a time when the quality of evidence is sufficient to change direction; we think this time has arrived for the research on donor pregnancy and patient mortality. The next section on future perspectives therefore lacks further validation studies of this research question, but instead focuses on novel study designs investigating personalized transfusion strategies over population effects.

Future perspectives

Future perspectives for causal inference in clinical transfusion research should focus on defining research questions with high clinical relevance and investigating important knowledge gaps. In this regard, we conclude that:

- The association between donor parity and subgroups of causes of death in young men is of interest, and the causal mechanisms could be investigated in a matched cohort study. This, however, would require the collaboration of blood banks and hospitals to be successful, and the formation of a consortium with representatives from hematology, blood banks and epidemiology. The size of such a study should involve a large national or international collaboration of research groups. Probing the interest and building the foundation for such a study would be an excellent first step.

- The incorporation into clinical studies, of assays quantifying potential pregnancy induced micro-chimerism and leukocyte transfer from female donors to patients, is required for complete understanding of the biological mechanisms underlying the observed epidemiological associations.
- The role of storage of blood products and its association with patient outcomes – as it is still incompletely understood – should be studied using g-methods (for which we refer to Chapter 6), or alternatively with well-designed randomized controlled trials investigating different storage thresholds.
- The effect of convalescent plasma for the treatment and prevention of respiratory viral infections in immunosuppressed people[10] or for prophylaxis[37] is still not clear and should be investigated to aid pandemic preparedness.

We expect the use of thorough epidemiological methods can help elucidate these research questions and are confident to have contributed to their future development with the research described in this thesis.

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