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Hemovigilance, heterogeneity, and hyperfibrinolysis : evaluating the Netherlands' switch to solvent/detergent plasma

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Citation
Saadah, N. H. (2018, September 11). *Hemovigilance, heterogeneity, and hyperfibrinolysis : evaluating the Netherlands' switch to solvent/detergent plasma*. Retrieved from https://hdl.handle.net/1887/65502

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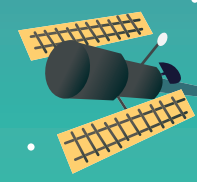
Author: Saadah, N.H.
Title: Hemovigilance, heterogeneity, and hyperfibrinolysis : evaluating the Netherlands' switch to solvent/detergent plasma
Issue Date: 2018-09-11

Hemovigilance, heterogeneity, and hyperfibrinolysis

Evaluating the Netherlands' switch to solvent/detergent plasma

Nicholas H. Saadah



Hemovigilance, heterogeneity, and hyperfibrinolysis - N.H. Saadah

Hemovigilance, heterogeneity, and hyperfibrinolysis – evaluating the Netherlands' switch to solvent/detergent plasma

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof.mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op
dinsdag 11 september 2018, klokke 11:15 uur

door
Nicholas H. Saadah

geboren te Oklahoma City, USA

PROMOTOR

Prof. dr. J.G. van der Bom (Leiden University Medical Center)

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Cover photo inset: Hubble Deep Field 1995 image
source: NASA public archive

In December of 1995, the Hubble Space Telescope held its orientation fixed for ten days and focused on an unremarkable, seemingly empty point of space – smaller than the period at the bottom of this page with this book held at arm's length. The resulting long exposure, the famous Hubble Deep Field image, is the cover image inset. Each of the thousands of light clusters in the picture is a galaxy with hundreds of millions of stars, most of which have orbiting planets.

Earning a PhD in clinical epidemiology and becoming good at one specific part of it has left me feeling smaller in the grand scheme and in awe of how much more there is to learn.
That in mind, consider that
the scene on the front
cover surrounds us
24 million
times
over
...
.

Author's notes
Statistical notation and language (American vs. British English) differ between chapters, corresponding to the linguistic and formating styles of their journals of publication. Supplemental material referenced in unpublished chapters is available upon request from the author (saadah@alumni.stanford.edu).

Printing costs generously subsidized by Sanquin and LUMC

This research is the result of a working partnership between Sanquin, the nation's blood supplier, and TRIP (Transfusie- en transplantatieReacties In Patiënten), the national bio- and hemovigilance office of the Netherlands. It was made possible by a grant from Sanquin, funding four years of independent PhD research at Sanquin's Center for Clinical Transfusion Research (CCTR) in partnership with the department of Clinical Epidemiology at the Leiden University Medical Center (LUMC). Advisorship was provided by Prof. J.G. (Anske) van der Bom (head, CCTR; professor, LUMC) and Dr. Martin Schipperus (hematologist, Haga teaching hospital; chairman, TRIP).

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Acknowledgments

“On January 1, 2014, Sanquin, the national blood bank of the Netherlands, replaced fresh frozen plasma with Omniplasma, a solvent/detergent plasma...”

Introduction

1

General introduction

Plasma transfusion is indicated in a range of medical situations involving replenishment of coagulative proteins (e.g. massive bleeding, liver disease) or removal of an insulting entity via plasma exchange (e.g. thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome)[1]. Two commonly used types of plasma for human transfusion are fresh frozen plasma (FFP) and pathogen-inactivated pooled plasmas (e.g. solvent/detergent treated [SD] pooled plasmas like Octaplas and OctaplasLG)[2].

Though plasma transfusions are generally safe, they may result in adverse events or *transfusion reactions* (table 1)[3]. Various steps involving the unit of plasma may be taken to reduce the risk of these adverse events. Untreated fresh frozen plasma is typically used after the unit, the donor, or both are tested for a set of pathogens, with the donor sometimes being re-tested following a quarantine period to guard against false negatives owing to the window period of some viral and bacterial infections[1].

Alternatively, steps can be taken to reduce the pathogenicity of the plasma unit. This is the case for SD plasma, where a pathogen inactivation process involving a solvent, tri(n-butyl) phosphate (TNBP), and a detergent, octoxynol, is performed on a pool of hundreds of FFP units to break open lipid-coated viruses before the pool is separated into individual units for transfusion.

Alongside the viral inactivation of the SD process, pooling has the additional effect of diluting antigens and antibodies, normalizing plasma protein levels, and neutralizing of some pathogens by antibodies present in the pool[4]. However, the SD process sharply reduces plasma levels of the pro-coagulative protein α_2-antiplasmin[5,6], leading some reports to link SD plasma with an increased risk of hyperfibrinolysis in massively transfused patients[7–9]. As pooling theoretically increases the risk of prion transmission, the pool may further be passed through a prion affinity ligand gel designed to safeguard against this (as is the case with OctaplasLG)[10].

On January 1, 2014, Sanquin, the national blood bank of the Netherlands, replaced fresh frozen plasma with Omniplasma, an SD plasma made from plasma donations of non-enumerated Dutch donors, as its distributed plasma for human transfusion[11]. The expected results of a national switch to SD plasma were a reduction in the risk of TRALI and allergic reactions as well as of viral and prion transmission[12]. Despite the smaller volume of SD-plasma units (200mL vs. ~300mL), the number of transfused plasma units was expected to remain consistent in patients transfused in surgical settings (where plasma is often ordered per unit), rising only in patients treated by plasma exchange where a specific plasma volume is transfused based on an estimate of body volume.

Choice of outcomes – effectiveness and transfusion reaction risk
Excepting for the small portion of plasma units used in plasma exchange (<1% of the units transfused in the Netherlands; chapter 2 – FROSTED study), plasma is transfused to replace coagulative proteins, the intended effect being stoppage or prevention of bleeding by aiding in the formation of a fibrin clot. Corresponding unintended side effects of concern when transfusing plasma are the transfusion reactions noted in table 1. This in mind, we set out to compare these two plasma types with regard to effectiveness at stoppage of bleeding and risk of transfusion reactions.

Given the rare nature of most plasma transfusion reactions (e.g. the risk of TRALI for mixed-sex plasma is around 1 event per 100,000 units transfused; chapter 4 – meta analysis), randomized clinical trials are not a realistic option for comparing risk among plasma types. Rather, comparison of transfusion reaction risk among blood products is typically performed by comparing of number of transfusion reaction cases and units transfused between products in large observational cohort studies.

Table 1: Plasma transfusion reactions and definitions

Transfusion reaction	*ISBT* definition*
allergic reaction	mucocutaneous allergic symptoms only within 4 hours of transfusion
anaphylactic reaction	mucocutaneous allergic symptoms with airway compromise or severe hypotension
febrile non-hemolytic transfusion reaction (FNHTR)	fever (≥39ºC oral or equivalent and a change of ≥2ºC from pretransfusion value) and chills/rigors within 4 hours of transfusion
hypotensive reaction	drop in systolic blood pressure of ≥ 30 mm Hg and a systolic blood pressure ≤ 80 mm Hg within one hour of transfusion
transfusion associated circulatory overload (TACO)	any four of (1)acute respiratory distress (2)tachycardia (3)Increased blood pressure (4)acute or worsening pulmonary edema on frontal chest x-ray (5) evidence of positive fluid balance - within 6 hours of completion of transfusion.
transfusion related acute lung injury (TRALI)	acute onset of hypoxia absent left arterial hypertension with bilateral infiltrates on chest x-ray within 6 hours of transfusion

* International Society of Blood Transfusion

Conversely, evaluation of blood product effectiveness is itself an active area of research in which different outcome measures can be reasonably chosen as measures of effectiveness[13]. Given the goal of plasma transfusion is to stop bleeding, the ideal measure of plasma effectiveness would involve precise measurement of the plasma's effect on formation of a cross-linked

fibrin clot within the vasculature. While current technology does not allow for this to be measured intravascularly, thromboelastography analysis estimates this very effect using a drop of the patient's blood in a small plastic cup designed to mimic the endothelium[14].

On the clinical front, investigation of plasma effectiveness may be performed using red blood cell units (RBCs) concurrently transfused as a proxy for bleeding[15]. Were one plasma less effective than the other, we might expect to see an increase in the number of red blood cell units transfused alongside that plasma. Given red blood cell and plasma units are often transfused in fixed ratios in the surgical setting, we might expect to see this increase without a concurrent change in the plasma/RBC units ratio. Thus a comparison of plasma and concurrent red blood cell use allows for a clinical estimation of plasma effectiveness at a population level.

This in mind, we compared use and effectiveness of the plasma products using two studies. In our ***FROSTED study (chapter 2)***, we collected patient-level transfusion data from six Dutch hospitals and compared plasma and concurrent RBC use between FFP and SD plasma. In our laboratory ***TEG study (chapter 3)***, we used thromboelastography (TEG) to compare the fibrinolytic states of whole blood reconstituted with the two products. After a short discussion of methodological considerations, we present our ***meta-analysis (chapter 4)*** comparing transfusion reaction risks for various plasma products, performed by studying plasma use and outcomes in other countries, followed by our ***ISTARE study (chapter 5)***, analyzing unpublished international hemovigilance data. Methodological considerations culminated in a comparative ***methodological study (chapter 6)***.

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“..around 20% of the transfusion episodes involved transfusion of only plasma, without concurrent RBCs. This is not in line with current evidence-based indications for plasma transfusion...”

FROSTED study

2

Transition from fresh frozen plasma to solvent/detergent plasma in the Netherlands: comparing clinical use and transfusion reaction risks

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(*submitted for publication*)

SUMMARY

We compared blood product usage and transfusion reaction risks before and after the Netherlands' transition from fresh frozen plasma (FFP) to solvent/detergent (SD) plasma. Using diagnostic data from six Dutch hospitals, national blood bank data, and national hemovigilance data for 2011-2017, we compared mean number of RBC units transfused per episode and the mean plasma/RBC units ratio for patients receiving FFP vs. SD plasma for various patient groups and calculated odds ratios (ORs) comparing transfusion reaction risks. Analyzing 13,910 transfusion episodes, SD plasma was associated with fewer RBC units transfused per episode in gynecological ($\Delta\mu_{RBCs(gyn)}$ = -1.66 [95%CI: -2.72, -0.61]) and aneurysm ($\Delta\mu_{RBCs(an)}$ = -0.97 [-1.59, -0.35]) patients. Difference in mean plasma/RBC ratio (*f*) was not significant for any patient group. SD plasma was associated with fewer allergic (other) (OR = 0.19 [0.11, 0.33]; p<0.01) and allergic (anaphylactic) (OR = 0.37 [0.18, 0.77]; p<0.01) reactions than FFP. The switch from FFP to SD plasma did not lead to notable changes in clinical plasma use. Despite the smaller size of SD plasma units, the plasma/RBC units ratio remained consistent across all patient groups. SD plasma is associated with fewer allergic (other) and allergic (anaphylactic) reactions than FFP.

INTRODUCTION

Plasma transfusion is indicated in a range of medical situations involving replenishment of coagulative proteins to stop or prevent bleeding (e.g. surgery, liver disease), or for removal of an insulting entity via plasma exchange (e.g. thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome [TTP/HUS])[1,2]. On January 1, 2014, Sanquin Blood Bank, the national blood bank of the Netherlands, replaced quarantined fresh frozen plasma (FFP) with Omniplasma, a solvent/detergent treated pooled plasma (SD plasma). Omniplasma is made from plasma donation of non-remunerated Dutch donors and is functionally equivalent to OctaplasLG[3]. As FFP can be stored for up to two years, FFP distribution and use continued in a decreasing fashion during the period 2014-2015. As of 2016, excepting for a few patient groups for which FFP remains indicated, SD plasma is the only plasma type available for transfusion in the Netherlands[4].

The expected results of the switch to SD plasma were a reduction in the risk of TRALI and allergic reactions as well as (theoretically) in viral and prion transmission[5] as observed in other countries switching to SD plasma[6–14]. Plasma and red blood cell units are often transfused in fixed ratios in the surgical setting (e.g. two units of plasma for every three units of red blood cells), however SD plasma units are smaller than FFP units (200mL vs. ~300mL, respectively). Of interest was thus whether this ratio of blood product use changed with the switch from FFP to SD plasma. Since the purpose of plasma in the surgical setting is to stop active bleeding, the number of red blood cell units transfused alongside the plasma serves as a measure of effectiveness of plasma transfusion at the population level. We compared this for the two plasma types as well.

Analysis objectives

To compare plasma use and transfusion reaction risks for SD plasma and FFP in the Netherlands in the period before and after the national switch to SD plasma on January 1, 2014.

METHODS

Data Sources

With approval from the medical ethical committee of the Leiden University Medical Center (protocol number P13.251), we submitted our study plan to the Dutch national blood bank (Sanquin), six Dutch hospitals, and the Dutch National hemovigilance and biovigilance office (TRIP; Transfusie-

en transplantatieReacties In Patiënten). The study, funded by a grant from Sanquin (PPOC-13-RvB-04), was reviewed and approved by representatives at each hospital, three academic hospitals (Leiden University Medical Center; Maastricht University Medical Center; University Medical Center Utrecht) and three general hospitals (Maasstad hospital, Rotterdam; Isala hospital, Zwolle; OLVG location East, Amsterdam), which altogether account for roughly 20% of the plasma transfused per annum in the Netherlands. Data from these sources were used to examine change in blood product use (blood bank data and hospital data) and transfusion reaction risk (hemovigilance data) in the years before and after the national switch to SD plasma in 2014.

National blood bank data: For the analysis of blood product use at the national level, we chose units issued as our parameter as this represents the true national demand for plasma. From Sanquin Blood Bank we collected the number and type (i.e.: FFP or SD plasma) of plasma units distributed to each of the 94 Dutch hospitals during the period 2012-2017, along with hospital type (academic medical center or general hospital).

Hospital data: From each of the six participating hospitals, we collected the following data on all blood products transfused for transfusion episodes involving plasma transfusion during all or part of the period 2010-2016: coded patient ID; patient sex; patient year of birth; diagnostic code and associated treatment description described using codes as defined by the Dutch healthcare authority[15]; treating ward; transfusion start and end times; type and unique Unit Identification Number of the blood product transfused.

Hemovigilance data: From TRIP, the Dutch national hemovigilance and biovigilance office, we collected the number and type of transfusion reactions reported by all Dutch hospitals, along with the potentially associated blood products as provided by the reporting hospitals. Each transfusion reaction reported to TRIP is reported with an imputability of 'certain', 'probable', 'possible', 'unlikely', or 'certainly not', describing the certainty with which the transfusion reaction can be diagnosed [16]; here we analyzed data on reactions with imputability levels of 'certain', 'probable', and 'possible'.

Grouping of transfusions into transfusion episodes and patient subpopulations

Transfusions were grouped into *transfusion episodes,* with a *transfusion episode* defined as a series of consecutive transfusions for which the time interval between transfusions did not exceed 72 hours. In order to be able to perform the comparisons in relatively homogeneous patient groups, transfusion episodes were subdivided based on the ward specified by their

diagnostic code(s), the four analyzed wards being (1) cardiothoracic surgery + cardiology (CTsurg+cardio); (2) general surgery (gs); (3) gynecology (gyn); (4) all others (oth), with this last group including TTP/HUS patients. To create further homogenous groups, within each of the analyzed wards we selected transfusion episodes coded with the most commonly occurring diagnostic codes. Within the cardiothoracic surgery + cardiology group, we selected episodes involving patients undergoing cardio arterial bypass grafting (CABG), valve replacement (VR), or maze procedure. Within the general surgery group, we selected episodes involving patients with any type of aneurysm. Within the gynecological group, we selected obstetric episodes. We analyzed episodes involving plasma exchange for TTP/HUS patients separately.

As each transfusion was coded with multiple diagnostic codes, often from different wards, we assigned each transfusion episode to only one ward with a hierarchy of cardiothoracic surgery + cardiology > general surgery > gynecology > other. This ensured each episode was analyzed in one ward group, and potentially one diagnosis group. These four ward groups (cardiothoracic surgery + cardiology; general surgery; gynecology; other) and the three diagnosis groups (CABG+VR+maze; aneurysm; obstetric; TTP/HUS) were used throughout the analysis.

Blood product use analysis

National plasma use during study period

For visualization of blood product use at the national level, we plotted number of FFP and SD plasma units distributed by the Dutch blood bank (Sanquin) to (1) all hospitals, (2) academic hospitals, and (3) general hospitals, for the period 2011-2017.

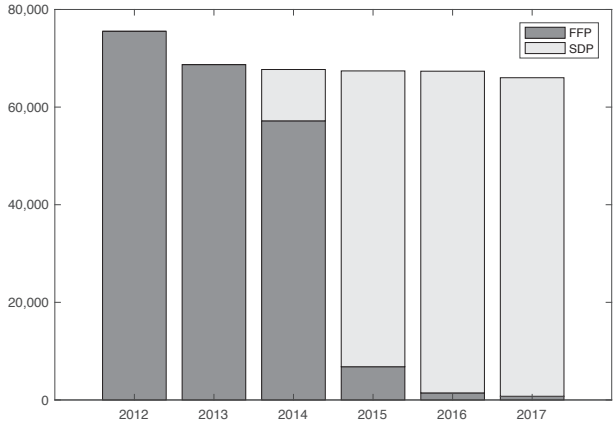
Patient-level blood product use

For each of the analyzed groups, we selected episodes involving transfusion of both plasma and RBCs and calculated mean plasma units per episode, mean RBC units per episode, and the mean ratio thereof (plasma/RBC units). For this analysis, we excluded the TTP/HUS group as plasma-exchange for TTP/HUS does not typically involve RBC transfusion and the plasma is not given to stop bleeding. We used bootstrapping with 10,000 iterations to model distributions for these three means for SD plasma and FFP and calculated mean differences and 95% confidence intervals using a two-tailed t-test on the bootstrap estimates assuming unequal variance.

Sensitivity analyses

As a first sensitivity analysis, we repeated the plasma/RBC ratio analysis on each group using only those patients receiving ≥5 red blood cells during the

Figure 1: Number and type of plasma units distributed to all Dutch hospitals between 2012 and 2017. The national switch from FFP to SD plasma occurred on January 1, 2014, but FFP units can be stored for up to two years prior to use, hence a gradual transition to SD plasma is observed.



transfusion episode to additionally compare use in patients experiencing heavy bleeding. As a second sensitivity analysis, to ensure the chosen hierarchy did not affect our results, we re-ran our analyses using two other hierarchies for group selection (general surgery > cardiothoracic surgery + cardiology > gynecology > other; gynecology > general surgery > cardiothoracic surgery + cardiology > other).

Comparison of transfusion reaction risk for FFP and SD plasma

We compared the probability of transfusion reactions between the two plasma types for the seven plasma transfusion reactions reported during the study period. Definitions used for these transfusion reactions[16] are a modified version of the International Society of Blood Transfusion (ISBT) Haemovigilance Working Party's *Proposed Standard Definitions for Surveillance of Non Infectious Adverse Transfusion Reactions*[17]. Risk ratios comparing SD plasma and FFP with regard to these transfusion reactions were calculated and tested against the null hypothesis of no difference using Fisher's exact test. The resulting risk ratios (RRs) compare the risks of experiencing the given transfusion reaction for SD plasma vs. FFP (i.e. RR<1 indicates fewer transfusion reactions are associated with SD plasma than FFP).

RESULTS

Comparison of blood product use

National plasma use during the study period

Figure 1 shows plasma use in the Netherlands for the period 2012 through 2017,

with the national switch to SD plasma occurring on January 1, 2014 (the date as of which Sanquin began distributing SD plasma to hospitals as the standard plasma product). As FFP can be stored for up to two years, stocks continued to be distributed and transfused in the Netherlands until the end of 2015. Total plasma use decreased by 13% in the course of the six-year study period. This trend was not reversed by the switch to the smaller SD plasma units.

Patient-level plasma use

Figure 2 shows our data flow. From the six participating hospitals, we collected data on 18,053 transfusion episodes involving plasma transfusion. Together, these episodes involved transfusion of 85,768 plasma units (65,160 FFP; 20,608 SD plasma), 91,318 red cell units, and 26,290 platelet units, and were coded by 891 unique diagnostic codes. The numbers of transfusion episodes per ward were: cardiothoracic surgery + cardiology (9,420) episodes; general surgery

Figure 2: Data flow diagram showing categorization of episodes into sub-cohorts for patients receiving plasma transfusion in (all or part of) 2010-2016

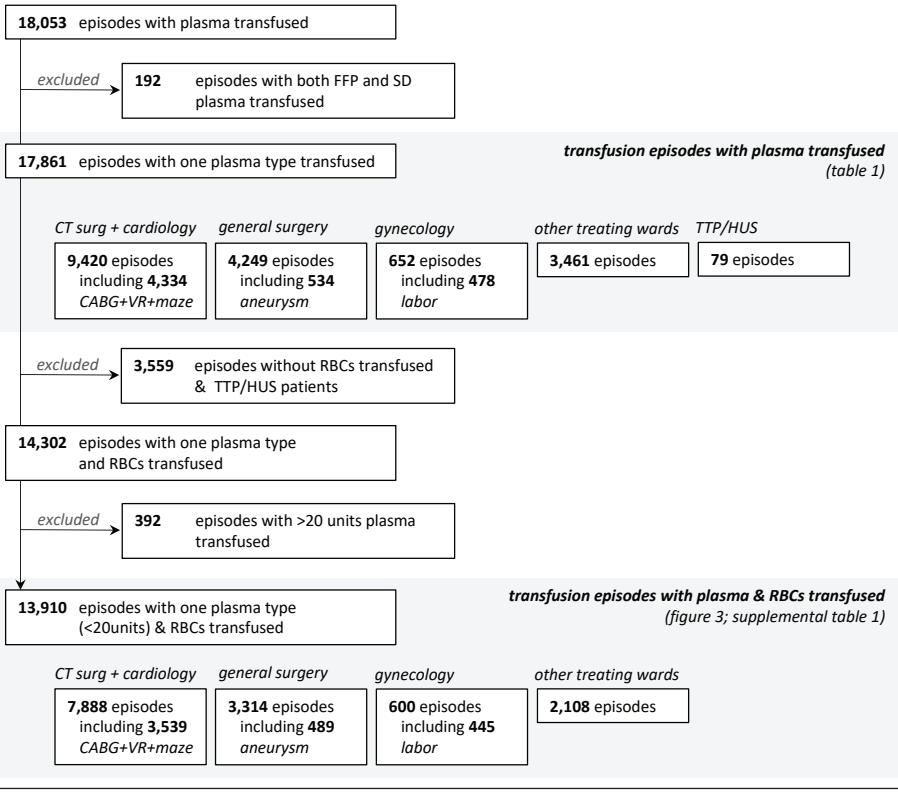


Table 1: Blood product details for the different cohorts (episodes involving plasma transfusion)

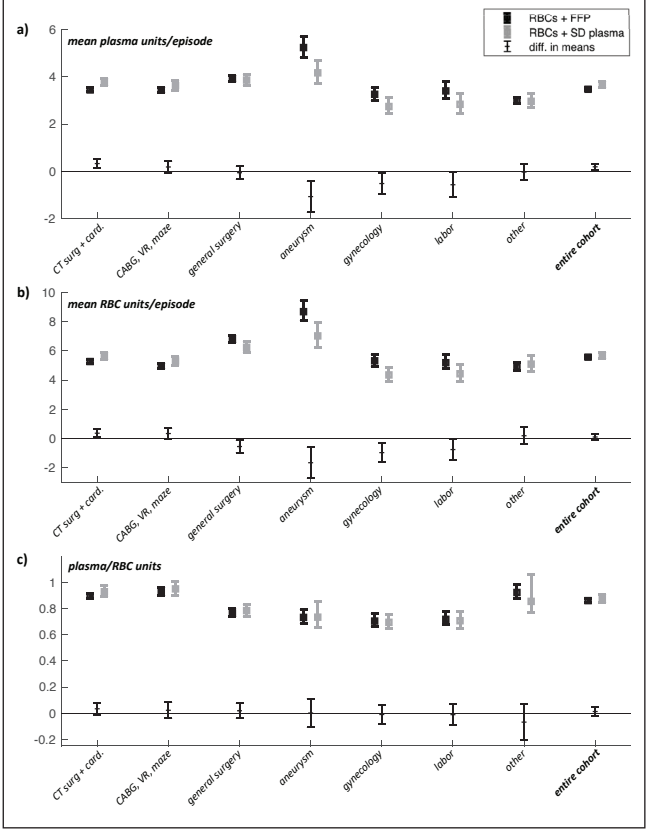
	CT surgery + cardiology		*general surgery*		*gynecology*		*other*	*TTP/HUS*	*entire cohort*
Episode characteristics:	***all***	***CABG,VR,maze***	***all***	***non-elective aneurysm***	***all***	***labor***			
transfusion episodes	9,420	4,334	4,249	534	652	478	3,540	79	17,861
median age in years (IQR)	62 (35-73)	68 (57-75)	58 (36-70)	74 (67-79)	34 (29-39)	33 (29-36)	46 (11-65)	53 (43-60)	58 (27-71)
proportion male	0.64	0.68	0.58	0.79	0	0	0.54	0.18	0.59
Blood products (units transfused):									
FFP									
total:	31,073	12,156	16,985	2,364	1,795	1,384	14,707	3,211	64,560
avg per ep (sd):	4.30 (7.98)	3.72 (5.19)	5.10 (9.72)	6.06 (6.18)	3.55 (5.14)	3.82 (5.81)	5.04 (13.95)	47.93 (69.28)	4.62 (9.79)
SD plasma									
total:	10,704	4,256	4,652	748	395	325	3,155	979	18,906
avg per ep (sd):	4.89 (5.16)	3.99 (3.17)	5.07 (4.98)	5.19 (3.94)	2.71 (1.46)	2.80 (1.59)	5.06 (6.36)	81.58 (37.58)	4.88 (5.30)
Red Blood Cells									
total:	50,066	20,229	25,356	4,719	3,094	2,261	10,815	57	89,331
avg per ep (sd):	5.31 (8.08)	4.67 (6.73)	5.97 (7.47)	8.84 (8.16)	4.75 (4.41)	4.73 (4.16)	3.06 (5.02)	0.72 (1.26)	5.00 (7.38)
Platelets									
total:	15,489	6,395	5,135	653	549	290	4,521	3	25,694
avg per ep (sd):	1.64 (4.41)	1.48 (2.13)	1.21 (4.33)	1.22 (1.81)	0.84 (3.56)	0.61 (1.17)	1.28 (6.50)	0.04 (0.19)	1.44 (4.86)

(4,249); gynecology (652); other (3,540). Number of transfusion episodes per diagnosis were: CABG, valve replacement, or maze procedure (4,334); aneurysm (534); labor (478); TTP/HUS (79) (table 1). The average number of plasma units transfused was higher for SD plasma than for FFP (81 vs. 48 plasma units/episode) in the TTP/HUS cohort where a prescribed plasma volume is exchanged.

During analysis, we found outliers greatly skewed the results of our plasma/RBC ratio calculations as some plasma exchange patients with diagnoses other than TTP/HUS (who were thus not excluded from analysis) received a unit of red blood cells within 72 hours of a plasma exchange episode. This then gave them into an 'active bleeding' status in our analysis of plasma/RBC ratio and units of RBCs transfused in conjunction with plasma. As plasma exchange patients, their plasma/RBC ratio was enormous as the amount of plasma they received was aimed at exchange, rather than stoppage of active bleeding. To eliminate these outliers for both SD plasma and FFP, we excluded episodes involving more than 20 units of plasma from our use analysis (392 out of 14,302, or about 3% of the episodes – figure 2).

Figure 3 shows the results of our comparison of SD plasma and FFP with regard to (a) mean plasma units transfused, (b) mean RBC units transfused and (c) mean plasma/RBC ratio for episodes involving transfusion of both RBCs and plasma (13,910 episodes). For all three outcomes (mean plasma units/episode; mean RBCs/episode; mean plasma/RBC ratio), a positive difference in means indicates a higher mean value for SD plasma. Changes in mean plasma and RBC units transfused per episode with the switch were negative for some groups, indicating a decrease with the switch to SD plasma (aneurysm: $\Delta\mu_{Pl(an)} =$

Figure 3: (a) mean plasma units, (b) mean RBC units, and (c) mean plasma/RBC units ratio for FFP (thick black) and SD plasma (thick grey) along with mean differences (thin black) for all three values. Note that mean differences are calculated as meanSD - meanFFP such that a positive value indicates a higher value for SD plasma, and vice versa



-1.06 [95% confidence interval: -1.71, -0.41], $\Delta\mu_{RBCs(an)}$ = -1.66 [-2.72, -0.61]; gynecology: $\Delta\mu_{Pl(gyn)}$ = -0.52 [-0.95, -0.08], $\Delta\mu_{RBCs(gyn)}$ = -0.97 [-1.59, -0.35]) and higher for others, indicating an increase (cardiothoracic surgery + cardiology: $\Delta\mu_{Pl(cts)}$ = 0.33 [0.15, 0.51], $\Delta\mu_{RBCs(cts)}$ = 0.36 [0.08, 0.64]). For the group as a whole, the mean number of plasma units transfused per episode decreased slightly with the switch to SD plasma ($\Delta\mu_{Pl(cohort)}$ = 0.19 [0.06, 0.32]).

The mean plasma/RBC ratio (f) for the group as a whole (13,910 episodes) involving transfusion of both plasma and RBCs was 0.86 [0.84, 0.88] for FFP and 0.87 [0.85, 0.91] for SD plasma. The difference in means ($f_{SD} - f_{FFP}$) was 0.01 [-0.02, 0.05]; p=0.48 indicating no significant change in the number of plasma units transfused per unit of red blood cells when SD plasma is transfused. For all wards (cardiothoracic surgery + cardiology, general surgery, gynecology) and diagnoses (CABG+valve replacement+maze procedure, aneurysm, labor), $f_{SD} - f_{FFP}$ remained consistently close to zero with none of the differences being statistically significant at the α=0.05 level.

Sensitivity analyses

Supplemental figure 1 shows the results of our first sensitivity analysis, the plasma/RBC (*f*) ratio comparison for those episodes involving transfusion of plasma and five or more RBC units. The ratios for both plasma types were lower in this group of massive transfusion patients as compared to the patient cohort as a whole: f_{FFP} = 0.56 [0.55, 0.57]; f_{SD} = 0.57 [0.55, 0.59]; $f_{SD} - f_{FFP}$ 0.02 [-0.01, 0.04]; p=0.19. Here too none of the ward or diagnostic based sub-cohorts returned a statistically significant result for the difference in means $f_{SD} - f_{FFP}$. In supplemental table 1 the numeric results of our entire blood product use analysis are shown.

In our second sensitivity analysis, involving use of varying hierarchies for cohort selection, we found changing the hierarchy yielded nearly identical results as only a few transfusion episodes were coded with diagnostic codes from two different treatment wards (data not shown).

Comparison of plasma transfusion reaction risk

During the period 2012-2016, Sanquin distributed 209,681 units of FFP and 137,028 units of SD plasma. During the same period, the national hemovigilance office received reports of 46 allergic (anaphylactic) reactions, 128 allergic (other) reactions, 10 mild non-hemolytic febrile reactions (mild NHFR), 9 Non-Hemolytic Transfusion Reactions (NHTRs), 4 cases of Transfusion Associated Circulatory Overload (TACO), 2 cases of Transfusion Related Acute Lung Injury (TRALI), and 29 'other' plasma transfusion reactions in association with transfusion of one or more plasma units. Table 2 shows risk ratios comparing SD plasma and FFP for the seven plasma transfusion reaction types reported during the study period with an imputability of 'certain', 'probable', or 'possible'. SD plasma was associated with fewer allergic (other) reactions (RR=0.19 [0.11, 0.34]; p<0.01) and allergic (anaphylactic) reactions

Table 2: Comparison of number of transfusion reactions and transfusion reaction risk for FFP and SD plasma using national hemovigilance data

Transfusion reaction	FFP (209,681 units)	SD plasma (137,028 units)	Risk Ratio (95% CI) $risk_{SD}/risk_{Q\text{-}FFP}$	sig.
Allergic (other) rxns.	114	14	0.19 [0.11 to 0.33]	p<0.01
Allergic (anaphylactic) rxns.	37	9	0.37 [0.18 to 0.77]	p<0.01
NHTR	9	1	0.17 [0.02 to 1.34]	p=0.10
FNHTR	8	1	0.19 [0.02 to 1.53]	p=0.10
TACO	2	2	1.53 [0.22 to 10.86]	p=0.65
TRALI	1	1	1.53 [0.10 to 24.46]	p=0.71
Other	24	5	0.32 [0.12 to 0.84]	p=0.01

(RR=0.38 [0.18, 0.79]; p<0.01), as well as fewer 'other' plasma transfusion reactions (RR=0.33 [0.13, 0.86]; p=0.02) than FFP.

DISCUSSION

Using nationwide blood bank and hemovigilance data, we compared nationwide plasma use and transfusion reaction risks for FFP and SD plasma in the years surrounding the Dutch switch to SD plasma from 2014. Using patient data from six Dutch hospitals, we compared plasma use for transfusion episodes involving the two plasma types for the same period. The mean number of plasma and RBC units transfused per episode decreased significantly in the aneurysm and gynecological groups, and the decrease in overall plasma use continued despite the switch to the smaller SD plasma units. The plasma/RBC ratio remained constant across all patient cohorts. The risk of most plasma transfusion reactions decreased.

In the Netherlands a 200mL unit of SD plasma is smaller than a unit of FFP which typically contains between 300 and 330mL of plasma[3], meaning transfusing equal volumes of the two plasma products requires transfusing more units of SD plasma. At the national level, we observed no such increase in units issued, with the switch to SD plasma not interrupting the downward trend in plasma use over the period. At the transfusion episode level, we observed only a small increase in mean plasma units transfused per episode for the cohort as a whole($\Delta\mu_{Pl(cohort)}$ = 0.19 plasma units [0.06, 0.32]). Rather than a large increase of SD plasma being transfused – an increase of units by $^1/_3$ could have been expected - this small increase is likely due to plasma exchange patients who, being exchanged with a specific volume, were transfused with more units of SD plasma (table 1). The changes in the plasma use for the ward-based patient groups and diagnosis-based sub-cohorts varied and were likewise often statistically significant, but did not show the trend we would expect to see were the number of plasma units transfused systematically different for FFP vs. SD plasma. Given that the change in plasma/RBC ratio (*f*) was not significant for any of the cohorts, we interpret these results as showing continued transfusion of SD plasma in the same proportion to RBC units as FFP plasma. We find no previous studies comparing the plasma/RBC ratio for FFP and SD plasma.

In broad terms, plasma is transfused to replenish plasma proteins during active bleeding (e.g. during surgery) or to remove a harmful entity via plasma exchange (e.g. in TTP/HUS patients). By creating cohorts of transfusion episodes involving transfusion of both RBCs and plasma, we aimed to capture episodes where plasma was used in cases of active bleeding. The further

stratification of these episodes by ward and diagnosis was intended to create progressively more homogeneous cohorts for comparison. Given the practice of transfusing plasma and red blood cells in a fixed ratio, if one plasma type more effectively stopped active bleeding than the other, we might expect a change in the mean number of RBCs transfused per episode with the switch to SD plasma[18]. We observed such a change in the general surgery and gynecological groups, where the number of RBCs transfused alongside plasma was around half a unit (general surgery) and one unit (gynecology) lower for SD plasma than for FFP. Confounding our results, however, is the trend of decreased red blood cell transfusion within the Netherlands[19]. Within our analysis, the mean number of concurrently transfused RBCs was generally similar to or lower for SD plasma, transfused after 2014, than for FFP, transfused before 2016 (figure 3). While we cannot separate the effects of plasma efficiency from those of this trend, our data suggest no differences in effectiveness of stoppage of bleeding between the two plasma types.

The results of our transfusion reaction risk analysis, showing a lower incidence of allergic (other) and allergic (anaphylactic) reactions for SD plasma as compared to FFP, are in line with those of several other studies in which SD plasma was consistently found to lead to fewer transfusion reactions in general[6–14]. Of note, however, is the TRALI case associated with transfusion of SD plasma in 2016. The imputability of this TRALI case was listed as 'possible' and the patient, a pediatric stem-cell transplant recipient, had multiple other risk factors for acute lung injury (ALI), and had received transfusions of RBCs and platelets outside the six-hour interval after transfusion of the plasma unit in question[20]. Upon review of the case, the expert panel tasked with evaluating debatable cases could not rule out TACO.

Limitations

In our analysis of blood product use and plasma transfusion safety, around 20% (3,559 of 17,861 episodes – see figure 1) of the transfusion episodes involved transfusion of only plasma, without concurrent RBCs. This is not in line with current evidence-based indications for plasma transfusion which (if followed) would lead to plasma being transfused with RBCs except in cases of plasma exchange (plasma exchange episodes comprise less than 1% of the transfusion episodes analyzed in our study)[21]. After review of a sample of these patients' transfusion data we confirmed that data was not missing (i.e. that only plasma was transfused during these episodes). Previous studies have likewise pointed out a high rate of plasma transfusion outside the context of evidence-based indications[2,22,23]. As an example, in some of the reporting hospitals, plasma is transfused prophylactically prior to biopsy procedures.

Further, we matched patients only on ward or diagnosis without correcting for other predictors, as this was not the goal of our analysis. The conclusions are thus to be interpreted at a population level, and not at the level of the individual patient. Finally, given the rare nature of many of the transfusion reactions analyzed, even six years of data from a country performing only 60,000 plasma transfusions per year yields datasets too small for solid hemovigilance comparisons. Meta-analyses or large-scale observational trials are better equipped to address comparative safety of blood products with regard to rare adverse events.

Conclusions

Using national blood bank and hemovigilance data, as well as transfusion data from six large hospitals in the Netherlands, we compared FFP and SD plasma with regard to blood product use and transfusion reaction risk in the period surrounding the national switch from FFP to SD plasma in 2014. We found some small differences in average number of RBCs transfused alongside SD plasma vs. FFP, but no systemic changes in mean RBCs transfused or the mean plasma/RBC ratio when comparing the two products. This suggests the two plasmas were transfused in the same ratio to RBCs and that they do not differ significantly in their effectiveness at stopping bleeding. SD plasma is associated with fewer allergic (other) and allergic (anaphylactic) transfusion reactions.

ACKNOWLEDGEMENTS

The authors wish to thank Yavanna van Oostveen and the data management team at Sanquin's Center for Clinical Transfusion Research (CCTR) for their collection and cleaning of the patient data used within this study and the Scientific Committee of the CCTR for their analytical advice.

Co-author contributions to the manuscript are as follows. For contributions provided by multiple co- authors, names are listed alphabetically.

- J. van der Bom and M. Schipperus (PhD advisors), and N. Saadah (PhD student) conceived the study
- N. Saadah performed the analysis and wrote the manuscript under guidance from J. van der Bom and M. Schipperus
- J. van der Bom provided clinical and epidemiological review of the manuscript
- J. Wiersum-Osselton and M. Schipperus provided clinical and hemovigilance review of the manuscript
- M. van Kraaij provided clinical and blood product usage review of the manuscript

- C. Caram-Deelder confirmed all results via independent parallel analysis and provided statistical review of the manuscript
- E. Beckers, A. Leyte, J. Rondeel, K. de Vooght, F. Weerkamp, and J.J. Zwaginga provided data from the six participating hospitals and clinical review of the manuscript

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“Our experiment showed a roughly linear relationship between S/D plasma fraction and decrease in clot lysis time as the plasma compartment was diluted with S/D plasma...”

TEG study

3

Effect of solvent/detergent treated pooled plasma on fibrinolysis in reconstituted whole blood

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(2017) Transfusion, 57(10), pgs. 2381–2389

ABSTRACT

Background
Hyperfibrinolysis has been observed in patients heavily transfused with Solvent/Detergent-treated pooled Plasma (SD-plasma). We set out to compare coagulation and fibrinolytic parameters in blood containing SD-plasma with blood containing fresh frozen plasma, with and without α_2-antiplasmin or tranexamic acid (TXA) supplementation.

Methods
Whole blood samples were reconstituted from red cell concentrates, platelet concentrates, and varying mixtures of Fresh Frozen Plasma (FFP) and SD-plasma. Hematocrit and platelet count of reconstituted blood samples were varied. For a subset of runs, α_2-antiplasmin or TXA were added to SD-plasma whole blood samples. Thromboelastography (TEG) analysis was performed to assess 50% clot lysis time ($CLT_{50\%}$), maximum amplitude (MA), and initial clotting time (R-time).

Results
The change in $CLT_{50\%}$ of whole blood as the plasma compartment transitions from FFP to SD-plasma was -52%[95%CI:-60% to -45%], p<0.001. Platelet count strengthened the effect, leading to an additional change in $CLT_{50\%}$ of -8% [-14% to -2%], p=0.012 as platelet count increased from 10 to 150 x 10^9/L. MA and R-time were not associated with fraction of SD-plasma in whole blood plasma compartment. α_2-antiplasmin and TXA restored clot lysis time in SD-plasma whole blood.

Conclusion
Whole blood with SD-plasma has shorter clot lysis times *in vitro* as compared to whole blood with FFP. Clot strength and initial clotting time appear not to be affected by SD-plasma fraction. α_2-antiplasmin and TXA supplementation can restore clot lysis time of SD-plasma whole blood to that of FFP whole blood. Clinicians should be aware of the decreased clot lysis time associated with SD plasma transfusion.

INTRODUCTION

Solvent detergent (SD) treatment for pooled plasma donations to inactivate pathogens[1] may alter the fibrinolytic potential of blood following plasma transfusion.[2,3] SD treatment has been shown to decrease plasma levels of both α_2-antiplasmin, an inhibitor of fibrinolysis[4], and protein-S, a coagulation inhibitor[5], altering the dynamic hemostatic balance between pro- and anticoagulation processes and pro- and anti-fibrinolytic systems. It is theorized that the decrease in α_2-antiplasmin concentration may lead to the runaway fibrinolysis sometimes observed following massive transfusion with SD-plasma.[6]

Given the heterogeneity of the patient population requiring massive plasma transfusion as well the infrequent, though often deadly, occurrence of hyperfibrinolysis[3] following massive plasma transfusion, the association between SD-plasma and hyperfibrinolysis cannot easily be explored by clinical study. *In-vitro* studies exploring this effect have been carried out before, though to our knowledge never in whole blood. Both thromboelastography (TEG) and ROTEM analysis on pure fresh frozen plasma (FFP) and pure SD-plasma in the presence of phospholipids to initiate clotting and tPA to initiate fibrinolysis showed clot lysis time of SD-plasma to be significantly shorter than that of FFP.[7,8] However, given the role of platelets and red blood cells on hemostasis, further study in whole blood is warranted.

While the role of platelets in thrombin generation and clot formation is well documented[9], there is growing evidence that platelets too play a role in fibrinolysis. Platelets have been demonstrated to not only inhibit fibrinolysis by the release of plasminogen activator inhibitor 1 (PAI-1)[10,11], but to further augment clot lysis by supporting the conversion of plasminogen into plasmin.[12,13] Red blood cells, by aggregating to the center of vascular flow, push platelets to the periphery, thus increasing the subendothelium-platelet interaction.[14-16] They may actively participate in thrombin generation[17,18] and, under hemolytic conditions, red blood cells may further promote clot lysis.[19] By measuring the effect of SD-plasma on fibrinolysis in whole blood, we account for the effects of red blood cells and platelets on this association. Additionally, an *in-vitro* study allows varying of hematocrit (Hct) and platelet (PLT) count to simulate patients of various clinical (hemostatic) states, thus quantifying the effect of red blood cells and platelets on the association between SD-plasma and fibrinolysis. Finally, an *in-vitro* study allows reintroduction of α_2-antiplasmin to SD-plasma.

We performed a TEG-based analysis on whole blood samples with varying amounts of SD-plasma in their plasma compartment. Our three research goals were to determine (1) the association of increasing fractions of SD-plasma vs. FFP with fibrinolysis in whole blood, (2) whether this association depends on hematocrit and/or platelet count, and (3) whether supplementation of SD-

plasma with anti-fibrinolytics such as α_2-antiplasmin or tranexamic acid (TXA) can correct this effect.

METHODS

Study design
We investigated the association of SD-plasma fraction and fibrinolysis and the effect of hematocrit and platelet count on that association using thromboelastography (TEG) (TEG5000, Haemoscope Corp, Niles, IL, USA). Physiologically realistic whole blood samples were reconstituted by mixing red cell concentrates, platelet concentrates, and fresh frozen plasma (FFP) and/or SD-plasma (Omniplasma, Octapharma, Vienna, Austria) in various concentrations. Tissue factor (1 pM; Innovin®, Siemens Healthcare Diagnostics, Marburg, Germany) was used to initiate coagulation, and tissue Plasminogen Activator (tPA) (50-150 ng/mL; Boehringer Ingelheim GmbH, Ingelheim, Germany) to induce lysis. Two levels each of hematocrit (0.20 and 0.40) and platelet count (10 and 150 x 10^9/L) were used for the reconstituted samples, simulating patients with both normal and decreased hematocrit and platelet count. Four whole blood samples were prepared for each TEG run, differing only in SD-plasma fraction of the plasma compartment, which varied from 0% to 100% in four steps across each run's four samples. Thromboelastography analysis was run simultaneously on each four-sample set.

In an additional set of experiments, we investigated whether adding α_2-antiplasmin or TXA to SD-plasma corrects decreased clot lysis time in whole blood as compared to FFP. For each TEG run, one FFP whole blood sample and one (unaltered) SD-plasma whole blood sample were run alongside two SD-plasma samples supplemented with α_2-antiplasmin (ITK diagnostics, Uithoorn, the Netherlands; stock activity 9.04U/mg) or TXA (Sigma-Aldrich, Zwijndrecht, the Netherlands), typically given to patients to decrease fibrinolysis.[20] α_2-antiplasmin activity was measured with the Berichrom activity assay kit (Siemens).

Preparation of reconstituted blood samples
Whole blood samples were reconstituted using fresh red cell concentrates (<5 days after donation), platelet concentrates (3-5 days after donation), FFP and SD-plasma. Different red cell concentrates, platelet concentrates, and FFP units were used on each experimental day. All SD-plasma samples were derived from the same batch. Red blood cells and platelets were type A positive; plasma (both FFP and SD-plasma) was type AB.

FFP and SD-plasma were stored at <-20°C and thawed on the day of the experiment. Red cell concentrates were centrifuged at 2,100 x *g* for 10 minutes (Rotina 420R, Hettich, Geldermalsen, the Netherlands) to separate out SAGM storage medium which was pipetted away and discarded. The hematocrit of the

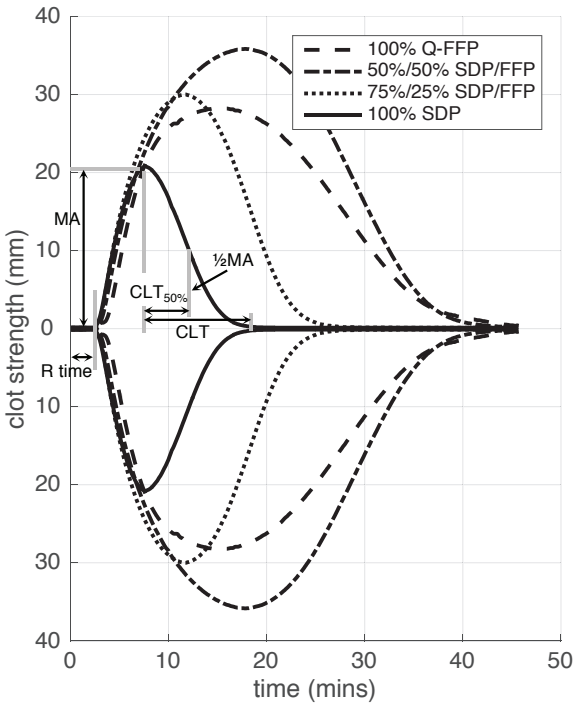
resulting erythrocyte preparation was measured using a cell counter (Sysmex XT2000i, TOA, Tokyo, Japan). Platelet concentrates were centrifuged at 940 x *g* for 10 minutes to separate out storage medium and were re-suspended in SD-plasma to make a platelet preparation with a target concentration of 4,000 x 10^9 platelets/L, with the actual concentration being measured using a cell counter. Four plasma mixtures were made by mixing FFP and/or SD-plasma, with SD-plasma fractions of 0 (pure FFP), 0.5 (50% SD-plasma, 50% FFP), 0.75 (75% SD-plasma, 25% FFP), and 1 (pure SD-plasma). Whole blood samples were prepared with a hematocrit of 0.20 or 0.40 by adding different amounts of red cell concentrate to the reconstituted blood samples; similarly, sample platelet counts were set to 10 and 150 x 10^9/L by adding different amounts of the platelet preparation.

Thromboelastography (TEG) analysis
Red cell preparations, all four plasma mixtures, and diluted tissue factor, tPA concentrates, α_2-antiplasmin, and TXA in a 1% v/v human serum albumin solution (Sanquin), were stored on ice during the course of the day while the platelet preparations were kept on a roll mixer at room temperature. One mL of each whole blood sample was reconstituted as follows: plasma from each of the plasma mixtures was added to test tubes 20 minutes before the TEG run was to begin; red cell and platelet preparations were slow-pipetted into the plasma mixtures and the mixtures left to warm to room temperature; tissue factor (1 pM) and tPA (50-150 ng/mL), α_2-antiplasmin and TXA were added just before the TEG run was to begin and the test tubes inverted five times gently to mix the samples. Per TEG protocol, 20 µL of 0.2M $CaCl_2$ (Haemoscope Corp, Niles, IL, USA), followed by 340 µL of the corresponding reconstituted whole blood sample was added to each channel. Samples were left to run in the TEG machine for 60 minutes.

Outcome measures
The TEG output is a plot of clot strength vs. time. Clot strength is defined as the torque-resistance on the pin turning within the liquid, blood in our case, in the sample cup, and is expressed as mm of TEG trace amplitude. Fibrinolysis was quantified using 50% clot lysis time ($CLT_{50\%}$), defined as time from maximum clot strength (maximum amplitude [MA] on the TEG plot) to 50% clot lysis; this served as our primary outcome measure. Secondary outcomes were standard TEG parameters MA and R-time. MA represents maximum clot strength, and is the maximum amplitude reached during the TEG run. R-time is the clot initiation time, representing elapsed time between experiment start and when the TEG trace amplitude first exceeds 2mm.[21] Figure 1 indicates how these values are defined.

To allow inter-experimental comparisons, the $CLT_{50\%}$ of samples was normalized by the $CLT_{50\%}$ of that run's FFP reconstituted whole blood sample (FFP was considered to be the physiologic control). The resulting normalized $CLT_{50\%}$ ($CLT_{50\%n}$) is thus the length of time needed for 50% of the formed clot

Figure 1: TEG traces from a representative run. TEG variable descriptions are detailed using the trace for the sample reconstituted with 100% S/D plasma. See text for explanation of measurements.



to lyse, relative to the time needed for the FFP reconstituted whole blood sample. MA and R-time were likewise normalized to MA_n and $R\text{-time}_n$.

Statistical analysis and definitions

Linear regression methods were applied to each of our three normalized outcome measures ($CLT_{50\%n}$, MA_n, $R\text{-time}_n$) with SD-plasma fraction of whole blood plasma compartment (0, 0.5, 0.75, or 1) as the independent variable and hematocrit (as fraction) and platelet count (in PLT x 10^9/L) as covariates. This quantified the associations between SD-plasma fraction and $CLT_{50\%n}$, MA_n, and $R\text{-time}_n$, as well as the effect of hematocrit and platelet count on these association. These associations were expressed as the regression derived slopes (β) of the $CLT_{50\%n}$, MA_n, or $R\text{-time}_n$ vs. SD-plasma fraction curve. Given both our normalized outcome measures (dependent variables) and SD-plasma fraction (independent variable) take values between 0 and 1, these slopes additionally correspond to the change in $CLT_{50\%n}$, MA_n, and $R\text{-time}_n$, respectively, due to the plasma compartment transitioning from FFP to SD-plasma. The influences of covariates hematocrit and platelet count on these associations were likewise estimated and presented.

Sensitivity analyses

The amount of tPA required to induce lysis varied between blood products used on different experimental days. Given that we varied tPA between experimental days, we ran sensitivity analyses on our primary outcome to test whether this experimental choice influenced our results. We ran two sensitivity analyses. (1)

We repeated our regression analysis on a set of runs whose samples differed only in tPA concentration (all had Hct=0.40, PLT count=150 x 10^9 PLT/L). Leaving hematocrit and platelet count out of the model (as they did not differ between samples) and instead adding tPA concentration as a variable, we used our regression model to both compare the results to those of our primary analysis and to quantify the effect (slope) of tPA concentration on $CLT_{50\%n}$. (2) We further ran our regression analysis on a subset of runs equivalent in tPA concentration (all had tPA=50ng/mL) with varying hematocrit and platelet counts and compared the results to those of the primary analysis. All calculations were carried out in MATLAB (R2015b, The MathWorks, Inc, Narick, Massachusetts, U.S.A.).

RESULTS

Effect of SD-plasma fraction, platelet count, and hematocrit on clot lysis time
We ran 16 TEG analysis runs with four samples each of reconstituted whole blood varying only in SD-plasma fraction. During three of the five experimental days, a tPA concentration of 50 ng/mL was sufficient to induce lysis while during two experimental days it was not. Following tPA calibration runs during these two experimental days, tPA levels leading to lysis within 1h of experiment start were chosen. The results of a sample tPA calibration run are shown in

Figure 2: Results of tPA calibration run. (a) TEG traces for samples reconstituted with S/D plasma only (Hct 0.40, PLTs 150 3 109/L) with various tPA concentrations (in ng/mL) indicated. (b) Plot of tPA concentration (ng/mL) versus time to MA for the same samples

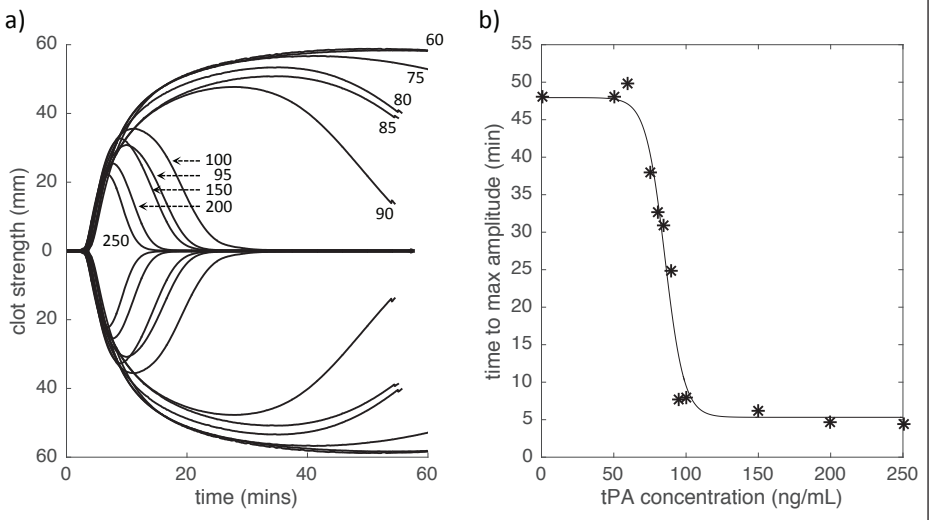


Table 1: Experimental run details and results used in regression analysis. Absolute time to $CLT_{50\%}$ and $CLT_{50\%}$ normalized by the 100% FFP sample's $CLT_{50\%}$ ($CLT_{50\%n}$, in parentheses) are provided for each of the four samples in each run.

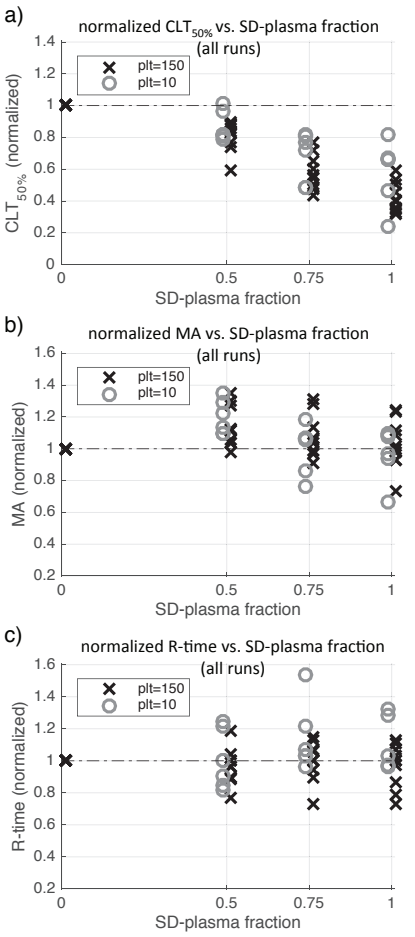
Sample characteristics						**Results:** $CLT_{50\%}$ in minutes (normalized $CLT_{50\%}$)			
						Plasma compartment (*% SD plasma/% FFP*):			
Run	Exp day	Hct	PLT count	tPA (ng/mL)	storage med(%)	*0% SD/100% FFP*	*50% SD/50% FFP*	*75% SD/25% FFP*	*100% SD/0% FFP*
1	1	0.4	150	50	16%	25.3 (1.00)	15.0 (0.59)	12.0 (0.48)	10.1 (0.40)
2	1	0.4	10	50	15%	12.7 (1.00)	10.3 (0.82)	6.2 (0.49)	3.0 (0.24)
3	1	0.2	150	50	12%	28.4 (1.00)	23.8 (0.84)	12.4 (0.44)	10.0 (0.35)
4	1	0.2	10	50	11%	15.0 (1.00)	12.2 (0.81)	7.3 (0.49)	7.0 (0.47)
5	2	0.4	150	50	18%	37.8 (1.00)	33.3 (0.88)	18.4 (0.49)	15.3 (0.40)
6	2	0.4	10	50	17%	16.1 (1.00)	12.7 (0.79)	12.4 (0.77)	13.2 (0.82)
7	2	0.2	150	50	13%	63.3 (1.00)	56.5 (0.89)	35.9 (0.57)	29.0 (0.46)
8	2	0.2	10	50	12%	21.8 (1.00)	17.8 (0.82)	15.7 (0.72)	14.3 (0.66)
9	3	0.4	150	50	17%	30.3 (1.00)	26.9 (0.89)	21.8 (0.72)	17.8 (0.59)
10	3	0.4	10	50	17%	12.3 (1.00)	11.8 (0.97)	9.9 (0.81)	8.3 (0.67)
11	3	0.2	150	50	11%	32.6 (1.00)	26.8 (0.82)	21.2 (0.65)	17.3 (0.53)
12	3	0.2	10	50	11%	18.3 (1.00)	18.5 (1.01)	15.0 (0.82)	12.2 (0.66)
13	4	0.4	150	100	12%	13.5 (1.00)	11.6 (0.86)	6.8 (0.51)	4.3 (0.32)
14	5	0.4	150	150	16%	9.8 (1.00)	8.6 (0.87)	7.6 (0.77)	4.9 (0.50)
15	5	0.4	150	150	15%	14.3 (1.00)	11.0 (0.77)	8.6 (0.60)	4.8 (0.33)
16	5	0.4	150	150	15%	14.8 (1.00)	10.8 (0.73)	8.0 (0.54)	5.3 (0.36)

figure 2, along with a plot of time to MA vs. tPA concentration, showing high hemostatic sensitivity of our whole blood samples to tPA concentration within a small range.

Table 1 shows experimental details for our TEG runs, along with absolute and normalized $CLT_{50\%}$, and the portion of each sample consisting of storage medium (SAGM [red cells], SD-plasma [platelets], 1% albumin + PBS [tissue factor and tPA]). Example TEG tracings (those of run 13) are shown in figure 1. We observed great inter-experimental variance in absolute $CLT_{50\%}$ between similar mixtures examined on different experimental days (see table 1). Concordance of traces occurs only when samples are made from the same erythrocyte and platelet preparations on the same experimental day (data not shown). Given that we use normalized data ($CLT_{50\%n}$, MA_n, R-$time_n$) as our outcome measures, our analysis is not affected by this inter-experimental variance.

Figure 3a shows the relationship between our main outcome, $CLT_{50\%n}$, and SD-plasma fraction for the 16 TEG runs detailed in table 1. $CLT_{50\%n}$ decreased with increasing SD-plasma fraction, indicating faster clot lysis when more SD-plasma was present in the sample. The change in $CLT_{50\%n}$ due to the plasma compartment transitioning from FFP to SD-plasma is statistically significant ($\Delta CLT_{50\%n}$ =-52%[95%CI:-60% to -45%], p<0.001). Platelet count is a statistically significant covariate, leading to a further change in $CLT_{50\%n}$ of -8%[-14% to -2%], p=0.012 as platelet count increases from 10 to 150 x 10^9/L. Hematocrit has no significant effect on $CLT_{50\%n}$.

Figure 3: (A) $CLT_{50\%}$ normalized by that of FFP whole blood versus S/D plasma fraction—all runs; (B) MA normalized by that of FFP whole blood (MA_n) versus S/D plasma fraction— all runs; (C) clot initiation time normalized by that of FFP whole blood ($R\text{-time}_n$) versus S/D plasma fraction—all runs. PLT count: (x) 150 x 10^9/L; (o) 10 x 10^9/L



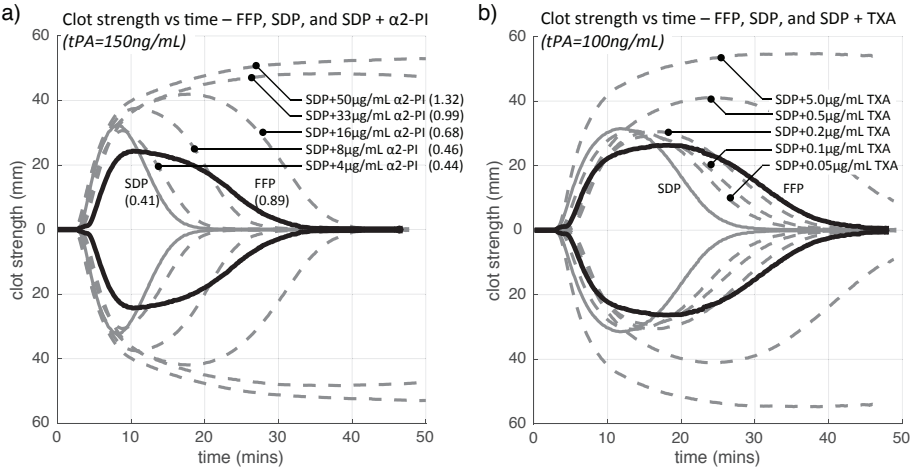
Effect of SD-plasma, platelet count, and hematocrit on maximum amplitude, R-time

Figures 3b and 3c show the relationship between the SD-plasma fraction and our secondary outcomes, MA_n and $R\text{-time}_n$, respectively. Our regression analysis showed SD-plasma, hematocrit, and platelet count all to have no statistically significant effect on MA_n as the plasma compartment transitioned from pure FFP to pure SD-plasma. However, as shown in figure 3b, there was a sinusoidal association between MA_n and SD-plasma fraction (i.e. MA_n was highest in the 50% SD-plasma samples). This pattern, whereby the 50% SD-plasma whole blood sample experiences a higher MA_n than the samples made with pure FFP or SD-plasma, was a regular finding during our experimentation (figure 1). Our regression likewise showed SD-plasma and hematocrit did not significantly affect $R\text{-time}_n$ (figure 3c), while platelet count's effect on $R\text{-time}_n$ was significant, leading to a mean change in $R\text{-time}_n$ of -13%[-22% to -4%], p=0.004 as platelet count increased from 10 to 150 x 10^9/L. Full experimental details for MA and R-time are found in supplemental table 1.

Correction of fibrinolytic state with α_2-antiplasmin or TXA

We performed seven additional TEG runs using α_2-antiplasmin (4 runs) or TXA (3 runs) supplemented SD-plasma (numeric data not presented). Figure 4a shows TEG traces for α_2-antiplasmin supplemented

Figure 4: Effect of α2-antiplasmin (a) and TXA (b) on S/D plasma clot lysis time. Experimentally measured α2-antiplasmin activity, in U/mL, is listed for S/D plasma (SDP) and FFP.



SD-plasma whole blood samples, along with control whole blood samples reconstituted with only FFP and only SD-plasma in the plasma fraction. As can be appreciated from the TEG traces, adding α_2-antiplasmin to SD-plasma whole blood resulted in a dose-dependent increase in MA with concomitant increase in CLT to values exceeding those observed in FFP whole blood.

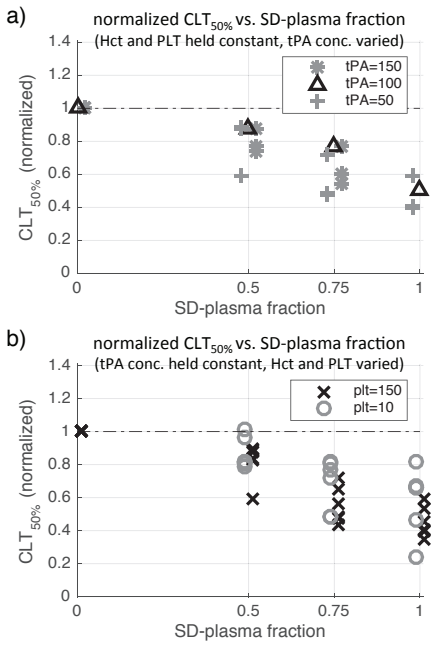
Figure 4b shows TEG traces for TXA supplemented SD-plasma whole blood samples, along with control samples reconstituted with only FFP and only SD-plasma in the plasma fraction. Adding TXA to SD-plasma whole blood likewise increased clot lysis time and MA dose dependently to values exceeding those in FFP whole blood, though at plasma concentrations far below those typically reached *in vivo* following administration of TXA at typical therapeutic doses[22].

Sensitivity analyses

Figure 5a shows the relationship between the SD-plasma fraction and $CLT_{50\%n}$ for a subset of runs differing only in tPA concentration (runs 1,3,5,7,9,11,13-16). As hematocrit and platelet count were constant for these runs (all runs in this subset had hematocrit=0.4, PLT=150 x 10^9/L), they were left out of the regression model. Here, the change in $CLT_{50\%n}$ due to the plasma compartment transitioning from FFP to SD-plasma is -59%[-69% to -48%], p<0.001, close to that of the main analysis, -52%[95%CI:-60% to -45%], p<0.001. The effect of tPA concentration on $CLT_{50\%n}$ was not statistically significant.

Figure 5b shows the relationship between the SD-plasma fraction and $CLT_{50\%n}$ for a subset of runs differing only in hematocrit and platelet count (runs 1-12).

Figure 5: Results of sensitivity analyses: (A) $CLT_{50\%n}$ versus S/D plasma fraction for various tPA concentrations; here Hct level 0.4, PLT count 150 x 10^9/L for all runs; effect of tPA is not significant. tPA: (*) 150 ng/mL; (Δ) 100 ng/mL; (+) 50 ng/mL.
(B) $CLT_{50\%n}$ versus S/D plasma fraction for all runs with tPA of 50 ng/mL and Hct level and PLT count varied; change in $CLT_{50\%}$ due to plasma compartment transitioning from FFP to S/D plasma (–0.50 [95%CI, -0.59 to -0.40]) matches very closely that of the main analysis performed on all runs (–0.52 [95%CI, -0.60 to -0.45]). (B) PLT count: (x) 150 x 10^9/L; (o) 10 x 10^9/L



As tPA concentration was constant for these runs at 50ng/mL, it was left out of the model. Here, the change in $CLT_{50\%n}$ due to the plasma compartment transition, -50%[-59% to -40%], p<0.001 was nearly identical to that of the main analysis, -52%[95%CI:-60% to -45%], p<0.001.

DISCUSSION

SD-treated plasma was associated with faster tPA-induced clot lysis in reconstituted whole blood as compared with FFP. The SD-plasma fraction of the whole blood plasma component correlated strongly with decreased time to 50% clot lysis. A higher platelet count strengthened the effect of SD-plasma on $CLT_{50\%}$, while hematocrit did not significantly influence this effect. Addition of either α_2-antiplasmin or TXA corrected clot lysis time of whole blood with SD-plasma to that of whole blood with FFP.

To our knowledge, this study represents the first *in vitro* test of SD-plasma's hemostatic influence in whole blood. Previous studies investigated the clot lysis time of SD-plasma vs. FFP in the absence of red cells and platelets.[7,8] Both studies showed SD-plasma to have significantly decreased clot lysis time as compared to FFP. Our study confirms the same effect in reconstituted whole blood samples, and additionally quantifies the effects of hematocrit and platelet count on this association. This effect has not been observed in plasmas pathogen inactivated via three other common methods – the Theraflex Methylene blue, Amotosalen Intercept, and Mirasol riboflavin based systems.[23]

In reconstituted whole blood, neither the hematocrit nor the platelet count significantly affected the normalized clot strength, as measured by MA_n, though their effect on absolute MA was clearly observed as higher hematocrit and platelet count led to a higher MA. The effects of plasma composition and hematocrit on normalized clotting time ($R\text{-}time_n$) were likewise not significant, while platelet count had a significant effect on normalized clotting time with $R\text{-}time_n$ decreasing as platelet count increased. Thus, whole blood with a higher platelet count experienced a greater relative drop in both clot lysis time and clot initiation time as the plasma compartment transitioned from FFP to SD-plasma.

α_2-antiplasmin supplemented SD-plasma whole blood TEG curves show longer clot lysis times than those of FFP whole blood when α_2-antiplasmin concentrations between the two plasma types are equalized. The SD process reduces not only the level of α_2-antiplasmin, an inhibitor of fibrinolysis, but also that of protein S, an inhibitor of thrombin generation and subsequent fibrin clot formation. In their comparison of FFP and SD-treated plasma, Pitkänen et al (2015) showed a decrease in free protein S from 0.93±0.10 in FFP to 0.82±0.01 IU/mL in SD-plasma, while the decrease in α_2-antiplasmin was much more pronounced, going from 0.96±0.12 in FFP to 0.43±0.04 IU/mL for SD-plasma.[7] In pure SD plasma, in the absence of cells, this results in a procoagulant and profibrinolytic phenotype.[7,8] In our study analyzing SD-plasma supplemented with platelets and red blood cells, we observed no such procoagulant phenotype as we found no association between SD-plasma fraction and R-time.

The role of protein S in coagulation however is complex and is not limited to the initiation phase of coagulation where it acts as a cofactor for tissue factor pathway inhibitor.[24,25] Protein S is also involved in reducing thrombin generation by activated protein C dependent and independent mechanisms in the propagation phase[26-28]. Clot structure and stability are dependent on the amount of thrombin generated, with clots being more resistant to fibrinolysis when formed at higher thrombin concentrations.[29] At low α_2-antiplasmin concentrations such as those in SD-plasma whole blood, active fibrinolysis begins prior to the clot reaching its maximum strength. As clot formation involves a balance between concurrent thrombin generation and fibrinolysis, by shutting down fibrinolysis via α_2-antiplasmin or TXA supplementation, clot formation becomes dependent primarily on the extent to which thrombin is generated. The concurrent decrease in protein S may explain lower α_2-antiplasmin concentrations than those found in FFP being sufficient to restore clot lysis time of SD-plasma to that of FFP. This may likewise explain TXA levels far below those observed following therapeutic TXA administration[22] being sufficient to restore clot lysis time.

Limitations

Our study was motivated by reports of hyperfibrinolysis following massive

SD-plasma transfusion in some patients[2,3], as well as *in-vitro* studies showing shorter clot lysis times in pure SD-plasma as compared to pure FFP[7,8].
Given the impracticality of a clinical study powered to quantify the risk of hyperfibrinolysis in patients heavily transfused with SD-plasma, an *in-vitro* study represents an informative first step. However our study, like any *in-vitro* study used to address a clinical issue, has limitations. While whole blood samples were dutifully reconstituted using red blood cells, plasma, and platelets, the samples ultimately contained a modified version of whole blood which was up to 18% storage medium by volume (table 1). The buffers, enzymes, and storage media in our samples and the handling of the blood products may introduce variance for which we cannot fully account. As we centrifuged and washed both RBCs and platelets in our experimental protocol, the potential role of microparticles generated during preparation and storage of these blood products was not evaluable. Accordingly, the full extent of the effect of SD-plasma transfusion on fibrinolysis can only be studied in a clinical trial.

Repeatability of TEG traces between different experimental days, and thus absolute (i.e. non-normalized) TEG parameters, proved a challenge, twice requiring an increase in the tPA level to observe any lysis at all. Our sensitivity analysis on runs with equivalent tPA concentration yielded a similar result for the association between $CLT_{50\%n}$ and SD-plasma fraction, suggesting this non-repeatability indeed did not affect our conclusions as they are based on analysis of normalized TEG parameters.

Clinical implications
Despite not being directly generalizable to the clinical world, our results may have clinical implications. In cases of massive transfusion, half or more of a patient's plasma compartment may be replaced by SD-plasma. Our experiment showed a roughly linear relationship between SD-plasma fraction and decrease in clot lysis time as the plasma compartment was diluted with SD-plasma, reaching a minimum of around 50% the original clot lysis time when the plasma compartment had completely transitioned to SD-plasma. Given that a 3% decrease in clot lysis at 30 minutes past MA is already considered hyperfibrinolysis[30], the association between SD-plasma and fibrinolysis warrants attention. The clinician should be aware of this effect and of the potentially reparative effect of TXA on the hemostatic state of a patient heavily transfused with SD-plasma.

Summary
In summary, this TEG-based analysis of whole blood samples reconstituted with various levels of SD-plasma fraction suggests that replacement of FFP in the plasma compartment of whole blood with SD-plasma decreases clot lysis time. α_2-antiplasmin and TXA can restore clot lysis time of whole blood with SD-plasma to that of whole blood with FFP. Physicians should be aware of the effect of SD-plasma transfusion on fibrinolytic state, especially in cases of

massive transfusion, and the potential corrective action of TXA.

ACKNOWLEDGEMENTS

The authors would thank Professor Joost Meijers (Sanquin Research, department of Plasma Proteins; Academic Medical Centre of Amsterdam, department of Experimental Vascular Medicine) for his expertise and helpful suggestions regarding α_2-antiplasmin, Mr. Carel Eckman (Sanquin blood supply) for his measurement of α_2-antiplasmin activity, and Ms. Jennifer Buchli (NASA Johnson Space Center, Houston, TX, USA) for her helpful advice on TEG measurement sensitivity.

Conflict of Interest statement
Sanquin Blood Supply is the distributer of Omniplasma within the Netherlands.

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“Extreme heterogeneity in reported rates of plasma transfusion reactions hinders our ability to provide reliable transfusion reaction rate estimates.”

Meta-analysis

4

Comparing transfusion reaction rates for various plasma types: a systematic review and meta-analysis/regression

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(2017) Transfusion, 57(9), pgs. 2104–2114

ABSTRACT

Introduction
We estimated rates for common plasma-associated transfusion reactions and compared reported rates for various plasma types.

Methods
We performed a systematic review and meta-analysis of peer-reviewed articles reporting plasma transfusion reaction rates. Random-effects pooled rates were calculated and compared between plasma types. Meta-regression was used to compare various plasma types with regard to their reported plasma transfusion reaction rates.

Results
Forty-eight studies reported transfusion reaction rates for Fresh Frozen Plasma (FFP, mixed-sex and male-only), Amotosalen INTERCEPT plasma (AI-FFP), Methelene-Blue treated plasma (MB-FFP), and Solvent/Detergent treated pooled Plasma (SDP). Random-effects pooled average rates for FFP were: allergic reactions ($92/10^5$ units transfused, 95% confidence interval [CI] 46-184); Febrile Non-Hemolytic Transfusion Reactions (FNHTRs) ($12/10^5$ units, 95%CI 7-22); Transfusion Associated Circulatory Overload (TACO) ($6/10^5$ units, 95%CI 1-30); Transfusion Related Acute Lung Injury (TRALI) ($1.8/10^5$ units, 95%CI 1.2-2.7); anaphylactic reactions ($0.8/10^5$ units, 95%CI 0-45.7). Risk differences (RD) between plasma types are not statistically significant for allergic reactions, TACO, or anaphylactic reactions. Methylene blue FFP led to fewer FNHTR than FFP (RD=-15.3 FNHTR/10^5 units transfused [24.7 to 7.1]); male-only FFP led to fewer TRALI cases than mixed-sex FFP (RD= -0.74 TRALI/10^5 units transfused [-2.42 to -0.42]).

Conclusion
Meta-regression shows the rate of FNHTR to be lower for Methylene blue FFP as compared to FFP and the rate of TRALI to be lower for male-only FFP than for mixed-sex FFP, while no statistically significant differences are observed between plasma types for allergic reactions, TACO, and anaphylactic reactions. Reported transfusion reaction rates suffer from high heterogeneity.

INTRODUCTION

Plasma transfusion indications and sources

Plasma transfusions are indicated in cases of massive bleeding, thrombotic thrombocytopenic purpura (TTP), hemolytic-uremic syndrome (HUS), liver disease, deficiencies in clotting factors for which concentrates do not exist (e.g. Factor V), and a few other disease states[1].

Plasma units may be prepared via apheresis or from whole blood[2]. As with any blood product, steps may be taken to prevent transfusion of harmful pathogens potentially contained within the unit. Various protective methods are used worldwide, falling generally into two categories – quarantine and pathogen reduction.

Securing human plasma

For plasma secured via a quarantine process, donors are tested for a set of pathogens at the time of donation, following which the plasma unit is frozen. The unit is cleared for use following a second negative screen, typically 4-6 months later[1,3].

The mid-eighties saw the introduction of chemical and irradiative processes used to reduce the pathogenicity of human plasma units[4]. The various so-named 'pathogen reduction' processes can be performed via single-unit pathogen reduction or pooled pathogen reduction techniques. Single unit pathogen reduction involves exposing units individually to chemicals and light (UV or visible) to disrupt transcription of pathogen DNA[5]. Pooled pathogen reduction utilizes a solvent/detergent treatment performed on pools of several hundred human plasma units before the pool is re-divided into units for transfusion[4]. This pooling process serves to dilute antibodies/antigens (e.g. IgA), which are not affected by the pathogen reduction process, to titers roughly a thousand times lower[6]. A subsequent filtration process filters out cell remnants.

Examples of commonly transfused plasma types are fresh frozen plasma (FFP), quarantined FFP (Q-FFP) (secured via the quarantine method), male-only FFP (MO-FFP), solvent/detergent treated pooled plasma (SDP) (a pooled pathogen reduced plasma), and Amotosalen INTERCEPT (AI-FFP) and THERAFLEX methylene blue (MB-FFP) plasmas (single-unit pathogen reduced plasmas)[7]. Processing details for these plasma types can be found in Table 1.

Transfusion reactions

Plasma transfusions may lead to adverse events (transfusion reactions

[TRs]), including minor allergic reactions, anaphylactic reactions, febrile non-hemolytic transfusion reactions (FNHTRs), transfusion related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), bacteremia, transfusion-related sepsis, and hemolysis[8]. Accurate estimates of transfusion reaction rates are necessary for effective comparisons of plasma products. However, reported rates of transfusion reactions suffer from high heterogeneity, likely the result of differences in definitions, observational vigilance during transfusions, and reporting practice, as well as pathophysiological differences between patient populations. In addition, the low rate of plasma transfusion reactions leads to many studies being underpowered for accurate comparison of products. This presents an issue for hospitals and national blood banks that must choose among several options for their human plasma needs based in part on their comparative safety.

We performed a systematic review and meta-analysis of plasma transfusion reaction rates to answer two research questions: (1) what are the most common transfusion reactions associated with plasma transfusion and the rates thereof in general patient populations, and (2) how do the various plasma products compare with regard to rates of these transfusion reactions?

Table 1: Details on various plasma types transfused

Plasma type	Classification	Process	Notes
FFP[1]	Single-donor, non-pathogen reduced	• Untreated plasma	Does not undergo pathogen-reduction process; considered control for this analysis
Q-FFP[1]	Single-donor, non-pathogen reduced	• Donors are screened for a set of pathogens at donation, and plasma unit is frozen • Unit cleared for use after a second negative screen 4-6 months (typically) later	Does not undergo pathogen-reduction process; Q-FFP data are pooled with FFP data for this analysis
MO-FFP	Single-donor, non-pathogen reduced	• FFP donated by male donors (in some cases, only those who have never received a blood transfusion) • Intended to reduce passive infusion of HLA/HNA antibodies found in high titers in plasma from multiparous women	Where only male plasma is transfused, female plasma is typically used for plasma product manufacture
AI-FFP[5]	Single-donor, pathogen reduced	• FFP units infused with amotosalen-HCl and exposed to UV light (320-400 nm) for 6-8 minutes, creating interstrand cross-links within pathogen DNA/RNA • Process can be performed in addition to or instead of quarantine process	In-hospital system; secures two or three units of plasma at a time
MB-FFP[5]	Single-donor, pathogen reduced	• FFP units infused with anhydrous methylene blue chloride and exposed to visible light (590 nm) for ~20 minutes, generating reactive oxygen species • Process can be performed in addition to or instead of quarantine process	In-hospital system; secures one unit of plasma at a time
SDP[4]	Pooled, pathogen reduced	• 1% TNBP + 1% octoxynol added to pool of ~1000 FFP units for 60-90 minutes to inactivate enveloped viruses • Pooled then passed through column filled with affinity ligand resin intended to bind prion proteins (PrPSc)	e.g., Octaplas, OctaplasLG

UV = ultraviolet; HLA/HNA = human leukocyte antigen/human neutrophil antigen; TNBP = tri(n-butyl) phosphate; PrPSc= prion protein scrape associated.

METHODS

Study protocol

This systematic review and meta-analysis was written to conform to the PRISMA (Preferred Reporting Items for Systemic reviews and Meta-Analyses) standards[9] as specified in Zorzela et al. *PRISMA harms checklist: improving harms reporting in systematic reviews*[10]. A study inclusion/exclusion protocol was agreed upon beforehand, in writing, with the first author serving as primary reviewer and the second author (FvH [M.D.]) acting as secondary reviewer (protocol found in appendix).

Eligibility criteria

Outcomes of interest: Our goal was to investigate reported rates of plasma transfusion reactions for the various plasma types in general patient populations. Additionally, we wished to investigate which, if any, study characteristics serve as good predictors of reported rates.
Types of studies: Observational studies and randomized clinical trials (RCTs) were included in our analysis while case-control studies, case reports, and case series were excluded as they cannot be used to derive population-based transfusion reaction rates. Articles of all languages were included; non-English articles were translated by fluent speakers into English.

Information sources

Peer-reviewed articles and conference abstracts detailing plasma transfusion reactions were identified within the PubMed, Embase, Web of Science, COCHRANE, Academic Search Premier, ScienceDirect and CINAHL (Cumulative Index to Nursing and Allied Health Literature) databases.

Search

Search parameters included the names of known plasma types and transfusion reactions as well as a set of generic terms which could be used to describe studies on plasma transfusion reactions. The search was performed in February of 2017 and the full search strategy is listed in the appendix.

Study selection

Initial screening: Figure 1 shows a flow diagram describing our article screening and exclusion protocol. Titles and abstracts were initially screened independently by first (NS) and second (FvH) authors. Disagreements on inclusion were resolved via discussion. Articles were read in full by first and second authors with articles further excluded as described in figure 1. Note that studies covering only plasma exchange were excluded, as these inevitably investigated transfusion reaction rates in specific (rather than general) patient

populations.

Data collection process
The following characteristics were recorded for each included study: number and types of transfusion reactions, units transfused, year of publication, study type (cohort or trial), plasma type, study period, and country in which study was performed. We additionally noted whether transfusion reactions were passively reported or actively assessed, assigning the label 'active' to studies of the latter type. Data extraction was performed by the first and checked by the second author. Our data extraction form is provided in the appendix.

Data items
The outcome of interest was rate of plasma transfusion reactions. Due to discrepancies in definitions of transfusion reactions and in methods of reporting, several assumptions were made in order to enable pooling of data. Both 'urticarial' and 'allergic' were assumed to refer to allergic reactions (which tend to comprise primarily dermatological symptoms). For anaphylactic reactions, the word 'anaphylactic' was a requirement (i.e. mention of allergy-related hypotension was not considered sufficient to denote anaphylaxis). The definition of FNHTR differs based on standard used but generally involves a body temperature rise of 1-2° with or without chills/rigors – reports of any of these were taken to mean FNHTR had been observed and the standard used noted. The formal definition of TRALI differs slightly between countries but is generally defined as non-cardiogenic bi-pulmonary edema on chest imaging within six hours of transfusion, absent other likely causes[11]. For our analysis, all cases designated as TRALI were accepted as such while those noted as 'possible TRALI' were omitted, as this standard was a common convention among the TRALI studies we found. For studies reporting an imputability measure (measure of confidence that the implicated blood product was indeed responsible for the specified transfusion reaction), transfusion reactions ascribed to a product with a 'definite' or 'likely' level of imputability were analyzed as cases while those with a 'possible' or 'unlikely' imputability level were not.

The term 'male-only' plasma is a misnomer – several countries denoting their plasma as male-only transfuse female plasma post-pregnancy following a negative screen for HLA/HNA antibodies (the suspected cause of immune-mediated TRALI). For consistency, we use the term 'male-only' but pool all low TRALI-risk plasmas together into this group. Male-only and mixed-sex FFP were analyzed separately for our TRALI analysis, and together for the remaining transfusion reactions.

Risk of bias assessment
To evaluate studies on their potential for bias in their reported plasma transfusion reaction rates, we used the Risk Of Bias In Non-randomised Studies of Interventions (ROBINS-I) tool as described in Sterne et al (2016) *ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions*. This tool consists of a series of questions pertaining to the article designed to evaluate seven forms of bias – bias due to (1) confounding, (2) deviations from intended interventions, and (3) missing data, as well as bias in (4) selection of participants into the study, (5) classification of interventions, (6) measurement of outcomes, and (7) selection of the reported result. The evaluations were performed in concert by the first and second authors.

Statistical analysis
Summary measure: The principal summary measure was transfusion reaction rate per 100,000 plasma units transfused.

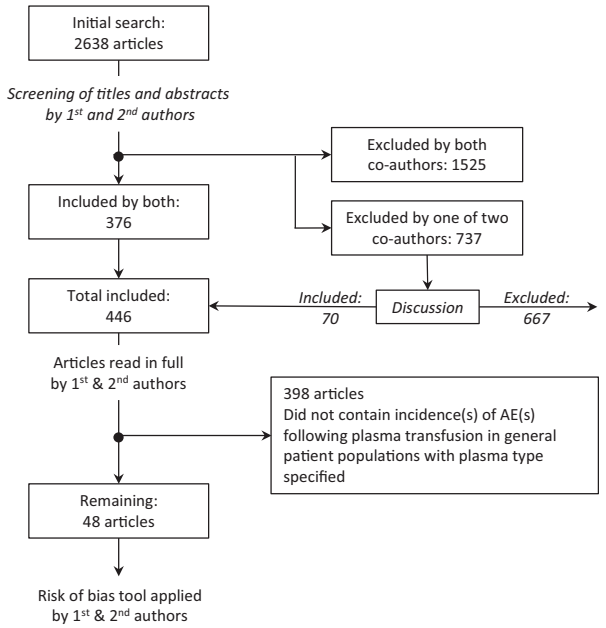
Synthesis of results: We constructed forest plots for each of the commonly reported transfusion reactions using binomial distribution-based exact methods to calculate 95 percent confidence intervals (CIs). These plots allow visualization of the heterogeneity within the reported rates for each of the transfusion reactions analyzed.

In order to estimate the expected rate of transfusion reactions (research question #1), pooled rates for the various plasma types were calculated for each transfusion reaction using the binomial-normal model as described by Stijnen et al. (2010)[12]. This method assumes a binomial distribution for number of cases and accounts for the heterogeneity between studies by assuming a normally distributed random effect. Risk differences (RDs) and their 95% confidence intervals were computed to compare the rate of transfusion reactions between plasma types.

Comparison of plasma types via meta-regression
Meta-regression techniques were used to compare the various plasma types as previously described[12].

Bias assessment (inter-study)
The forest plots also function as funnel plots as each study's vertical position indicates its size (total units transfused — note the scale along the y-axis). As such, the point distribution about the mean allows for observation of small-study related bias (such as publication bias[13]). To quantify the portion of the observed inter-study variation attributable to heterogeneity between the studies, we calculate and present the (un-weighted) I^2 value as described by

Figure 1: Flow diagram describing screening and exclusion of articles. Values represent numbers of articles. AE = adverse event.



DerSimonian and Laird (1986)[14].

Sensitivity analyses
To check our pooled averages and plasma type comparisons, we re-performed our meta-regression excluding those studies with the lowest risk-of-bias, according to the ROBINS-I risk of bias tool, and compared these results to the results of our primary analysis. Ideally, we would exclude the higher risk-of-bias studies for a sensitivity analysis. However this was not here an option, as explained in the results section.

RESULTS

Study selection

Figure 1 details the results at each step of our study selection process. Our search yielded 2,638 articles and conference abstracts. Review of titles and abstracts excluded 2,192 articles. The remaining 446 articles, written in nine languages (English, French, German, Dutch, Swedish, Italian, Russian, Serbian, and Czech), were translated into English as necessary. Based on the full text, we excluded an additional 398 articles for a lack of original transfusion reaction rate data following transfusion in general patient populations with a specified plasma type. We extracted the relevant data from the remaining 48 articles before applying the ROBINS-I risk of bias tool (Figure 1).

Study characteristics

A total of 48 studies reported rates for 5 types of transfusion reactions,

providing a total of 120 separately reported transfusion reaction rates — allergic transfusion reactions [35], febrile non-hemolytic transfusion reactions (FNHTRs) [18], transfusion associated circulatory overload (TACO) [9], anaphylactic transfusion reactions [12], and transfusion related acute lung injury (TRALI) [46]. The 48 studies, many of which reported on transfusion reaction rates for multiple plasma types, investigated Amotosalen INTERCEPT plasma (AI-FFP) [4], solvent/detergent pooled plasma (SDP) [8], male-only fresh frozen plasma (MO-FFP) [7], Methylene Blue treated fresh frozen plasma (MB-FFP) [3], and mixed-sex fresh frozen plasma (FFP) [44]. All 48 of the studies were cohort studies, though one involved active assessment of transfusion reactions (as opposed to passive collection of transfusion reaction reports). As only one study fell into the 'active' category, we were unable to test differences between studies based on this characteristic.

Using the ROBINS-I tool, we found all studies had a low risk of bias due to confounding, participant selection, intervention classification, deviations from intended interventions, and selection of reported result. We found all studies to have a moderate risk of bias in outcome measurement. All but three studies (19, 27, and 54) were found to have a low to moderate risk of bias due to missing data, while these three had a low risk in this bias category. The studies were performed between 2001 and 2016 in 20 different countries and observed in total over 25 million transfusions. Complete study characteristics and extracted data, along with their ROBINS-I evaluations, can be found in the appendix (supplemental table 1).

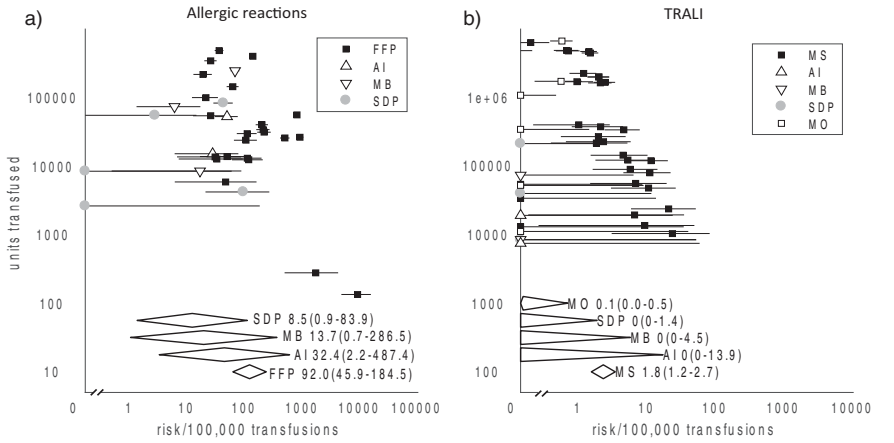
Transfusion reaction-specific results

Figure 2 shows forest plots of reported rates for the two transfusion reactions on which the most data were available, allergic reactions and TRALI, expressed as cases/100,000 units transfused. The forest plots for anaphylactic reactions, FNHTRs, and TACO are found in online supplemental figure 1. As study size is plotted on the (log)scaled y-axis, all forest plots additionally function as funnel plots for inspection of small-study related bias (such as publication bias).

Allergic transfusion reactions

Incidence and plasma type comparison: Figure 2a shows the forest plot for the 35 individually presented rates of allergic plasma transfusion reactions. The findings show considerable heterogeneity ($I^2_{allergic}$ for all plasma types = 0.91). FFP is associated with 92 allergic reactions/10^5 units transfused (95%CI 46-184), SDP with 32/10^5 unit transfused (95%CI 2-84), Methylene Blue FFP with 14/10^5 units transfused (95%CI 1-286), and Amotosalen INTERCEPT with 32/10^5 units transfused (95%CI 2-487). The differences in associated rate of allergic reactions between the plasma types do not meet the standards of statistical

Figure 2: Forest plots for reported rates of (a) allergic reactions and (b) TRALI per 100,000 units transfused showing means (squares) and binomial distribution-calculated 95% confidence intervals (line segments). Studies are arranged vertically according to size, allowing the plot to also function as a funnel plot. Pooled average rates are presented for each plasma type (open diamonds) for which more than two studies report a transfusion reaction incidence.



significance at the a=0.05 mark.

Publication bias: Figure 2a shows no overt signs of publication bias.

Transfusion Related Acute Lung Injury (TRALI)
Incidence and plasma type comparison: Figure 2b shows the forest plot for the 46 individually presented rates of plasma transfusion associated TRALI. The findings are moderately heterogeneous ($I^2_{TRALI} = 0.51$). Mixed sex FFP is associated with 1.8 TRALI cases/10^5 units transfused (95%CI 1.2-2.7) while male-only FFP is associated with 0.1 TRALI cases/10^5 units transfused (95%CI 0-0.5). No cases of TRALI were observed following transfusion with SDP (95%CI 0-1.4). This results in a statistically significant mean TRALI rate difference between male-only FFP as compared to mixed-sex FFP (RD= -0.74 TRALI/10^5 units transfused [-2.42 to -0.42]), while the smaller sample sizes of the studies investigating SDP lead to a non-significant rate difference for SD-plasma as compared to mixed-sex FFP (RD= -0.99 TRALI/10^5 units transfused [-2.65 to 0.44]).

Publication bias: Figure 2b shows no overt signs of publication bias, with zero-case studies balancing out studies reporting non-zero rates throughout the

range of study sizes.
Febrile Non-Hemolytic Transfusion Reactions (FNHTRs)
Incidence and plasma type comparison: Supplemental Figure 1a shows the forest plot for the 18 reported rates of plasma transfusion related FNHTR. These 18 studies included a mix of definitions (AABB definition [1], ISBT definition [2], Popovsky definition[2], temperature rise of 1° [3], not specified [10]) for FNHTR. Given the small number of data points corresponding to each definition and the relative consistency among FNHTR definitions, these studies were analyzed together.

The findings show substantial heterogeneity ($I^2_{FNHTR} = 0.83$). FFP is associated with 12 FNHTR cases/10^5 units (95%CI 7-22) while no cases of FNHTR were observed following transfusion with plasma pathogen inactivated via the Methylene Blue system or SDP. The difference in FNHTR rate for Methylene Blue plasma as compared to FFP is here significant (RD=-15.3 FNHTR/10^5 units transfused [24.7 to 7.1]).

Publication bias: Supplemental figure 1a shows no overt signs of publication bias with the point scatter roughly evenly dispersed on both sides of the mean.

Anaphylactic reactions and Transfusion Associated Circulatory Overload (TACO)
Incidence and plasma type comparison: Supplemental figures 1b and 1c show the forest plots for the 12 reported rates of plasma transfusion related anaphylaxis and 9 reported rates of plasma transfusion related TACO, respectively. The random effects pooled average rate for all FFP is 0.8 anaphylactic reactions/10^5 units transfused (95%CI 0-45.7) and 5.9 TACO events/10^5 units transfused (95%CI 1.2-29.7). Given the small number of data points available for these two transfusion reactions, calculation of heterogeneity measures and comparisons of pooled averages for various plasma types is here not statistically appropriate. Likewise, with so few data points, point scatter cannot reasonably be used to judge publication bias.

Sensitivity analysis
Calculation of pooled averages and comparison of plasma types for each of the five above-covered transfusion reactions was repeated while excluding the three studies judged to have a lower risk of bias via the ROBINS-I tool. Ideally, the studies with a higher risk of bias would be excluded for our sensitivity analysis. However, as 45 of the 48 studies were judged to have a low/moderate risk of bias with only three judged to have a low risk of bias, this was not here an option. Rather, we compared the results of our analysis with and without the higher quality (lower risk of bias) studies. This sensitivity analysis returned figures nearly identical to those calculated using all studies (data not shown).

DISCUSSION

Our meta-analysis combines the results of 120 individually reported rates gleaned from 48 studies to estimate rates of common plasma transfusion reactions and to compare various plasma products with regard to transfusion reaction rates. To our knowledge, this is the first meta-analysis presenting data for all plasma sorts and all reported transfusion reaction types in general patient populations to form a complete comparative picture of plasma products.

The most commonly reported plasma transfusion reactions are, in order of decreasing frequency: allergic reactions, febrile non-hemolytic transfusion reactions, transfusion associated circulatory overload, anaphylactic reactions, and transfusion related acute lung injury. Male-only FFP was associated with significantly fewer cases of TRALI than mixed-sex FFP. The types of the studies here analyzed were exclusively non-comparative, non-randomized, observational cohort studies performed by compiling descriptions of those transfusion reactions which were reported. They were thus susceptible to a low to moderate bias, though the conclusions derived by analysis of our data with and without the higher quality (lower risk of bias) articles were no different.

We suggest the most significant result in our analysis is the heterogeneity shown by the forest plots displaying reported transfusion reaction rates. All five forest plots (two in the main body, three in the appendix) show the extreme variance characteristic of reported transfusion reaction rates. Our analysis shows that differences in plasma types leads in part to this variance. However, observing only FFP plasma (the most transfused plasma type within this analysis) shows that even within data on the same plasma type, variance in reported transfusion reaction incidences is high. Though the rare nature of transfusion reactions partly explains the large variance, this variance is likely too large to attribute to sample-error only. We address this extreme variance below in our limitations section, however in addition to the limitations it imposes, it is a sign of how important standardization in both diagnosis of and reporting of transfusion reactions is.

Regarding our conclusions on TRALI, our meta-analysis joins a growing chorus of studies showing male-only plasma leads to significantly fewer TRALI cases than mixed-sex plasma[15-18], including a recent meta-analysis by Schmickl et al.[18] Our analysis differs from theirs in two important ways. Firstly, we used binomial methods to calculate pooled averages as opposed to the oft-applied Dersimonian-Laird technique. In addition to properly modeling count data as binomially distributed (as opposed to approximating count data as normally

distributed on the log scale as per DerSimonian-Laird), binomial methods avoid the inaccuracies introduced by the use of the zero-cell correction factors required by the Dersimonian-Laird technique in cases of studies observing no events – a significant advantage given that 15 of the 46 studies we included observed no TRALI cases. Secondly, we included both comparative and single-product studies in our analysis while Schmickl et al. included only comparative studies (i.e. studies comparing mixed-sex to male-only FFP) and thus excluded several large-scale studies reporting TRALI rates for only mixed-sex FFP. Though we agree with the conclusion of Schmickl et al., we believe our analysis adds to the evidence base on this topic.

Our analysis was not statistically powered to show a significant difference in TRALI rate between SDP and mixed-sex FFP. Despite the fact that no case of TRALI was ascribed to SDP transfusion within our study, the small sizes of the SDP studies broadens the confidence interval, making the calculated rate of 0 not statistically significantly lower than that of mixed-sex FFP.

As with most hemovigilance analyses, our calculated rates are presented per unit rather than per volume despite the difference in sizes between plasma products (e.g. ~330mL [apheresis derived FFP] versus ~200mL [whole blood derived FFP] versus 200mL [SDP]). This practice is based on the assumption that plasma-transfusion protocols typically specify number of units, rather than volume of plasma needed.

Limitations

As with any hemovigilance-based review, the clinical value of pooled averages is limited by the inter-study heterogeneity of the data. Inter-study heterogeneity can result from differences in definitions of outcomes, differences in methods used for data collection, and from real pathophysiological differences between the populations. We suspect differences in observational vigilance during transfusion and consistency in reporting of transfusion reactions to be the most influential factors.

We provide overviews of the reported rates for five common plasma transfusion reactions and confirm earlier reports that male-only plasma leads to significantly fewer cases of TRALI than mixed-sex FFP. Extreme heterogeneity in reported rates of plasma transfusion reactions hinders our ability to provide reliable transfusion reaction rate estimates.

Funding

This study forms part of an epidemiological PhD study. No outside funding was provided or requested for this study.

ACKNOWLEDGEMENTS

The authors would like to thank F. van Hout (FvH), A. Lysakowska (AL), S. Mahinrad (SM) and G. Ogliari (GO) for their gracious help translating German (FvH), Swedish (AL), Czech (AL), Serbian (AL), Russian (SM), and Italian (GO) articles.

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“...regression models comparing transfusion reaction incidence rates between countries can return accurate results, provided multiple measurements are available for each country...”

ISTARE study

5

Comparing transfusion reaction incidences of various plasma products – an analysis of seven years of ISTARE haemovigilance data

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(2018) British Journal of Haematology, 180(5), pgs. 727–734

SUMMARY

Plasma transfusions may result in transfusion reactions. We used the International Surveillance of Transfusion-Associated Reactions and Events (ISTARE) database, containing yearly reported national annual aggregate data on transfusion reactions from participating countries, to investigate incidences of plasma transfusion reactions and compare transfusion reaction risks for different plasma types. We calculated incidences for plasma transfusion reactions and compared transfusion reaction incidences between plasma types using random effects regression on repeated measures. The ISTARE database contains data from 23 countries reporting units issued and/or transfused and transfusion reactions observed for some portion of seven years (2006-2012). Interquartile ranges (IQRs) of plasma transfusion reaction incidences are: allergic reactions (5.6-72.2 reactions/10^5 units transfused); febrile non-haemolytic transfusion reactions [FNHTR] (0-9.1); transfusion associated circulatory overload [TACO] (0-1.9); transfusion related acute lung injury [TRALI] (0-1.2); and hypotensive reactions (0-0.6). Apheresis plasma was associated with more allergic reactions (OR=1.29 [95% Confidence Interval (CI):1.19-1.40]) and hypotensive reactions (OR=2.17 [1.38-3.41]) than whole blood derived plasma. Pathogen inactivated plasma was associated with fewer transfusion reactions than untreated plasma. Apheresis plasma is associated with more allergic and hypotensive reactions than whole blood derived plasma. Pathogen inactivated plasma is associated with fewer transfusion reactions than untreated plasma.

INTRODUCTION

Plasma transfusion is indicated in cases of Thrombotic Thrombocytopenic Purpura/Haemolytic-Uremic Syndrome (TTP/HUS), massive bleeding, liver disease, deficiencies in clotting factors for which concentrates do not exist (e.g. Factor V), and a few other disease states[1]. Though plasma transfusions are generally safe, they may result in adverse events (transfusion reactions), including minor allergic reactions, febrile non-haemolytic transfusion reactions (FNHTRs), transfusion-related acute lung injury (TRALI), transfusion associated circulatory overload (TACO), hypotensive reactions, anaphylactic reactions, venous thromboembolism (VTE), bacteraemia, transfusion-related sepsis, hyperfibrinolysis, and hemolysis[2].

Various plasma types are available for transfusion, differing both in their method of acquisition and in which, if any, treatments are performed on the plasma units pre-transfusion. Plasma products can be generally split into three categories – (1) untreated single-donor plasmas (e.g. Fresh Frozen Plasma [FFP], quarantined or un-quarantined), (2) pathogen-inactivated single-donor plasmas (e.g. Amotosalen INTERCEPT plasma, Methylene-blue plasma), and (3) pathogen-inactivated pooled plasmas (e.g. Solvent/Detergent [SD] treated pooled plasmas like Octaplas and OctaplasLG). Untreated single-donor plasma is used after the unit, the donor, or both are tested for a set of pathogens, with the donor sometimes being re-tested following a quarantine period[1]. Pathogen-inactivated single donor plasma is generated by performing a pathogen inactivation process on individual bags of FFP. The various pathogen inactivation processes generally target either nucleotides or the lipid envelope present on many viruses[3]. For SD plasma, the pathogen inactivation process is performed on a pool of hundreds to thousands of FFP units, which is then separated into individual units for transfusion. This has the additional effect of diluting antigens and antibodies and normalising plasma protein levels, in addition to the neutralising of pathogens by antibodies present in the pool[4]. As pooling theoretically increases the risk of prion transmission, the pool may further be passed through a prion affinity ligand gel designed to safeguard against this (as is the case with OctaplasLG)[5].

Comparison of blood products with regard to their risk of transfusion reactions is hindered by the low incidence of transfusion reactions and the high heterogeneity in reported transfusion reaction incidences (e.g. the reported incidence of allergic transfusion reactions following plasma transfusion varies from 2 to 20,000 reactions per 100,000 units transfused[6,7]). The rarity of transfusion reactions makes comparisons in trials difficult as most trials will observe few if any adverse events. Analysis of large-scale observational

haemovigilance data likewise poses a challenge as these data are often collected at a national level by agencies receiving reports of transfusion reactions (so called national aggregate data). The variance between countries in the diligence with which transfusion reactions are diagnosed and reported contributes to differences in reported incidences, making inter-country comparisons of two different plasma products without accounting for this variance statistically inappropriate.

Given this large inter-country heterogeneity in reported transfusion reaction incidences, repeated measurements from each country are needed to compare blood components accurately with regard to their associated transfusion reaction incidences. With this in mind, in 2008 the European Haemovigilance Network (EHN – since 2010 the *International* Haemovigilance Network [IHN]) took on the development of an '*international web database for reporting and analysing all adverse reactions and events that threaten the recipient's and donor's health status and quality of life.*'[8] The resulting International Surveillance of Transfusion-Associated Reactions and Events (ISTARE) database contains incident count data (number of cases, number of units issued and/or transfused) on adverse events following transfusions of red blood cells, platelets, plasma, and other blood components, reported voluntarily by haemovigilance organizations in 25 countries[9].

We analysed the ISTARE plasma transfusion and transfusion reaction data to answer the research questions: (1) what are the reported incidences of common plasma transfusion reactions? (2) how do transfusion reaction incidences differ between the various plasma types?

METHODS

ISTARE database - data collection and export

The ISTARE database is a repository of voluntarily submitted annual aggregate data collected by national haemovigilance organizations on complications of blood donation and adverse reactions associated with transfusion of human blood components. It is maintained by a working group of the International Haemovigilance Network (IHN). Data is anonymized with countries identified only by a code number. Users provide incident count data (number of units issued and/or transfused and number of reactions reported) for the various transfusion reactions and blood products. Transfusion reactions classified according to IHN definitions[10] and deemed to be possibly, probably, or certainly attributable to the transfused component in question are recorded within the database.

A protocol describing this study and a formal data request were submitted to the ISTARE committee requesting use of their data on plasma transfusion reactions. Following approval of the protocol, incident count data covering transfusion of all plasma types within the database as well as all associated transfusion reactions was exported and delivered to us.

Incidences of common plasma transfusion reactions (research question #1)
From each annual report, we extracted the reported units issued and (when available) transfused, anonymised country code, and the reported number of allergic reactions, FNHTRs, TRALI, TACO, and hypotensive transfusion reactions for each plasma type. For countries reporting number of units transfused, we used this as our denominator, otherwise using number of units issued. The denominator type (units issued vs. units transfused) was noted. We calculated incidences and interquartile ranges (IQRs) for each of these transfusion reactions.

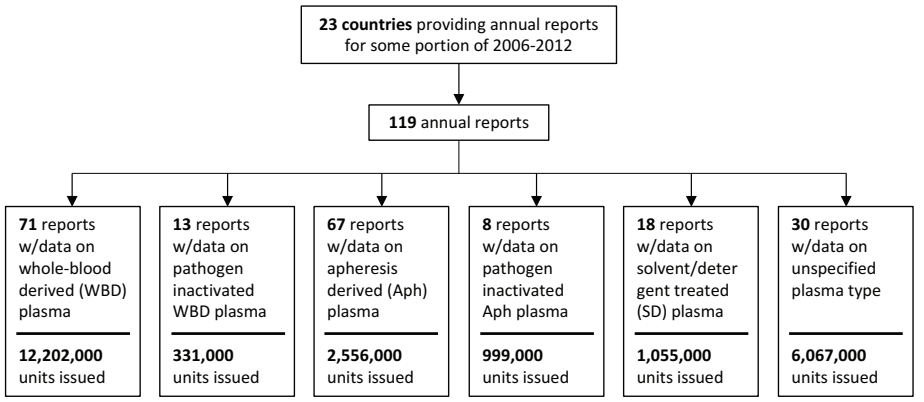
Comparison of transfusion reaction risk for various plasma types (research question #2)
To compare the various plasma types with regard to transfusion reaction incidence, we ran univariate regression analyses for each of the five transfusion reactions explored. Given the nature of the data (incident count data) with repeated measures from each country, we assumed the number of cases was binomially distributed [cases~Binomial(n,p)] with n=number of units issued (or transfused) and the log-odds (logit p) modelled linearly using a random effect model (binomial-normal mixed model). A categorical variable was used to denote the plasma types being compared and a country-specific random effect intercept added to the model, which was thus:

$$\text{logit}(p_{i,j}) = \beta_0 + \beta_i pltype_i + b_i$$

where p is the probability of the transfusion reaction in question (allergic reaction, FNHTR, TRALI, TACO, or hypotensive reactions) for plasma type *i* in country *j*, *pltype* is the plasma type, β_0 is the fixed effect intercept and b_j is the random effect intercept. By using the anonymised country code as a grouping variable in the random effects model, we allow between-country variance to be more accurately modelled.

We performed four comparisons across the five transfusion reactions: (1) untreated apheresis derived vs. untreated whole blood derived plasma, (2) untreated whole blood derived vs. SD plasma, (3) untreated apheresis vs. SD plasma, and (4) all untreated plasmas vs. all pathogen-inactivated plasmas (both single-donor and pooled). For our main analysis, we ran our regression

Figure 1: Annual reports analysed and units transfused for each plasma type. Aph, apheresis-derived; SD, solvent/detergent treated; WBD, whole blood-derived



model using data from all countries using any of the plasma types being compared. Because of the large heterogeneity in reported transfusion reaction incidence rates between countries, for each comparison we additionally ran our regression model using only data from countries using both plasma types being compared as a sensitivity analysis. The results of this sensitivity analysis can be interpreted as pooled ORs comparing the plasma types within countries using both.

As an additional sensitivity analysis, we re-performed our regression analysis separately on data from countries reporting number of units transfused vs. number of units issued. All calculations were carried out in MATLAB (R2015b, The MathWorks, Inc, Narick, Massachusetts, U.S.A.).

RESULTS

ISTARE plasma transfusion reaction data

Despite having started in 2008, the ISTARE database contains data from 2006 onwards due to provision of historical data by some countries. Twenty-three countries provided data on plasma transfusion within their borders for some part of the seven-year period covering 2006-2012, totalling 119 annual reports. Thirteen countries reported number of plasma units transfused for all or most of their reporting period, while ten countries reported number of plasma units issued. The incidence count data cover 17 types of transfusion-related adverse reactions recorded in the course of the transfusion of over 23 million units of five different plasma types (whole blood derived plasma [both untreated

and pathogen inactivated], apheresis plasma [both untreated and pathogen inactivated], and solvent/detergent treated pooled plasma). Details of the plasma-related data within the ISTARE database are provided in figure 1.

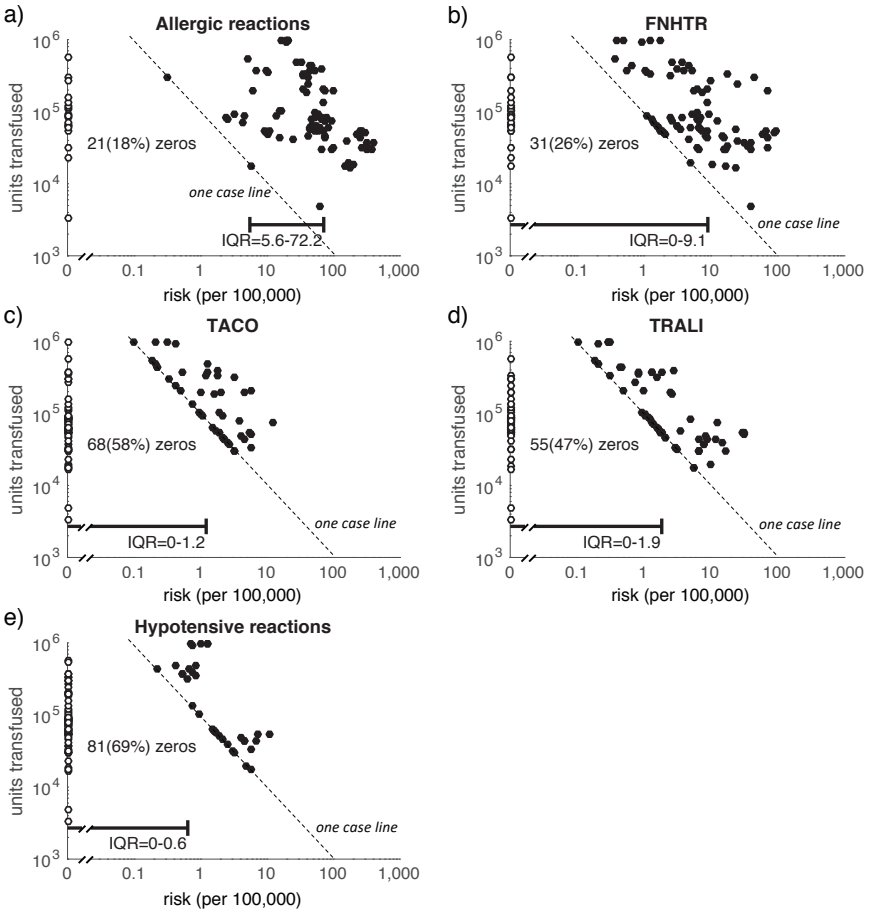
The total number of transfusion reactions assigned to each level of imputability, 'definite', 'probable' or 'possible', are reported for each transfusion reaction. However, as these totals do not distinguish between the implicated blood products (erythrocytes, platelets, plasma), we were unable to include imputability measures in our analysis of plasma transfusion reaction incidences. As no data on donor sex were provided, given the data were anonymised by country, we were unable to compare male-only plasma to mixed-sex plasma. Likewise, as the method of single-donor pathogen inactivation was generally not specified in annual reports, we could not calculate ORs comparing transfusion reaction risks between specific single-donor pathogen inactivated plasmas and untreated plasmas, leaving us instead to compare all pathogen inactivated plasmas to all untreated plasmas.

Selection of adverse events for analysis
Figure 1 shows the total number of annual reports detailing each of the plasma types used by countries participating in the ISTARE database, along with how

Table 1: Reactions associated with transfusions of plasma, recorded within the ISTARE database

	Reported cases:		incidence
Transfusion reaction:	**n**	**%**	**(per 10^5)**
Allergic reaction:	6412	76.49%	27.62
Febrile Non-hemolytic Transfusion Reaction (FNHTR):	1058	12.62%	4.56
Transfusion Associated Circulatory overload (TACO):	180	2.15%	0.78
Transfusion associated dyspnea (TAD):	159	1.90%	0.69
Other transfusion reaction:	159	1.90%	0.69
Unclassifiable complication of transfusion (UCT):	121	1.44%	0.52
Transfusion related acute lung injury (TRALI):	109	1.30%	0.47
Hypotensive reaction:	102	1.22%	0.44
Delayed Serologic Transfusion Reactions (DSTR):	24	0.29%	0.10
Transfusion transmitted viral infection – HBV:	19	0.23%	0.08
Acute Hemolytic Transfusion Reaction (AHTR):	17	0.20%	0.07
Delayed Hemolytic Transfusion Reaction (DHTR):	11	0.13%	0.05
Transfusion transmitted viral infection - Other:	4	0.05%	0.02
Transfusion transmitted viral infection – HCV:	3	0.04%	0.01
Transfusion transmitted viral infection – HIV:	2	0.02%	0.01
Transfusion transmitted bacterial infection:	2	0.02%	0.01
Hyperkalemia:	1	0.01%	0.004
Total:	**8383**		**36.12**

Figure 2: Funnel plots (units transfused versus reported risk) for (A) allergic reactions, (B) febrile non-haemolytic transfusion reactions (FNHTR), (C) transfusion-associated circulatory overload (TACO), (D) transfusion related acute lung injury (TRALI), and (E) hypotensive reactions for all plasma types together. Interquartile ranges (IQR) of reported risks are shown, along with number and percentage (in parentheses) of studies reporting zero cases for that transfusion reaction. Studies along the dotted line reported one case of that transfusion reaction. Note that the (log) x-axis is broken in order to display studies with zero cases.



many units of each were issued/transfused within those countries. Table 1 shows total reported cases for each of 17 plasma transfusion reactions recorded within the ISTARE database. Our study analysed allergic reactions [6412 cases], Febrile Non-Haemolytic Transfusion Reactions (FNHTR) [1058 cases],

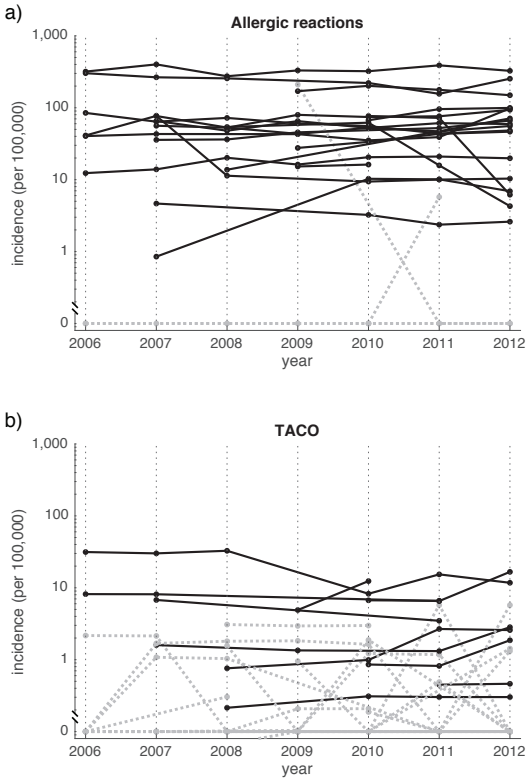
Transfusion Associated Circulatory Overload (TACO) [180 cases], Transfusion Related Acute Lung Injury (TRALI) [109 cases], and hypotensive reactions [102 cases]. We chose not to analyse Transfusion Associated Dyspnoea (TAD) [159 cases] as not all countries report incidences of this transfusion reaction. Further, we chose not to analyse 'other' transfusion reactions [159 cases] and Unclassifiable Complications of Transfusion [121 cases] as we could not reasonably assume that they represent similar adverse reactions across the various reporting countries. The remaining 14 transfusion reactions for which data are collected were observed too rarely or not at all, and are thus excluded from our analysis which requires incidence estimates of each adverse event from several countries to enable proper estimation of the random effect coefficients.

Incidences of plasma transfusion reactions
Figure 2 shows funnel plots[11] of $\log_{10}$(sample size) vs. $\log_{10}$(incidence) for all plasma types together for each of the five adverse events analysed. The linear formations assumed by the points are a result of plotting sample size vs. incidence (i.e. cases/sample size) on a log-log plot. Each linear formation corresponds to a specific number of cases, with the line closest to the plot origin corresponding to observation of one case, as indicated on the figures. The interquartile ranges for the reported incidences of each analysed transfusion reaction are: allergic reactions (5.6 to 72.2 cases/10^5 units transfused), FNHTR (0 to 9.1), TACO (0 to 1.9), TRALI (0 to 1.2), hypotensive reactions (0 to 0.6). Additionally, as it pertains to our discussion, the proportion of annual reports recording no case of each transfusion reaction is indicated.

Figure 3 shows $\log_{10}$(incidence) vs. year, grouped by country, for all plasma types together for allergic reactions and TACO. Countries reporting no cases or one case of the transfusion reaction being analysed are shown in grey while those reporting at least two cases are shown in black. The variance in incidence between countries is visibly larger than the within-country variance, as evidenced by the generally parallel course of the black incidence lines. Countries reporting no cases or one case (i.e. smaller countries) of the given transfusion reaction (grey lines) are subject to large swings in incidence from year to year.

Plasma type comparisons (regression analysis)
Table 2 shows the results of our regression analyses, comparing (1) apheresis derived vs. whole blood derived plasma, (2) untreated whole blood derived vs. SD plasma, (3) untreated apheresis derived vs. SD plasma, and (4) all untreated plasmas vs. all pathogen-inactivated plasmas (both single-donor and pooled), with regard to the odds of each of the five transfusion reactions analysed.

Figure 3: Reported risk versus time, grouped by country, for (A) allergic reactions and (b) transfusion-associated circulatory overload (TACO). Black solid lines represent countries reporting at least two cases of the adverse event per year; grey dotted lines represent coun- tries reporting zero or one case per year (and thus liable to large swings in reported risks)



(1) Apheresis derived vs. whole blood derived plasma (untreated): Transfusion of apheresis derived plasma was associated with a significantly increased risk of allergic reactions (OR = 1.29 [95% confidence interval (CI) 1.19 to 1.40]) and hypotensive reactions (OR = 2.17 [1.38 to 3.41]) compared to whole blood derived plasma. The odds of FNHTR, TRALI, and TACO were not significantly different between these two plasma types.

(2) Untreated whole blood derived vs. SD plasma: Transfusion of SD plasma was associated with a decreased risk of allergic reactions (OR = 0.27 [0.21 to 0.36]) and FNHTR (OR = 0.29 [0.15 to 0.54]) compared to untreated whole blood derived plasma.

(3) Untreated apheresis derived vs. SD plasma: Likewise, transfusion of SD plasma was associated with a decreased risk of allergic reactions (OR = 0.18 [0.14 to 0.24]) and FNHTR (OR = 0.30 [0.14 to 0.65]) compared to untreated apheresis derived plasma.

(4) All untreated plasmas vs. all pathogen-inactivated (single-donor and pooled) plasmas: Transfusion of pathogen-inactivated plasmas was associated with a

Table 2: For each transfusion reaction analysed, regression-derived odds ratios are presented for (i) untreated apheresis versus untreated WBD plasma, (ii) untreated WBD versus SD plasma, (iii) untreated apheresis versus SD plasma and (iv) all un-treated versus all pathogen-inactivated plasmas. As a sensitivity analysis, all analyses are repeated using only countries using both plasma types in question. 95%CI, 95% confidence interval; FNHTR, febrile non-haemolytic transfusion reaction; OR, odds ratio; PI, pathogen-inactivated; SD, solvent/de- tergent treated; TACO, transfusion associated circulatory overload; TRALI, transfusion related acute lung injury; WBD, whole blood-derived. Values in bold are significant at the α=0.05 level.

	Allergic reactions	FNHTR	TRALI	TACO	Hypotensive reactions
Main analysis					
Odds ratios - countries using *either* untreated WBD *or* untreated Apheresis plasma (19):					
untreated WBD:	ref	ref	ref	ref	ref
untreated apheresis:	**1.29 (1.19 - 1.40)**	0.78 (0.58 - 1.05)	1.00 (0.50 - 1.98)	1.11 (0.66 - 1.87)	**2.17 (1.38 - 3.41)**
Odds ratios - countries using *either* untreated WBD *or* SD plasma (20):					
untreated WBD:	ref	ref	ref	-	ref
SD plasma:	**0.27 (0.21 - 0.36)**	**0.29 (0.15 - 0.54)**	0.21 (0.02 - 1.92)	-	0.22 (0.02 - 2.18)
Odds ratios - countries using *either* untreated apheresis *or* SD plasma (20):					
untreated apheresis	ref	ref	ref	-	ref
SD plasma:	**0.18 (0.14 - 0.24)**	**0.30 (0.14 - 0.65)**	0.11 (0.01 - 1.00)	-	0.11 (0.01 - 1.00)
Odds ratios - countries using *either* untreated *or* pathogen-reduced plasmas (20):					
untreated plasmas	ref	ref	ref	ref	ref
all PI plasmas	**0.54 (0.48 - 0.60)**	**0.35 (0.26 - 0.48)**	0.50 (0.23 - 1.08)	**0.45 (0.23 - 0.90)**	**0.19 (0.04 - 0.93)**
Sensitivity analysis					
Odds ratios - countries using *both* untreated WBD *and* untreated Apheresis plasma (15):					
WBD:	ref	ref	ref	ref	ref
Apheresis:	**1.29 (1.18 - 1.40)**	0.77 (0.57 - 1.04)	1.07 (0.54 - 2.10)	1.09 (0.64 - 1.85)	**2.16 (1.37 - 3.40)**
Odds ratios - countries using *both* untreated WBD *and* SD plasma (7):					
untreated WBD:	ref	ref	ref	-	ref
SD plasma:	**0.27 (0.21 - 0.35)**	**0.28 (0.15 - 0.52)**	0.19 (0.02 - 1.74)	-	0.19 (0.02 - 1.85)
Odds ratios - countries using *both* untreated apheresis *and* SD plasma (7):					
untreated apheresis:	ref	ref	ref	-	ref
SD plasma:	**0.18 (0.14 - 0.23)**	**0.27 (0.13 - 0.57)**	0.13 (0.01 - 1.25)	-	0.09 (0.01 - 0.73)
Odds ratios - countries using *both* untreated *and* pathogen-reduced plasmas (10):					
untreated plasmas	ref	ref	ref	ref	ref
all PI plasmas	**0.54 (0.48 - 0.60)**	**0.35 (0.26 - 0.48)**	0.49 (0.23 - 1.06)	**0.46 (0.23 - 0.91)**	**0.20 (0.04 - 0.97)**

decreased risk of allergic reactions (OR = 0.54 [0.48 to 0.60]), FNHTR (OR = 0.35 [0.26 to 0.48]), TACO (OR = 0.45 [0.23 to 0.90]), and Hypotensive reactions (OR = 0.19 [0.04 to 0.93]) compared to untreated plasmas.

Sensitivity analyses:
For all four plasma type comparisons performed, our first sensitivity analysis, which pools ORs using data only from countries using both plasma types, returned almost identical results (table 2). Our second sensitivity analysis, performed separately on data from countries reporting number of units issued vs. number of units transfused, likewise returned nearly identical results to our primary analysis for all four comparisons (data not shown).

DISCUSSION

We evaluated seven years of annual aggregate haemovigilance data from 23 countries on plasma transfusion reactions and compared incidences of reported transfusion reactions between various plasma types. The most commonly reported plasma transfusion reactions are allergic reactions, FNHTR, TRALI, TACO, TAD, and hypotensive reactions. Inter-country variance in reported incidences of transfusion reactions collected within the ISTARE database is large while within-country variance tends to be small. Pathogen inactivated plasmas as a group were associated with fewer allergic reactions, FNHTR, TACO, and hypotensive reactions than untreated plasmas as a group. Apheresis derived plasma was associated with more allergic reactions and hypotensive reactions than whole blood derived plasma; SD plasma was associated with fewer allergic reactions and FNHTR than both untreated whole blood derived and untreated apheresis derived plasma.

Our conclusions on SD plasma are in line with those of previous publications showing SD plasma leads to fewer allergic reactions[12,13] as well as FNHTR[14] than whole blood derived plasma. However, we found no earlier studies presenting our finding that apheresis plasma is associated with more adverse reactions (allergic and hypotensive reactions) than whole blood derived plasma. This observation may be attributed to differences in the patient populations receiving apheresis vs. whole blood derived plasma (e.g. specific patient populations being treated exclusively with one plasma type), differences in cytokine activation due to the different processes experienced by these two components, or other differences introduced by processing these plasma components. We found no studies investigating the role of these potential mechanisms in adverse reaction aetiology in plasma - further research is needed on this front.

Aggregate annual data is the primary method by which transfusion-related adverse reactions are reported. However, heterogeneity in the reported incidence rates may be the result of differences in the vigilance with

which countries diagnose and report transfusion reactions to a centralized haemovigilance organization. As an example, within this database, reported incidences of allergic reactions following transfusion with whole blood derived untreated plasma vary from 13 to 421 reactions per 100,000 units transfused. However transfusion reaction incidences from any given country tend to vary little from year to year (figure 3). As such, regression models comparing transfusion reaction incidence rates between countries can return accurate results, provided multiple measurements are available for each country, as was the case here (a first to our knowledge). This emphasizes the need for committees like the ISTARE working group of the IHN which encourage countries to not only submit data on transfusion-related adverse reactions, but to improve the procedures by which those reactions are reported to a national haemovigilance agency.

Limitations

A major hindrance to any haemovigilance analysis is the rarity of transfusion reactions. Given that the most commonly reported transfusion reaction within the database (allergic reactions) had an incidence of below 0.1%, our analysis had to deal with an abundance of zero cells. As our regression analysis assumed a binomially distributed fixed effect, we avoided the inaccuracies introduced by zero cells as the binomial distribution is defined at zero. Nonetheless, the rare nature of the events being analysed make comparisons difficult in the absence of massive datasets.

Conclusion

Our findings support the notion that pathogen-inactivated plasmas as a whole lead to fewer allergic reactions, FNHTR, TACO, and hypotensive reactions than untreated plasmas as a whole, and that apheresis plasma leads to more allergic and hypotensive reactions than whole blood derived plasma. Between-country variance in reported plasma transfusion reaction incidences is high, while within-country variance is generally low, necessitating analysis of multiple years of data from each to allow proper comparison of plasma types across countries. Continued vigilant diagnosis and reporting of transfusion reactions to national haemovigilance agencies is needed to compare blood components properly via national annual aggregate data.

ACKNOWLEDGEMENTS

The authors would like to thank the ISTARE committee for their provision of these data and their help in the writing of this article.

Co-author contributions to the manuscript are as follows. For contributions provided by multiple co-authors, names are listed alphabetically.

- J.G. van der Bom, N. Saadah, and M. Schipperus conceived the research study.
- C. Politis, as head of the ISTARE committee, approved the protocol , provided the data, and served as an advisor on the project.
- R. Middelburg, C. Richardson, N. Saadah and J. Wiersum-Osselton designed the methodology.
- N. Saadah analysed the data and wrote the first draft of the manuscript with guidance from PhD advisors J.G. van der Bom and M. Schipperus.
- J.G. van der Bom, P. Renaudier, C. Richardson, P. Robbilard, N. Saadah, M. Schipperus, and J. Wiersum-Osselton contributed original content in the submitted manuscript.
- J.G. van der Bom, R. Middelburg, C. Politis, P. Renaudier, C. Richardson, P. Robbilard, N. Saadah, M. Schipperus, and J. Weirsum-Osselton critically reviewed the submitted manuscript.

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“As bivariate binomial-normal methods allow inclusion of studies reporting on only one treatment arm, they have the potential to increase the accuracy of the effect size estimate.”

Methodological study

6

Comparison of various methods in meta-analysis of sparse count data – addressing the challenges of analyzing hemovigilance datasets

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ABSTRACT

Background
Meta-analysis of count data (number of cases, number of exposed), is commonly used to compare event risks between blood products. Hemovigilance meta-analysis datasets often have high variance in reported event risks, studies reporting no events, and studies reporting on only one of the two treatments being compared. We compared various methods for meta-analysis of sparse hemovigilance count data.

Methods
We applied the DerSimonian-Laird (DL) method both (1) without and (2) with Hartung-Knapp (HK) correction, and the binomial-normal (BN) method, both in (3) univariate (uBN) and (4) bivariate form, on dual-armed studies (bBN_{dual}). We additionally applied (5) bivariate BN methods while including single-arm studies (bBN_{all}). We demonstrated their use in pooled odds ratio (OR) calculation using data from a recent hemovigilance meta-analysis and compared them via simulation study on similar datasets with known OR, varying study sizes and number of studies.

Results
The univariate BN method yielded the most accurate OR_{pred} more often than both DerSimonian-Laird methods and the bivariate BN method in meta-analysis of five dual-arm studies (p_{best_uBN}=0.25; $p_{best_bBN(dual)}$=0.18; p_{best_DL}=0.13; $p_{best_DL(HK)}$=0.10). Inclusion of single-armed studies with bivariate BN methods yielded more accurate OR_{pred} when number of studies (n) was small, but decreased the accuracy of the bBN_{all} predicted OR as n increased (n=15: p_{best_bBNall}=0.34, p_{best_uBN}=0.25; n=20: p_{best_bBNall}=0.25, p_{best_uBN}=0.27; n=30: p_{best_bBNall} =0.12, p_{best_uBN}=0.31).

Conclusions
Univariate BN methods are best used in meta-analysis of sparse hemovigilance count data. Bivariate BN methods with inclusion of single-arm studies may increase accuracy when number of studies is small.

INTRODUCTION

Count data (i.e. data reported as number of events and number exposed) are common to transfusion-medicine research[1]. Meta-analysis of count data to estimate pooled comparative measures (e.g. risk differences, odds ratios) comparing two or more treatment arms is a common method of summarizing treatment risks over studies. Transfusion medicine researchers face a specific set of challenges when comparing risks of rare-events among blood products via meta-analysis.

Characteristics of transfusion medicine meta-analysis datasets

Given the rarity of the events of interest, small studies may observe no events, resulting in so-called 'zero-cells' (data with many zero-cells are said to be 'sparse'). Given the variance in blood products used from country to country, studies comparing the two specific blood products of interest may be few in number, meaning researchers may pool data from studies exploring only one of the two blood products in question; the result is then a combination of studies exploring both study arms and studies reporting on only one (e.g. studies without a comparator; studies with a comparator other than the comparator of interest). Finally, reported incidences of transfusion-related adverse events are often highly heterogeneous with reported event incidences varying by several orders of magnitude between studies.

Methods of meta-analysis on count data

Pooling data in meta-analysis involves fitting a distribution as closely as possible to the effect estimates reported by the individual studies. Common effect estimates for count data are risk differences, relative risks, and odds ratios. If the heterogeneity in the effect estimates between studies is small, it is reasonable to assume the effect size (θ) is the same ('fixed') for all studies and that the differences observed are caused by natural variation or 'within-study variance' only – a so-called 'fixed effect' analysis. If the heterogeneity is large, as is typically the case in transfusion medicine research, it is more appropriate to assume each study has its own effect size, denoted for the i-th study as θ_i. These true but unobserved treatment effects θ_i are the effects we would expect to observe in studies with infinitely large sample sizes and are assumed to be a random sample from a normal distribution – a so-called 'random effect' analysis. Thus $\theta_i = \theta + \delta_i$ where θ is the mean population effect size and δ_i the deviation of the i-th study's effect size from that mean. The values reported by the individual studies become the estimates for θ_i, the variance of which is a sum of natural (within-study) and (random) between-study variance.

Two common methods of random effect analysis are the DerSimonian-Laird and the binomial-normal methods. The DerSimonian Laird method fits a normal distribution to the log-scaled effect-estimate (e.g. log of odds ratio or 'log(OR)') and assumes the random effect is likewise normally distributed. Alternatively, methods based on exact distributions can be used. Hamza et al.

(2008)[2] describe the binomial-normal (BN) method which takes advantage of the fact that the number of cases will be binomially distributed in n (number of exposed), with the random effect again assumed to be normally distributed. This method can be applied as a univariate analysis, where the outcome is log(OR), or as a bivariate analysis, in which the outcomes are the log(odds) of the two treatment arms, the difference of which is the log(OR). The bivariate model can thus include studies reporting on only one of the two treatment arms.

Once a methodology is selected, various choices can be made for (1) the method used to find values for θ_i defining a distribution likely to produce the observed dataset and for (2) the summary test statistic used to calculate the standard error for the test of whether the distributions in the two treatment arms are significantly different. The DerSimonian-Laird method models the between-study variance using the Cochrane test statistic and works reasonably well if the number of cases in each of the studies is not too small (>5). However, studies reporting no events must be adjusted with a correction factor (typically 0.5 is used instead of 0 cases). The now often-applied Hartung-Knapp correction for the DerSimonian-Laird technique uses a modified test statistic. The binomial-normal method uses iterative computation using the method of restricted maximum likelihood estimation (REML). The various statistical software packages offer different options for the specific method of REML used, meaning they will return slightly different results for the log(OR) and its 95% confidence interval.

Study motivation

The advantages of the binomial-normal model over the DerSimonian-Laird method for meta-analysis of count data are well demonstrated[2,4]. Despite this, DerSimonian-Laird remains a common methodology in the field of hemovigilance[5–7]. We find no study comparing univariate analysis of the dual-arm studies within a dataset and bivariate *binomial-normal* analysis of all studies. As bivariate binomial-normal methods allow inclusion of studies reporting on only one treatment arm, they have the potential to increase the accuracy of the effect size estimate. Given the aforementioned characteristics common to hemovigilance datasets, a demonstration of these methodologies on a real-world dataset and comparison of the methodologies via simulation study are warranted.

Using data from a recent meta-analysis on sparse hemovigilance count data including studies reporting on both or only one treatment arm, we demonstrated and compared various forms of the DerSimonian-Laird and binomial-normal techniques for calculating pooled odds ratios via meta-analysis. We then performed iterative simulation of meta-analyses using the same methodologies and compared their performance in analysis of randomly-generated datasets characteristic to transfusion-medicine meta-analysis with known OR. Syntax and explanations are provided for all methodologies in R,

SAS, and Stata.

METHODS

Demonstration of methodologies on real-world data

Using data from a recent meta-analysis comparing risk of transfusion-related acute lung injury (TRALI) between mixed-sex and male-only plasma, we demonstrated various techniques used to calculate the random effect pooled odds ratio comparing the two treatment-arms, and compared the results. On this example dataset, using those studies reporting on both treatments, we applied the *DerSimonian Laird method,* both (1) as described in their seminal 1986 publication[8] and (2) using the more recently suggested Hartung-Knapp correction for estimation of the between-study variance, the (3) *univariate binomial-normal method*, and the (4) *bivariate binomial-normal method*[4]. We additionally used the (5) *bivariate binomial-normal method* to analyze studies reporting on any (either one or both) treatments. Syntax for all methods is provided for SAS, Stata, and R in the online supplement.

Comparison of methodologies via iterative simulation of meta-analyses

Via iterative simulation using *SAS*, we compared these methods in meta-analyses of randomly generated sparse count data simulated to mimic characteristics of the example dataset. We varied (1) number of studies, (2) study sizes, and (3) heterogeneity in reported rate and compared the resulting predicted odds ratios to the known true odds ratio for the simulation. Details of simulation methods are summarized here and presented in supplemental figure 1.

Generation of Random data

Each simulation generated a dataset of some number of studies (15, 20, or 30) with some portion thereof reporting on both treatment arms (n_{both}), and an equal number reporting each on only the control arm or only the intervention arm (n_{one}): 15 study simulation (n_{both} = 5, n_{one}= 5); 20 study simulation (n_{both} = 10, n_{one} = 5); 30 study simulation (n_{both} = 20, n_{one} = 5). Study sizes were chosen randomly from uniform distributions with varying ranges - either 10^4-10^7 (to simulate a mix of small, medium, and large studies), 10^5-10^7 (to simulate a mix of medium and large studies), or 10^6-10^7 (to simulate only large studies).

To simulate data similar to the example data, we modelled correlated bivariate normal distributions using the covariance matrix reported by the *bivariate binomial-normal* method applied to studies reporting on both treatment arms (the fourth result in our demonstration). Thus, every call to the routine yielded a pair of odds (one for each treatment arm) from correlated distributions with means and variance (both within-study and between-study) equal to those estimated by analysis of the example data, making the resulting log(OR) from that example $\log(OR_{true})$. For each of the two resulting odds, we chose random

Table 1: Studies investigating incidence of TRALI for mixed-sex and male-only FFP from a recent meta-analysis we published. These were the data used as the example dataset and as a base for the datasets generated in our simulation study. Abbreviations: # units (number of units); FFP (Fresh Frozen Plasma); OR (odds ratio); TRALI (Transfusion Related Acute Lung Injury)

		control group		intervention group		
		# units		# units		
First author	*Year*	*mixed-sex FFP*	*TRALI*	*male-only FFP*	*TRALI*	
Michlig	2003	13033	0	-	-	studies investigating only control group
Silliman	2003	19411	1	-	-	
Norda	2006	745500	4	-	-	
Eder	2007	4864144	24	-	-	
Flesland	2007	901435	22	-	-	
Jutzi	2008	120000	11	-	-	
Keller-Stanislawski	2009	2000000	30	-	-	
Blumberg	2010	143945	5	-	-	
Funk	2010	15601000	84	-	-	
Keller-Stanislawski	2010	2000000	30	-	-	
Ozier	2011	337980	12	-	-	
Lin	2012	2250000	20	-	-	
Porretti	2012	90000	4	-	-	
Odaka	2013	55861	3	-	-	
Politis	2014	217173	3	-	-	
Harvey	2015	400213	3	-	-	
Hussain	2015	13620	1	-	-	
Kumar	2016	23800	4	-	-	
Eder	2010	3340474	38	1729128	7	studies investigating both study arms
Arinsburg	2012	147742	4	52230	0	
Eder	2013	1664598	31	6695037	28	
Jimenez-Marco	2014	10386	2	11122	0	
Funk	2015	4700000	50	4840000	2	
Funk	2012	-	-	1080000	0	studies investigating only intervention group
Bux	2013	-	-	343831	0	

zero-cells

n_a (# exposed, control group)

$cases_a$ (# events, control group)

n_b (# exposed, intervention group)

$cases_b$ (# events, intervention group)

variables from binomial distributions with p=odds and n=the randomly selected study size. For studies reporting only one arm, we dropped the unused study arm, resulting in a dataset with 15, 20, or 30 studies reporting cases and exposed for either one or both treatment arms.

Application of described methodologies

We applied the five methodologies described above to each dataset. For the univariate analyses – (1) DerSimonian-Laird, (2) DerSimonian-Laird with

Hartung-Knapp correlation, and (3) univariate binomial-normal method – we provided no starting guesses for the odds in the two treatment arms and the between- and within-study variances. For the bivariate binomial-normal methods – both applied to studies reporting on (4) both treatment arms or (5) only one treatment arms – we first ran a fixed effect analysis and used the resulting predictions for these quantities as starting guesses for the random-effect model. Random-effect models failing to converge on solutions thus returned the fixed-effect estimates.

We repeated our simulation 250 times on each of five datasets of (1) 15 small, medium, and large studies; (2) 15 medium and large studies; (3) 15 large studies; (4) 20 small, medium, and large studies; (5) 30 small, medium, and large studies. All calculations were performed on the log-scale. For each simulation run, we collected the five resulting predicted log(OR)s and standard errors of the log(OR) ($se_{log(OR)}$), along with a flag indicating whether each method had successfully converged on a solution for each.

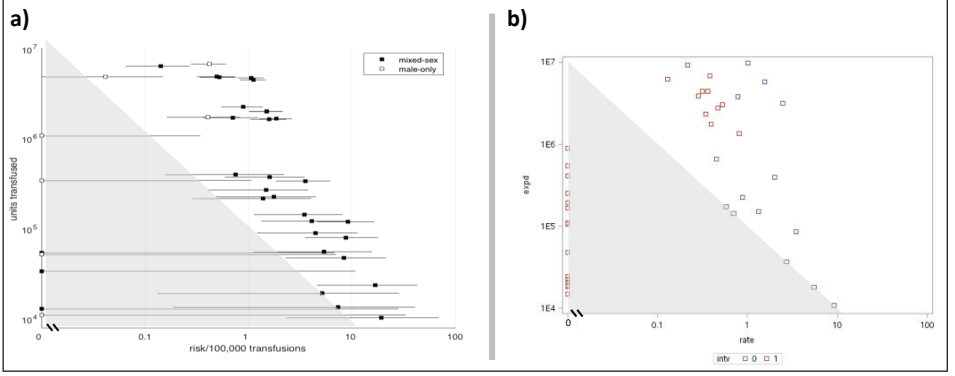
Comparison of methodologies
For each of the five methodologies, we calculated the following outcomes for comparison.

mean $\varepsilon_{log(OR)}$:	using those simulation runs which returned a result for log(OR), we calculated the mean absolute error in the estimated log odds ratio $\varepsilon_{log(OR)} = \lvert log(OR_{true}) - log(OR_{pred} \rvert$
mean $se_{log(OR)}$:	using those simulation runs which returned a result for $se_{log(OR)}$, we calculated the mean standard error in the estimated log odds
p_{conv}:	proportion of the simulated runs for which that methodology converged on a solution for log(OR)
$p_{success}$:	proportion of the simulation runs for which the true log(OR) lay within the confidence interval of the predicted log(OR). Here a method failed if $se_{log(OR)}$ was not returned)
p_{H1}:	proportion of the simulation runs for which H_0 was rejected at the α=0.05 level (i.e. the portion of the runs for which the 95% confidence interval did not include 1 resulting in a p value of less than 0.05). Here again a method failed if $se_{log(OR)}$ was not returned
p_{best}:	proportion of the simulated runs for which that methodology reported the lowest absolute error in log(OR)

RESULTS

Comparison of methodologies on real-world data
Table 1 shows data from our recent meta-analysis comparing risk of TRALI (a severe acute pulmonary condition associated with antibodies in the serum

Figure 1: Comparison of (a) real-world dataset and (b) example of simulated dataset used in simulation study. Data shown in (a) are the example dataset from a comparison of risk of an adverse event for two plasma types (mixed-sex and male-only) from Saadah et al. (2017) and were used as a base for modelling of simulated data. Note that the x-axis is broken to show 0 and as both axes are log10 scaled, no studies can populate the gray area (this would require reporting a non-integer number of cases between 0 and 1).



of ever-pregnant women) following transfusion for two types of fresh frozen plasma (FFP) – plasma from mixed-sex donors (mixed-sex FFP) and plasma from only male donors (male-only FFP). 25 studies reported cases of TRALI and number of plasma units transfused, with 20 reporting only on mixed-sex plasma only, five reporting on both mixed-sex and male-only plasma, and two reporting only on male-only plasma. By choice, we consider studies on mixed-sex plasma as the control analysis arm and studies on male-only plasma as the intervention group.

The studies vary in size from 10,386 to 15.6 million units of plasma transfused and report a total of 423 cases of TRALI. The unweighted pooled odds of TRALI are 0.97 and 0.25 cases per 100,000 units transfused in the control and intervention arms, respectively. Breaking these into three categories (small studies: studies observing $<10^5$ plasma units transfused; medium studies: 10^5-10^6; large studies: $>10^6$), the control and intervention groups each have a mix of all three study categories. Given the rarity of the event being studied, five of the studies reported no cases of TRALI (resulting in zero-cells)

Figure 1a shows this data as a plot of TRALI odds vs. study size (both axes are $\log_{10}$-scaled and in order to show zero-cells, the x-axis is broken). The reported odds are highly heterogeneous, spanning three orders of magnitude from less than 0.1 to more than 10 cases of TRALI per 100,000 plasma units transfused. Additionally, there is a strong correlation between reported odds and study size, with smaller studies reporting lower odds, and vice versa. Note that the log-log nature of the plot means no studies can populate the gray-shaded area

(as this would require observing a non-integer number of cases between 0 and 1).

Table 2 shows the results of meta-analysis on this dataset using the (1) *DerSimonian Laird,* (2) *DerSimonian-Laird (Hartung-Knapp),* (3) *univariate binomial-normal*, and (4) *bivariate binomial-normal* methods performed on the five studies reporting both arms, and (5) *bivariate binomial-normal* method performed on all 25 studies. For each methodology, the predicted $\log(OR_{pred})$ comparing the treatment to the control group and 95% confidence intervals calculated using SAS, Stata, and R, are presented.

The five methodologies return different values for the OR_{pred} when analyzing the five dual-armed studies, with the DerSimonian-Laird techniques yielding $\log(OR_{pred}) \approx -1.6$, and both univariate and bivariate binomial-normal techniques yielding $\log(OR_{pred}) \approx -1.9$. Analyzing all studies together, the bivariate binomial-normal technique yielded an even lower $\log(OR_{pred}) \approx -3$. For each give methodology, the three statistical programs return nearly identical results for $\log(OR_{pred})$, with small differences in the confidence intervals due to differences in how they calculate the standard error.

Table 2: Results of calculation of $\log(OR_{pred})$ via five statistical methodologies on example meta-analysis dataset. Calculations were performed in three statistical packages (Stata, SAS, R) with the name of function used provided in italicized text. Numbers in () are 95% confidence intervals. All syntax is found in the online appendix. Abbreviations: OR_{pred} (predicted odds ratio)

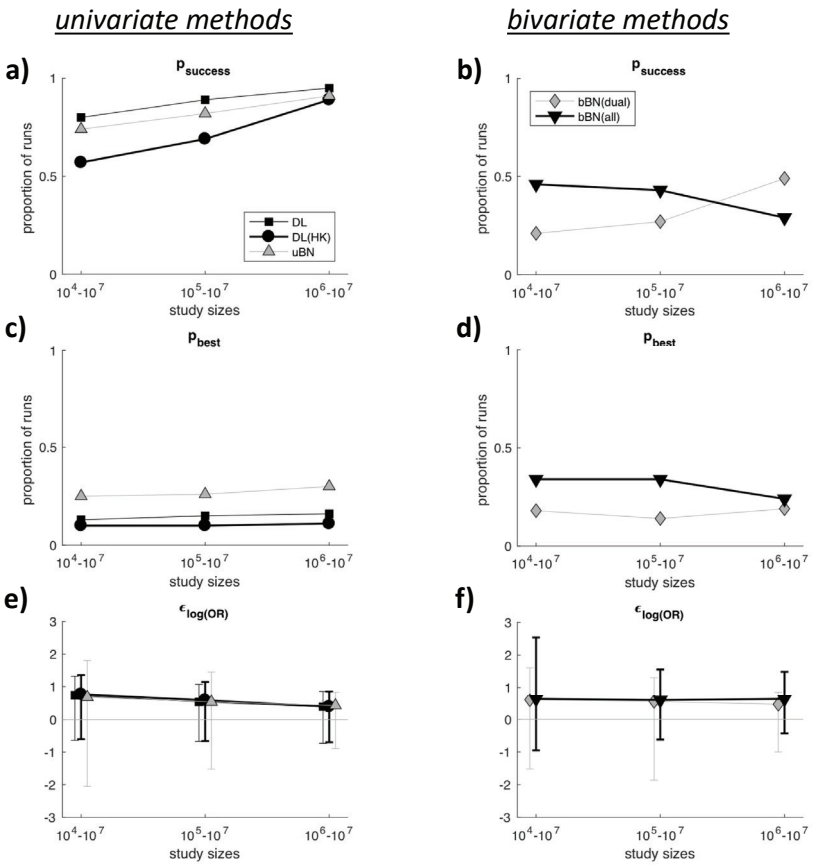
Method	*Stata*	*SAS*	*R*
DerSimonian-Laird (analytic)	*metan:* -1.626 (-2.347, -0.906)	*analytic eqs:* -1.626 (-2.347, -0.906)	*metabin:* -1.626 (-2.347, -0.906)
DerSimonian-Laird (Hartung Knapp)	*metareg:* -1.662 (-2.876, -0.448)	*proc mixed:* -1.662 (-2.876, -0.448)	*metabin:* -1.626 (-2.694, -0.558)
univariate BN (studies w/both arms)	*xtmelogit:* -1.941 (-2.907, -0.974)	*proc nlmixed:* -1.941 (-3.310, -0.572)	*rma.glmm:* -1.941 (-2.907, -0.974)
bivariate BN (studies w/both arms)	*xtmelogit:* -1.805 (-2.707, -0.904)	*proc nlmixed:* -1.806 (-3.274, -0.339)	*glmer:* -1.805 (-3.269, -0.659)
bivariate BN (studies w/any arms)	*xtmelogit:* -3.011 (-5.128, -0.894)	*proc nlmixed:* -3.013 (-5.253, -0.773)	*glmer:* -3.050 (-5.257, -0.841)

* all results presented as log(OR)

Comparison of methodologies via simulation study

Figure 1b shows odds vs. study size for a sample dataset generated by our simulation, in this case one of the datasets with 20 small, medium, and large studies. Visual inspection of the plot shows the heterogeneity in reported odds and the correlation between odds and study size are similar to those of the

Figure 2: Comparison of methodologies with 15 studies, 5 dual-arm and 10 single-arm, with sizes chosen from three ranges: (1) 10^4-10^7, (2) 10^5-10^7, (3) 10^6-10^7. Univariate methodologies (figures 2a, 2c) applied are (1) DerSimonian-Laird [DL], (2) DerSimonian-Laird (Hartung-Knapp) [DL(HK)], and (3) univariate binomial normal methods [uBN], while the bivariate binomial-normal method is applied to (4) dual-arm studies [bBN(dual)], and (5) all studies [bBN(all)] (figures 2b, 2d). Portion of simulation runs for which the odds ratio lay within that method's reported 95% confidence interval ($p_{success}$; figures 2a, 2b), portion of runs for which that methodology had the lowest error in predicted odds ratio (pbest; figures 2c, 2d), and mean absolute error in predicted log(OR) ($\varepsilon_{log(OR)}$; figures 2e, 2f) are plotted for each study size range.



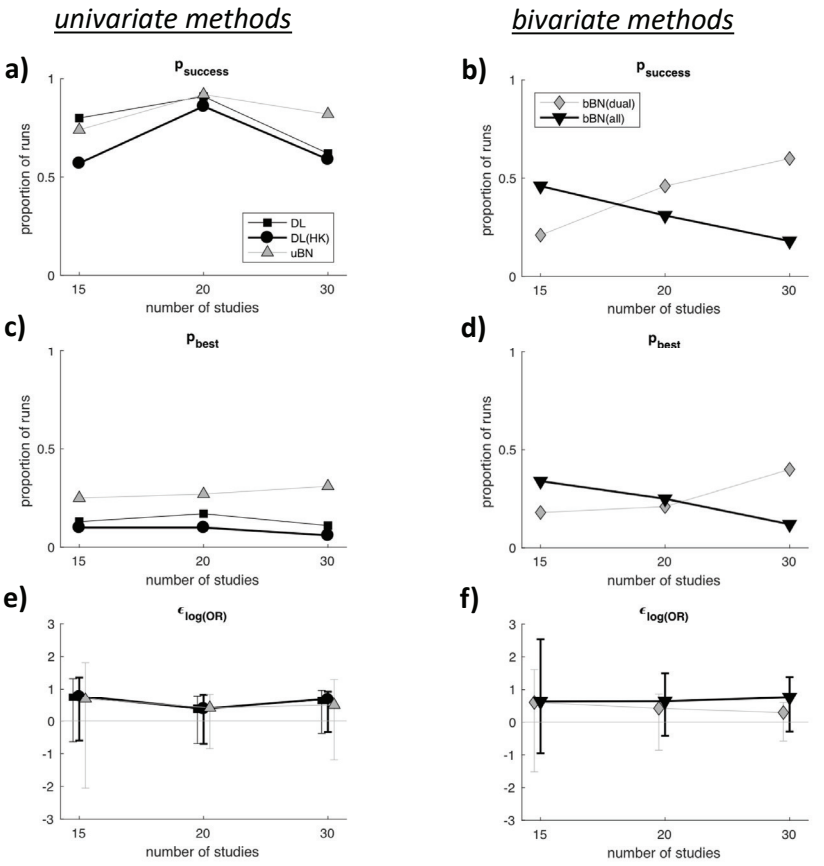
TRALI example dataset. For this sample dataset, the unweighted log(odds) in the two control and intervention arms were -11.64 and -12.98, making OR_{true} -1.34 (compared to -11.54 and -12.90 with OR_{true} = -1.36 for the TRALI example dataset).

Supplemental table 1 shows the numeric results of five 250 run simulations, with varying study sizes and numbers of studies, comparing the five methodologies in prediction of the true odds ratio, with the results visually presented in figures 2 and 3. Successful convergence on a solution was an issue for several methods. The uncorrected DerSimonian-Laird and univariate binomial-normal methods always or nearly always converged on a solution, as did the fixed effect bivariate methods used to calculate a starting guess for the random-effect bivariate methods (p_{conv_uBN} = 0.95-1; p_{conv}=1 for univariate and fixed-effect bivariate methods). However, the Hartung-Knapp corrected DerSimonian Laird and random-effects bivariate methods often or always failed ($p_{conv_DL(HK)}$ = 0.67-0.92; p_{conv_bBN} = 0.27-0.64 when analyzing dual-arm studies, and 0 when analyzing all studies). As such, many or all (in the case of bivariate BN analysis all studies) of the results used to calculate $\varepsilon_{log(OR)}$ for the bivariate methods are those of fixed-effect analysis.

Comparison methods for various study sizes
Comparing the results of the first three simulations (sim #1: 15 small, medium, and large studies; sim #2: 15 medium and large studies; sim #3: 15 large studies) allows observation of the effect of study sizes on each methodology. Figure 2, comparing univariate and bivariate methodologies for these three study size ranges, shows the univariate methods outperform the binary methods in successfully calculation of 95% confidence intervals including the true odds ratio (figure 2a, 2b). The five methods compared to do not differ greatly in mean $\varepsilon_{log(OR)}$, though the variance the estimate errors is smaller for the DerSimonian-Laird methodologies (figures 2e, 2f). Despite this, the DerSimonian-Laird methodologies rarely yielded the best estimate for log(OR), being outperformed by both univariate and bivariate BN methods (figures 2c, 2d). Importantly, in all but our simulation of 15 large studies (sim #3), inclusion of the single-armed studies in the bivariate BN method yielded a more accurate estimate of the log(OR), despite these results being fixed-effect, rather than random-effect estimates owing to the non-convergence of this method discussed above.

Comparison methods for various numbers of studies
Comparing the results of simulations one, four, and five (sim #1: 15 small, medium, and large studies; sim #4: 20 small, medium, and large studies; sim #5: 30 small, medium, and large studies) allows observation of the effect of number of studies analyzed on each methodology. Figure 3, comparing univariate and bivariate methodologies for three numbers of studies, shows the univariate methodologies continue to outperform the bivariate methodologies in calculation of confidence intervals including the true odds ratio (figures

Figure 3: Comparison of methodologies with 15, 20, or 30 studies with study sizes chosen from the range 10^4-10^7. Univariate methodologies (figures 3a, 3c) applied are (1) DerSimonian-Laird [DL], (2) DerSimonian-Laird (Hartung-Knapp) [DL(HK)], and (3) univariate binomial normal methods [uBN], while the bivariate binomial-normal method is applied to (4) dual-arm studies [bBN(dual)], and (5) all studies [bBN(all)] (figures 3b, 3d). Portion of simulation runs for which the odds ratio lay within that method's reported 95% confidence interval (psuccess; figures 3a, 3b), portion of runs for which that methodology had the lowest error in predicted odds ratio (pbest; figures 3b, 3c), and mean absolute error in predicted log(OR) ($\varepsilon_{\log(OR)}$; figures 2e, 2f) are plotted for each study size range.



3a, 3b), with univariate BN here clearly outperforming the DerSimonian-Laird methodologies as number of studies increases. The univariate BN method most often returns the best estimates, especially as number of studies increases (figures 3c, 3d). Of interest is the comparative performance of the bivariate methods, where inclusion of single-arm studies seems to provide a clear advantage where number of studies is small, and a clear advantage as number of studies increases. While here again mean $\varepsilon_{\log(OR)}$ differs only marginally between the methods, despite its outperformance by univariate BN methods, bivariate analysis of dual-armed studies stands out for its low mean $\varepsilon_{\log(OR)}$ (figure 3f).

DISCUSSION

In this simulation-based comparison of five methodologies in meta-analysis of sparse hemovigilance count data, univariate binomial-normal methodolodies most often provided the best estimate of log(odds ratio) comparing the two treatment groups, clearly outperforming DerSimonian-Laird methodologies. Inclusion of single-arm studies using bivariate binomial-normal methods can slightly increase the estimate accuracy when number of studies is low, though their accuracy decreases quickly as study number increases and more single-arm studies are added to the dataset. While the advantages of binomial distribution-based techniques in analysis of sparse count data over the commonly applied DerSimonian-Laird technique are well documented, we find no previous study comparing of error in predicted odds ratio between univariate BN methods on dual-armed studies and bivariate BN methods with inclusion of single-arm studies.

Inclusion of single-arm studies has as its advantage a larger analyzed dataset. However, given the high heterogeneity of reported risks characteristic of hemovigilance datasets, inclusion of studies without a comparator means an unmatched increase in the heterogeneity of the reported odds for one treatment arm. Whether inclusion of single-arm studies ultimately increases or decreases the accuracy of the resulting estimate depends on the dataset in question and cannot be prospectively determined. That the overall effect is one of increased accuracy where study size is small is encouraging, as hemovigilance meta-analysis datasets often have as many or more single-arm studies than dual-arms studies. However, even here the increase in accuracy was small ($\varepsilon_{\log(OR)\ uBN} = 0.68$ [95%CI (confidence interval): -2.05, 1.81]; $\varepsilon_{\log(OR)\ bBN(all)} = 0.63$ [95%CI: -0.95, 2.54]). Univariate BN methods on dual-arm studies additionally outperformed bivariate BN methods on only dual-arm studies – an expected result given the high heterogeneity – leading us to our finding of univariate BN as the safest choice for clinicians performing meta-analysis on hemovigilance datasets.

Convergence of methodologies
Successful convergence on a solution was a problem for several of the methodologies, most predominantly the random-effect bivariate BN method when single-arm studies were included (bBN_{all}) which never successfully improved upon its fixed effect estimate. To ensure this was not an issue with SAS, the statistical program in which the simulation study was run, we analyzed sample datasets from our simulation in R and Stata, neither of which were able to find solutions. Given the high heterogeneity of the data analyzed, this is not a necessarily surprising result as inclusion of uncoupled studies increases the variance in one arm without a concurrent increase in the other, making it more difficult for the software to find a distribution which could realistically yield the observed data. The low convergence of the random-effects models ultimately meant comparison of random effects estimates with a mix of fixed- and random-effects estimates. We felt this was the most honest method to compare these techniques, though we note the results would be different were we to compare methodologies using only those datasets for which they successfully converged on a solution.

Standard errors of predicted odds ratios
A key issue we faced in comparison of these methodologies was a failure to return a standard error for the log(OR), or in some cases the returning of a negative value (a mathematical impossibility). In these cases, we were unable to calculate a confidence interval for the log(OR) estimate, the result of which is a failure of that method to include the true log(OR) in it's confidence interval. This was particularly common among the binomial-based methods, explaining their low $p_{success}$ values despite their tendency to provide the best estimates and have high p_{best} values.

Limitations
Our simulation study comparing these five methodologies did so for datasets created from one covariance matrix, making the true odds and odds ratios the same for all our simulations, and did so for five combinations of study size and number of studies. The absolute errors on the log(OR) estimates, being characteristic of data with this specific covariance matrix, are here much less relevant than their comparative values between the methods. Additionally, our decision to use proportion of runs for which a given methodology returned the lowest $\varepsilon_{log(OR)}$ (p_{best}) as our primary outcome measure is not the only logical choice, with p_{H1} or $p_{success}$ also being reasonable choices. We felt p_{best} was the most appropriate outcome measure given the ultimate goal of meta-analysis is calculation of an accurate pooled odds ratio comparing two study arms.

Conclusions
Clinicians would do well to use univariate binomial-normal measures on studies reporting both arms in meta-analysis of sparse hemovigilance count data, as they consistently outperform DerSimonian-Laird techniques and bivariate binomial-normal methods. Using bivariate binomial-normal methods

with single-arm studies included may provide slightly better odds ratio estimates when number of studies analyzed is small, but their results quickly lose accuracy as number of studies increases.

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“The key lies not in ditching the chain, but in learning to better spot the ball.”

Discussion

7

Goal of dissertation
The ultimate goal of this dissertation is the comparison of two plasma products with regard to use, effectiveness, and risk of transfusion reactions. Many of the points made here are done so using examples from studies on the safety of blood products (hemovigilance). However, the lessons learned apply largely to any analysis of sparse clinical count data.

Background
Plasma transfusion is indicated in a wide set of medical situations with its use falling broadly into two categories – replenishment of coagulative proteins or removal of an insulting entity via plasma exchange. Plasma transfusion carries risks, the most serious of which are rare, and which are reported with varying success. Various options exist for transfusion of human plasma, including fresh frozen plasma (FFP) and solvent/detergent treated pooled (SD) plasma. On January 1, 2014, the Netherlands switched from FFP to Omniplasma, an SD plasma – the comparison of these two plasma types with regard to use, effectiveness, and safety are the subjects of this dissertation.

Table 1: Plasma transfusion reactions and definitions

Transfusion reaction	*ISBT* definition*
allergic reaction	mucocutaneous allergic symptoms only within 4 hours of transfusion
anaphylactic reaction	mucocutaneous allergic symptoms with airway compromise or severe hypotension
febrile non-hemolytic transfusion reaction (FNHTR)	fever (≥39ºC oral or equivalent and a change of ≥2ºC from pretransfusion value) and chills/rigors within 4 hours of transfusion
hypotensive reaction	drop in systolic blood pressure of ≥ 30 mm Hg and a systolic blood pressure ≤ 80 mm Hg within one hour of transfusion
transfusion associated circulatory overload (TACO)	any four of (1)acute respiratory distress (2)tachycardia (3)Increased blood pressure (4)acute or worsening pulmonary edema on frontal chest x-ray (5) evidence of positive fluid balance - within 6 hours of completion of transfusion.
transfusion related acute lung injury (TRALI)	acute onset of hypoxia absent left arterial hypertension with bilateral infiltrates on chest x-ray within 6 hours of transfusion

* International Society of Blood Transfusion

We compared use and effectiveness of the plasma products using two studies. In our *FROSTED study*, we collected patient-level transfusion data from six Dutch hospitals and compared plasma and concurrent RBC use between FFP and SD plasma. In our laboratory *TEG study*, we used throboelastography (TEG) to compare the fibrinolytic states of whole blood reconstituted with the two products. Following a short

discussion of methodological considerations, we present our comparisons of transfusion reaction risk for various plasma products, performed by studying plasma use and outcomes in other countries via meta analysis of peer-reviewed studies (*meta-analysis*) and analysis of unpublished international hemovigilance data (*ISTARE study*). Methodological considerations culminated in a comparative methodological study (*methodological study*).

Estimating plasma effectiveness via use (FROSTED study):
Given the size difference between FFP (300-330mL) and SD plasma (200mL)[1], transfusing an equivalent volume of SD plasma would require an increased number of units transfused as compared to FFP. However, the expectation was that protocols would not be adjusted and that the number of administered plasma units would remain stable in all but plasma-exchange patients, who are treated with a specified plasma volume. This seems indeed to be the case as plasma use decreased by 13% from 2012-2017 (figure 1), a trend not reversed by the switch to SD plasma. In TTP/HUS patients, the number of administered units increased as expected.

As discussed earlier, we chose to analyze concurrently transfused red blood cell units and the plasma/red blood cell units ratio as our clinical outcomes representing effectiveness. Analyzing 14,000 patients treated in six Dutch hospitals receiving plasma and RBCs in the same transfusion episode (i.e. actively bleeding patients) stratified by treating ward and diagnosis, we found only minute changes in number of RBCs concurrently transfused among the cohorts (figure 2a) and no statistically significant differences in the plasma/RBC ratio with SD plasma as compared to FFP (figure 2b). The latter result suggests the switch to the smaller SD plasma units did not lead to significant changes in transfusion protocols. In those cohorts in which the differences in concurrently transfused RBCs are significant, the differences are small and typically indicate fewer RBCs were transfused alongside SD plasma. Bearing in mind that the SD plasma episodes are by definition more current and that blood

Figure 1: Use of FFP, SD plasma, and total plasma use for the years 2012-2017 in the Netherlands.

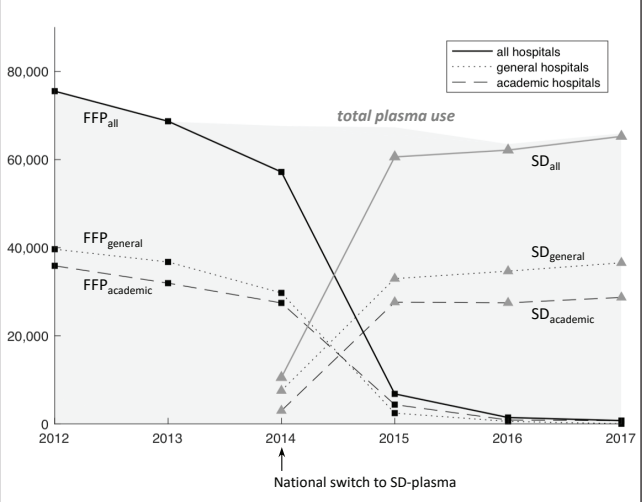
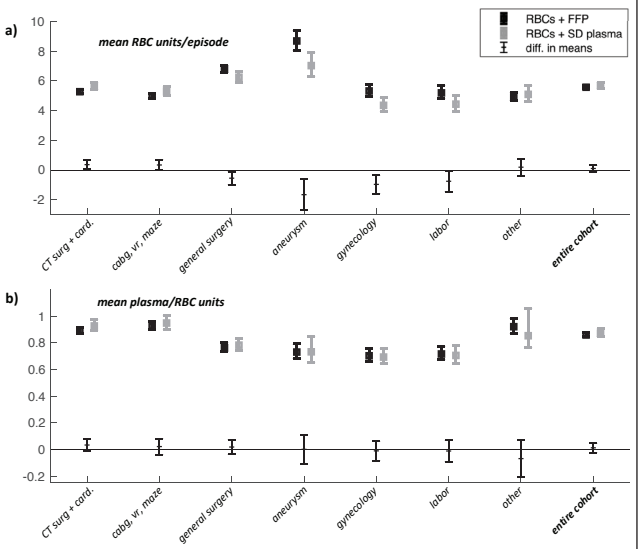


Figure 2: Mean (a) RBC units and (b) plasma/RBC units ratio for episodes involving transfusion of RBCs and either FFP or SD plasma, and difference in means for various patient groups (bars indicate 95%CIs)

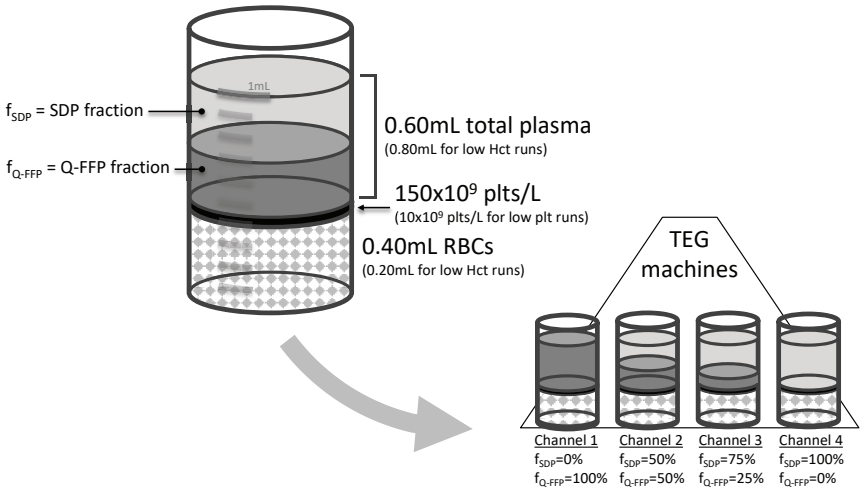


product use in the surgical setting is decreasing worldwide, our interpretation of these results is that SD plasma transfusion is not clinically associated with heavier bleeding than FFP.

Estimating plasma effectiveness via thromboelastography lab study (TEG study)

The potential association between SD plasma transfusion and the rare but often fatal outcome hyperfibrinolysis had been suggested by clinical study[2–4], though its association was not without counter argument[5]. Laboratory studies showed levels of the anti-fibrinolytic enzyme α_2-antiplasmin in SD plasma to be around half that of FFP[6–8]. Evidence suggests that if SD plasma is associated with an increased risk of hyperfibrinolysis, it is likely only in patients experiencing massive transfusion and the associated large-scale replacement of their native plasma with the α_2-antiplasmin-deficient SD plasma[2,3]. Given the rarity of massive transfusion and the likewise low incidence of hyperfibrinolysis within this patient group, the dataset needed to evaluate this association via clinical study was not realistically attainable. Instead, we studied this association as a measure of plasma effectiveness in the laboratory setting by reconstituting physiologically realistic whole blood samples and measuring clot lysis time using thromboelastography (TEG) analysis (figure 4). By filling the plasma fraction of the reconstituted blood samples with FFP, SD plasma, or a mixture thereof, we simulated progressive replacement of physiological plasma (FFP) with SD plasma. Our finding of a roughly 50% decrease in clot lysis time with SD plasma vs. FFP (figure 3) represents the most physiologically realistic lab study to date exploring this association. Additionally, by performing our study in reconstituted whole blood, we measured the influence of platelet count and hematocrit on this association. Our conclusions suggest a high SD plasma fraction leads to less effective clot maintenance and that anti-fibrinolytics should be considered when large volumes of SD plasma are transfused.

Figure 3: Schematic showing thromboelastography (TEG) study investigating association between SD plasma and fibrinolysis



Evaluating risk of transfusion reactions

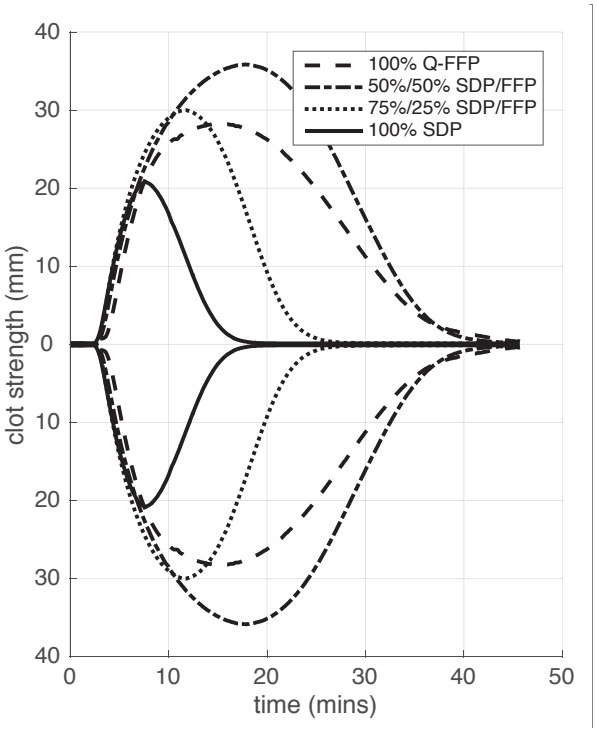
As Omniplasma is functionally equivalent to other SD plasmas, comparing other SD plasmas to FFP was a valid method of evaluating transfusion reaction risk for Omniplasma. We performed a systematic review and meta-analysis to compare various plasma types with regard to their risk of commonly reported transfusion reactions. Our systematic review on transfusion reaction incidences show the level of heterogeneity in reported transfusion reaction rates to be staggering and the number of zero cells high. These characteristics – sparse data with a high heterogeneity in reported effect – are common to hemovigilance data.

Consideration of how to analyze highly heterogeneous sparse data formed much of the work behind this dissertation, and as such is here thoroughly discussed. To demonstrate these characteristics, we present data from our systematic review and meta-analysis showing incident count data on TRALI collected from the peer-reviewed knowledge base. We refer to this dataset throughout this discussion. This is the same dataset used as demonstrative data in our methodological study.

Challenges of hemovigilance data

Figure 5 shows data from our systematic review on the risk of transfusion associated acute lung injury (TRALI) for two types of plasma, mixed-sex (control group) and male-only (intervention group). Each data point represents

Figure 4: Example TEG curves showing clot formation/lysis for differing SD plasmafractions



a reported risk, presented as incident count data (number of *cases* of TRALI reported by a study and number of *exposures* – in this example number of transfused plasma units reported by that study). Figure 6 on the next page shows these data visually as a forest plot with risk of TRALI per 100,000 units on the x-axis, and number of transfused plasma units on the y-axis (both axes are log scaled – note too that the x-axis is broken to show zero). Three data characteristics are clearly present: (1) the data are sparse (2) there is a high heterogeneity in reported outcome and (3) many studies reporting on only one of the two study arms (thus in this case studies reporting risk for mixed-sex plasma or for male-only plasma, but not both). Data with these three characteristics are representative of many datasets collected to answer hemovigilance related issues. We discuss them here individually.

Sparse data (zero-cells)

When many of the reported outcomes in a dataset are 0, the dataset is said to be 'sparse.' In this case, 6 of the 26 studies reported no cases of TRALI (2 in studies on mixed-sex plasma, and 4 in studies on male-only plasma) – these data are referred to as 'zero-cells'. When pooling the reported outcome (here risk of TRALI) as most meta-analyses do, zero-cells pose a problem if linear methods, such as the commonly applied DerSimonian-Laird method, are used.

Linear methods fit a normal distribution to the outcome, which may be a rate (e.g. risk) or a rate ratio (e.g. risk ratio, odds ratio). The distributions of these outcomes are not normal, but the log-scaled distributions are often approximately normal. For this reason, linear methods perform their analysis on

log(outcome). As the log of zero is undefined, studies reporting no cases of the outcome would lead to mathematical error. As such, a correction factor, typically 0.5, is added to zero-cells. While ½ a case makes no logical sense, it is mathematically valid and allows computation to continue. However, this represents addition of false cases into the analysis, thus decreasing the overall accuracy of the pooled result.

High heterogeneity in reported effect

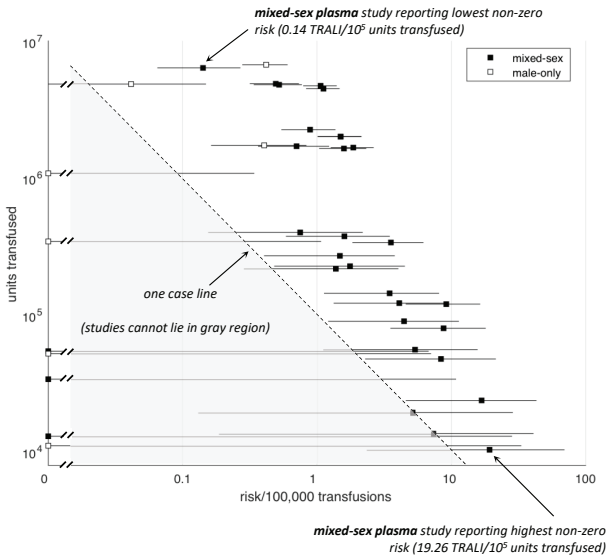
Observing figure 5, if we consider only those studies which observed at least one TRALI case (and could therefore calculate a non-zero TRALI risk), the range in reported risk is 0.14 to 19.26 TRALI/100,000 plasma units transfused. Thus, the study reporting the highest risk reports a risk *135 times higher* than the study reporting the lowest risk. In fact, the data are so spread that the x-axis of figure 6 needed to be log-scaled to allow convenient display. This is

Figure 5: Incident count data on risk of TRALI for mixed-sex and male-only plasma (meta-analysis)

		control group		intervention group		
		# units		# units		
First author	Year	mixed-sex FFP	TRALI	male-only FFP	TRALI	
Michlig	2003	13033	0	-	-	studies investigating only control group
Silliman	2003	19411	1	-	-	
Norda	2006	745500	4	-	-	
Eder	2007	4864144	24	-	-	
Flesland	2007	87000	22	-	-	
Politis	2007	54435	0			
Jutzi	2008	120000	11	-	-	
Keller-Stanislawski	2009	2000000	30	-	-	
Blumberg	2010	143945	5	-	-	
Funk	2010	15601000	84	-	-	
Keller-Stanislawski	2010	2000000	30	-	-	
Ozier	2011	337980	12	-	-	
Lin	2012	2250000	20	-	-	
Porretti	2012	90000	4	-	-	
Odaka	2013	55861	3	-	-	
Politis	2014	217173	3	-	-	
Harvey	2015	400213	3	-	-	
Hussain	2015	13620	1	-	-	
Kumar	2016	23800	4	-	-	
Eder	2010	3340474	38	1729128	7	studies investigating both groups
Arinsburg	2012	147742	4	52230	0	
Eder	2013	1664598	31	6695037	28	
Jimenez-Marco	2014	10386	2	11122	0	
Funk	2015	4700000	50	4840000	2	
Funk	2012	-	-	1080000	0	studies investigating only inter-vention group
Bux	2013	-	-	343831	0	

zero-cells

Figure 6: Forest plot, risk of TRALI per 100,000 plasma units transfused (meta-analysis)



an example of heterogeneity in reported outcome. High heterogeneity increases the standard error, and thus decreases the accuracy with which the pooled outcome can be reported. Mathematically, this results in wide confidence intervals on the pooled outcome measure (i.e. lower level of statistical significance or an increase in the p-value).

Heterogeneity in reported outcome arises from any number of causes – including genuine physiological differences in the patient populations being studied. In our case, there is little doubt that physiological differences alone cannot account for the large variance observed in reported TRALI risk. Rather this likely arises due to the realities of how hemovigilance data is collected. A chain requiring diagnosis, reporting to the hospital, followed by reporting to a national office must occur during treatment of a patient group sick enough to need plasma transfusion such that any transfusion related effects risk being masked. It may in hindsight be the natural expectation that a large variance would exist in the vigilance with which these events are dutifully diagnosed, reported to and collected by a central agency. It may too be a natural expectation that such a strong correlation would exist between study size and reported TRALI risk (note how smaller studies report higher risk and vice versa), as the size of the patient population observed could be reasonably expected to be associated with the vigilance with which transfusion associated adverse events can be dutifully diagnosed and reported.

Single armed studies

Note in figure 5 the propensity of studies reporting on only one study arm (19 studies report on mixed-sex plasma, 2 on male-only plasma, 5 on both). Traditional meta-analysis is performed on a comparative measure, such as risk ratio or odds ratio. Thus, using these methods, we could pool only the five studies reporting outcomes for both treatment arms. This would mean

discarding 21 of the 26 studies in analysis, yielding no useful data from them.

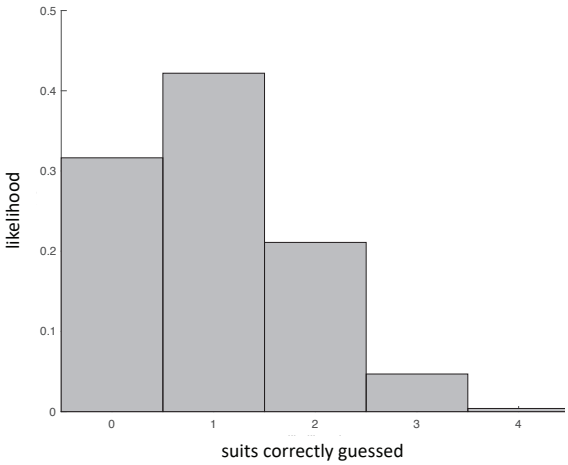
Taking these three data characteristics (sparse data, high heterogeneity, single-armed studies) into consideration, we chose to pool our incidence count data using *bivariate binomial-normal* methods. Doing so addresses all three of these characteristics common to hemovigilance data. We discuss how each is addressed in detail.

A description of binomial-normal methods

Pick a card, any card - logistic regression for sparse data

Imagine an experiment in which I ask individuals to guess the suits of four playing cards presented to them face down and record how many of the four were correctly guessed by each individual. Given there are four suits (♠,♥,♦,♣) and we allow each user to guess the suit of four cards, our expectation would be that people would, on average, guess the suit of one card correctly, for a mean proportion of 0.25 correct guesses. Were we to run this experiment on a thousand individuals and create a histogram of the results, we would expect to see something resembling figure 7.

Figure 7: Expected distribution of number of suits correctly guessed out of four attempts (binomial distribution with n=4 and p=0.25)



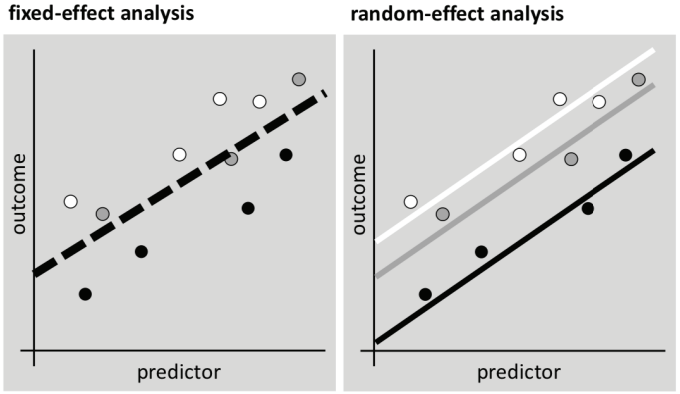
These data are clearly not normally distributed – rather they follow a binomial distribution, defined as the distribution resulting from a series of *Bernouli trials.* A *Bernouli trial* is an experiment with exactly two outcomes, *success* or *failure,* with a fixed *probability* of success (here ¼). Thus here, our data fit the distribution:

$$suits\ correctly\ guessed \sim Binomial(n,p)$$

where n=4 (number of Bernouli trials) and p = ¼ (probability of success). For the purposes of our TRALI data, a transfusion is likewise a *Bernouli trial*, where the two outcomes are *TRALI* or *no TRALI* and *p*, the *probability* of *success* is the *risk of TRALI per unit transfused*. In fact, hemovigilance research often centers on this type of data, *count data*, with the ultimate goal being calculation of

Figure 8: Subjective demonstration of fixed vs. random effect analysis

Example of fixed and random effect analyses, estimating the effect of a predictor on the outcome of interest, on a dataset with data from three independent groups. If the groups are analyzed together, the result is a poor curve-fit (large differences between predicted and observed outcome). When the grouping of the data is taken into account, we see the curve-fit is quite good. Thus the variance, and in turn the standard error of the effect estimate, are smaller, increasing the accuracy of the estimate.



the risk of a given event.

Pooling of data involves the fitting of a distribution to the dataset. As count data is not normally distributed, the convention is to work on the log scale, where log(p) is approximately normally distributed. As mentioned before, this requires the addition of correction factors (false ½ cases) to the dataset in the case of zero cells, as log(0) is undefined. Instead, we can fit the true binomial distribution to the number of cases reported, using the number of exposures (here number of plasma units transfused) as the number of *Bernouli trials*.

In fact, this is the technique of logistic regression, which fits a binomial distribution to the number of cases observed for a given number of exposures: *cases ~ Binomial(exposures, p)*. For reasons beyond the scope of this discussion, in logistic regression the dependent variable is logit(risk) which is the log(odds of the event). The binomial distribution is defined at zero (see figure 7 – in our card example, fully 30% of the individuals would be expected to guess 0 correct suits), thus no correction factors are needed for zero-cells when using binomial-distribution based techniques.

Random-effect models for heterogeneous data

The high heterogeneity of hemovigilance data provides the greatest challenge to successful comparison of blood products. No technique can completely correct for this, however using a random-effect technique can help.

If the risk of TRALI can be reasonable expected to remain consistent between patient groups, then we should model p, the risk, as a fixed value ($p=\theta$) – a

so called fixed-effect analysis. However, given the large range of reported values for TRALI risk and our knowledge of the pathophysiology of TRALI, this is clearly not here the case. Rather, we model p as a normally distributed variable – the sum of a constant (μ, the fixed-effect) and an intercept calculated separately for each study group (δ_i, the random-effect), or $p=\mu+\delta_i$. While a thorough mathematical exploration of the principles behind a random-effect model lie beyond the scope of this dissertation, a simplified example helps illustrate its use here to help cope with high heterogeneity in the reported outcome measure.

Figure 8 demonstrates the principles behind a random-effect analysis on an imaginary dataset comprising data from three sources using a plot of some arbitrary continuous predictor vs. a continuous outcome. The effect of the predictor on the outcome will be the slope of the line fit as closely as possible to the data, and the size of the standard error (the accuracy of the estimate, and thus the width of the confidence intervals) will depend on how close that curve-fit is. In a fixed effect model, the sources of the data are not taken into account and the line is fit to the entire dataset, leading to a poor curve fit (large distances between the line and the data points). In a random effect analysis, we see the curve-fit is very good when the sources of the data are taken into account.

While this example shows an analysis quite different to ours, the principle shown here holds too for our analysis. By allowing our outcome (risk of TRALI) to vary between studies, we can better account for the large heterogeneity in the reported TRALI risk. As the random effect is assumed to follow a normal distribution, a binomial model with a normally distributed random effect is referred to as a *binomial-normal* model.

Bivariate methods – inclusion of single-armed studies
Regardless of methodology used for pooling of the data, a study presenting data on only one of the two products of interest (as is the case for 21 of the 26 studies in our TRALI dataset) cannot be included if a comparative measure (e.g. risk ratio, risk difference, odds ratio) is the outcome measure the analysis pools. This leaves us with two options – univariate or bivariate analysis.

In a univariate analysis, a single variable must be chosen as the outcome measure – thus for comparison of two products, a *risk difference* or *odds ratio* would typically be used. Were we to use such an analysis, only those studies reporting on both treatment arms (5 of the 26 TRALI studies) could be included in the analysis.

A second option is to perform a bivariate analysis in which our two outcomes are the *odds of TRALI* (here odds≈risk) in the two treatment arms. By then comparing the odds, we can calculate an *odds ratio* (or alternatively, a *risk difference*). As the data in each arm are pooled separately, we can include

studies reporting on only one treatment arm. This has the potential to increase the accuracy of the estimate as we are able to pool more data. However, given we are pooling heterogeneous data, inclusion of data from a study in one arm without a corresponding result for the second likewise has the potential to decrease the accuracy of the effect estimate.

The bivariate binomial-normal method
These three methodological choices (binomial based distribution, random effect, bivariate) combine to form the *bivariate binomial-normal* method:

bivariate: indicates we model the odds in the two treatment arms separately and compare them to derive a comparative measure (*odds ratio* or *risk difference*)
binomial: indicates we assume the number of cases is binomially distributed [cases~binomial(n,p)], with n=number of plasma units transfused and p=risk of TRALI
normal: indicates we assume *logit(p)* is the sum of a fixed effect and a random effect and is normally distributed

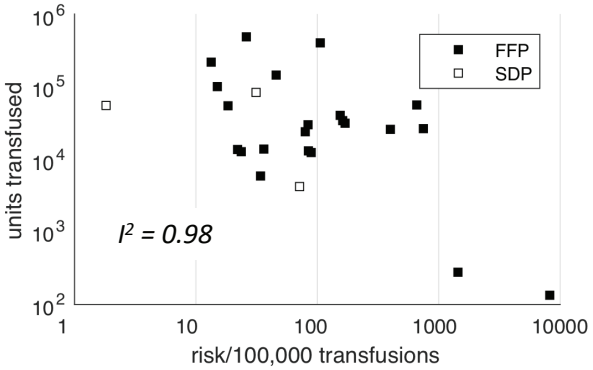
This is the model we used to analyze data in all three analyses of transfusion reaction risk (*meta-analysis, ISTARE study, FROSTED study*). The distribution based methodological choices (*binomial-normal*) are fairly obvious choices given the data characteristics and supported by a strong evidence base. However, the choice of whether or not to pool comparative (i.e. dual-arm) and non-comparative (i.e. single-arm) data in analysis of sparse count data (i.e. univariate vs. bivariate methods) has not been thoroughly explored. As such, we used the *bivariate* method for our analyses, while comparing univariate and bivariate methods in analysis of sparse count data became the subject of a methodological study discussed shortly.

Meta-analysis – comparing transfusion reaction risks using peer-reviewed data
Using our chosen methodology, we first compared risk of various transfusion reactions among plasma types using peer reviewed data in our meta-analysis. The example data used throughout this discussion is the TRALI dataset from our meta-analysis, analysis of which yielded a statistically significant result for the comparison of male-only to mixed-sex plasma with regard to TRALI risk ($RD_{SD/FFP}$ = -0.74 TRALI cases/10^5 plasma units transfused [95% confidence interval (CI) -2.42 to -0.42]). This is not a surprising result, given its evidence base[9–11].

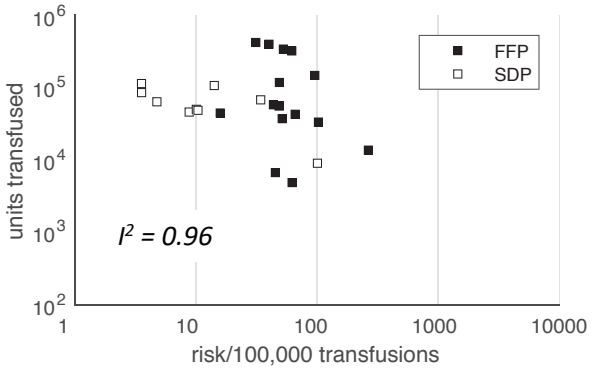
However, the large heterogeneity observed in the TRALI dataset persisted through analyses of every transfusion reaction analyzed. As an example, figure 9(a) shows risk vs. study-size (both log-scaled) for the FFP and SD plasma data on allergic plasma transfusion reactions from our meta-analysis. Note the extreme heterogeneity of the results, verified by the I^2 value of 0.98 (indicating

Figure 9: Risk of allergic reaction per 100,000 units transfused vs. study size for fresh frozen plasma and SD plasma - data from meta analysis (a) and ISTARE analysis (b). Note both axes are log-scaled.

(a) *Data from meta-analysis*



(b) Data from ISTARE analysis



only 2% of the observed heterogeneity could be reasonably attributed to natural variance). This heterogeneity led to a non-statistically-significant result for the comparison of TRALI risk between SD plasma and FFP despite the fact that no TRALI cases were ascribed to SD plasma transfusion. Note too that the comparison of SD plasma and FFP for risk of allergic reaction in our meta analysis was not significant (RD=0.10 [-0.23 to 0.45]; p=0.23), as we will reference this later. Here again, extreme heterogeneity kept us from reaching a conclusion for which a large evidence base exists.

ISTARE study – using repeated measures to battle heterogeneity

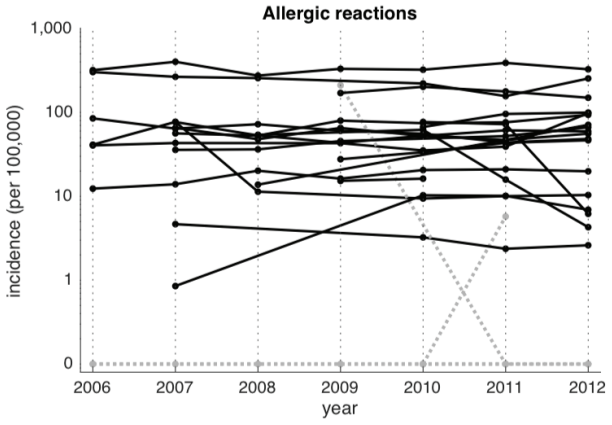
The ISTARE database, run by the International Hemovigilance Network (IHN), is a database of transfusion related adverse events reported annually by 24 countries with each country reporting cases and units transfused each year for a large set of transfusion reactions and blood products. We refer to this type of data as *repeated measures* data (such as the example data in figure 10). Recall that in our meta-analysis, most studies reported data from individual sources. As a dataset of *repeated measures,* the ISTARE database represents the type of data with which a random effect analysis can help increase estimate accuracies in spite of high heterogeneity. This in mind, we set out to answer the same research questions addressed in our meta-analysis, comparing risks of plasma

transfusion reactions for various plasma types, with the expectation of more accurate outcome estimates.

Figure 9(b) on the previous page shows the reported incidences of allergic reactions for FFP and SD plasma recorded in the ISTARE database, with all countries and years plotted together (x-axis, log scaled) vs. study size (y-axis, log scaled). As can be seen by comparing it to the plot above it (the same data for our meta-analysis), the data are likewise extremely heterogeneous. However, look at the same data grouped by country and plotted over time with incidence now on the (log-scaled) y-axis (figure 10). The heterogeneity is large, but within the sample of a given country, the reported incidences remain quite stable. Note how this data resembles that of the example data in figure 8 – giving us hope that the random effect analysis of the repeated measures of the ISTARE data might yield more accurate outcome estimates. Indeed, while our comparison of SD plasma to FFP with regard to allergic reactions risk did not yield the expected statistically significant result in our meta-analysis, in our ISTARE study it did ($OR_{SD/FFP}$ = 0.27 [0.21 to 0.36]), despite an almost equally high heterogeneity (I^2=0.96). We take this as a demonstration of the power of a random-effect model. Of note is the fact that despite the use of a random effect model, the large heterogeneity in reported TRALI risk was here again too large to yield the expected significant difference between SD plasma and FFP, despite the evidence base for this.

For that portion of the FROSTED study dealing with hemovigilance, we again analyzed repeated measures, though this time from a single data source, the Dutch hemovigilance office TRIP (Transfusie en transplantatieReacties In Patiënten). Here too we showed a statistically significant odds ratio comparing SD plasma to FFP with regard to allergic reactions ($OR_{SD/FFP}$ = 0.19 [95%CI: 0.11 to 0.34]). Here again the result for TRALI is insignificant.

Figure 10: Reported risk of allergic plasma transfusion reactions over time grouped by reporting country to the ISTARE database. Countries reported 0 or 1 case are connected with dotted lines.



Recall that two of the reasons given for the national switch to SD plasma were the expected reduction in the risk of allergic

reactions and TRALI[1], both of which are well documented in the knowledge base[12,13]. Of the three studies analyzed to compare these specific risks (*meta-analysis*, *ISTARE study*, *FROSTED study*), two showed a significant reduction in risk of allergic reactions while none could show the expected reduction in TRALI risk to a statistically significant level. Our TRALI analyses often involved comparing 0 cases to 1 case with a high standard error resulting from the extreme heterogeneity of the data. Proper modeling of the distribution and a random-effect model go only so far –at some point, heterogeneity wins over methodology.

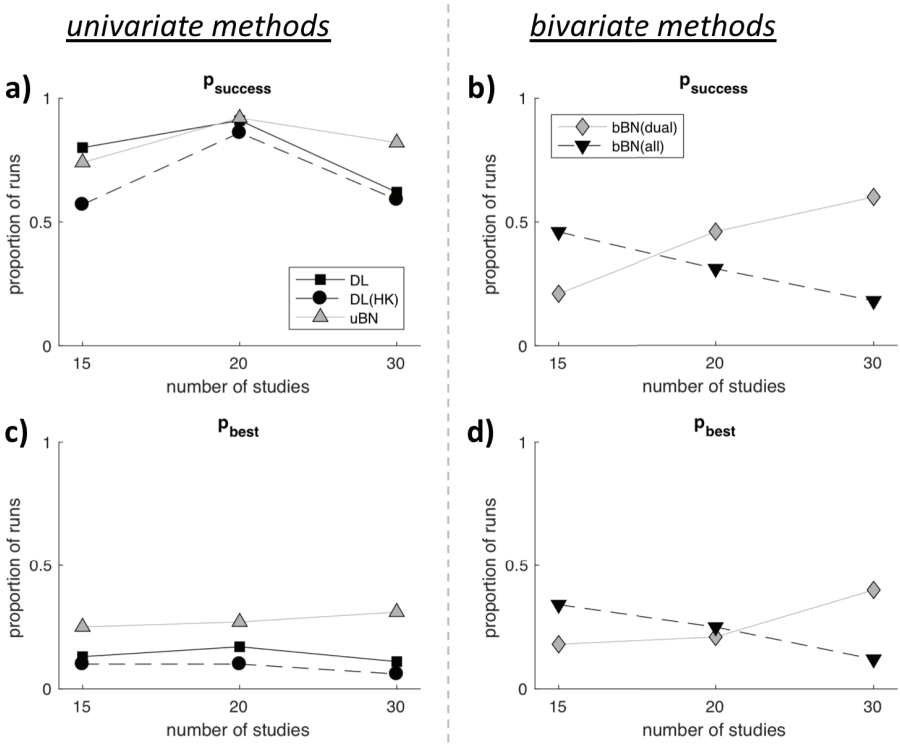
Methodological study – analyze single-arm studies too?
Recall figure 5 showing our meta-analysis data for risk of TRALI from mixed-sex and male-only plasma. Note again that of the 26 studies presented, 5 report on both treatment arms while 19 report on only mixed-sex and 2 report on only male-only plasma. Without an evidence base to guide our choice between a univariate analysis (using the union of the datasets) or a bivariate analysis (using the intersection of the datasets), we chose for the bivariate and designed a methodological study to address the issue.

To test the accuracy of univariate and bivariate methods, we began by using each to calculate the pooled odds ratio comparing risk of TRALI for SD plasma and FFP using the TRALI dataset. For completeness, we calculated the same OR using the DerSimonian-Laird technique. We found the three results (*DerSimonian-Laird, univariate binomial normal, bivariate binomial normal*) to be quite different but of course, not knowing the true OR, had no way of knowing which was more accurate.

To answer this question, we ran a simulation study in which we repeatedly simulated data similar to those in the TRALI example with a known OR and used each of the three methods to estimate the odds ratio and corresponding standard error. Comparing the predicted odds ratio to the (here known) true odds ratio allowed comparison of these methodologies in analysis of simulated sparse data with high heterogeneity and many non-comparative studies. We varied the range from which study sizes were randomly chosen and the number of studies in the analysis and compared, among others, univariate BN methods on dual-arm studies and bivariate BN methods on all (dual- and single-arm) studies in accuracy of the resulting predicted OR.

Figure 11 shows the results of three simulations, each of 1,000 meta-analyses on 15, 20, or 30 studies using five methods. While conclusions drawn from this specific simulation cannot be generalized, we show bivariate BN methods on all studies to be the most accurate method where there are few studies per meta-analysis, but they quickly lose accuracy as number of studies grows. Univariate BN methods on dual-arm studies returned more consistently accurate results and grew more accurate with rising number of studies. Given the risk to accuracy in addition of single-arm studies to the analysis, we

Figure 11: Comparison of methodologies in calculation of pooled odds ratio (OR) via meta-analysis of 15, 20, and 30 mixtures of dual- and single-arm studies. Abbreviations: DL (DerSimonian-Laird); DL(HK) (Hartung-Knapp corrected DL); uBN (univariate binomial-normal); bBN(both) (bivariate BN – dual arm studies); bBN(all) (bBN – all studies); $p_{success}$ (proportion of runs with OR_{true} in 95%CI of result); p_{best} (proportion of runs returning closest OR estimate)



recommended analysis of dual-arm studies only in highly heterogeneous sparse datasets such as those common to hemovigilance research.

Summarizing thoughts

We set out to compare FFP and SD plasma with regard to effectiveness and transfusion reaction risk. Our analysis of effectiveness, through lab study and concurrent blood product use, showed an association between SD plasma and fibrinolysis and demonstrated the switch to SD plasma was not associated with significant changes in clinical use. Our comparison of transfusion reaction risks, through meta-analysis and analysis of the ISTARE database, showed these to be lower for SD plasma for several transfusion reactions. Our methodological article showed inclusion of non-comparative studies in meta-analysis of count

data may decrease the accuracy of the resulting pooled comparative measure.

As important are the points made about hemovigilance data, as these points are generalizable to analysis of sparse clinical data. We discussed earlier how heterogeneity in reported risks arises from the reporting chain hemovigilance data must successfully complete, stretching from bedside to hemovigilance office with many steps along the way. We showed throughout this discussion how disruptive this high heterogeneity is to hemovigilance research. Our discussion of methodology is valid and the numerical methods applied appropriate, but exact statistical methods are ultimately only as useful as the data they analyze.

A look to the future

In American football, a team must advance the ball ten yards downfield within four attempts (downs) or lose possession of the ball to the other team. As such, how far downfield the ball has moved is of critical importance. If it can't be visually determined how far the ball has moved, a device consisting of two metal poles separated by a rigid chain is used to measure the distance with such precision that it takes a three-man team to do it and brings the game to a temporary halt.

This all sounds fine until you learn the process by which the ball is 'spotted' on the field for measurement. With play often ending in piles of a dozen large football players scrambling for the ball lying somewhere at the bottom, it is the job of the referee to determine where in 3-dimensional space the ball was when the carrying player's knees hit the ground, and 'spot' (place) the ball on the field directly underneath. And as statisticians shake their heads, a test of high precision is run against this woefully imprecise guess to determine who gets the ball.

This reminds me of where hemovigilance research stands. Investigation of rare events requires collection of large datasets to ensure enough cases are observed for analysis, often requiring pooling data from multiple sources and suffering the resulting heterogeneity. Yet we test the results of analysis on these extremely heterogeneous datasets against exact thresholds (e.g. α=0.05) and make clinical decisions based on the results. I will admit for much of my PhD studies, I've felt like a football official shaking my head while stretching a chain out along a field.

However, when investigating rare events, we don't have a viable alternative. Randomized clinical trials would require unrealistically large study sizes. Given the extreme heterogeneity in reported risk for these events, case control studies could only reasonably be used if adverse events for both blood products are reported by the same hemovigilance apparatus. Given most hemovigilance systems are nationally based and that most countries use a single plasma type, this is a rarity. Despite the challenges addressed here, large observational cohorts remain the most effective tool we have in hemovigilance analysis. The

key lies not in ditching the chain, but in learning to better spot the ball.

Fortunately, with regard to this issue, the future of all clinical research, including hemovigilance research, is bright. We live in the era of big data, and while it remains to be seen which parts of the research chain are eventually automated, the interconnected systems of the future will surely greatly increase the efficiency with which clinical data is stored and shared. Imagine a future in which clinical data is standardized in form like telephone numbers and web addresses are today – these data would have a low heterogeneity in spite of having been pooled from different sources.

Interconnection of clinical datasets (such as the ISTARE database) and standardization in reporting methods will aid with all three of the data challenges we've here discussed (sparse data, high heterogeneity, non-comparative studies). The era of big data has the potential to greatly reduce the heterogeneity in reported outcomes, though only if diagnostic and reporting procedures are concurrently standardized and applied. As is so often the case, communication and working partnerships are the solutions to this problem.

Summary of dissertation

Omniplasma vs. FFP:

The Netherlands switched from FFP to Omniplasma in 2014 with the expectation that the risks of allergic reactions and TRALI would decrease without a corresponding rise in units of plasma transfused per episode. We undertook a post-marketing study to compare the two products with regard to effectiveness and transfusion reaction risk. Plasma use did not change with the switch to Omniplasma – either on average or when normalized by concurrently transfused red blood cells. Omniplasma is associated with a reduced risk of allergic reactions. Comparisons of TRALI risk are inconclusive. Analyzing peer-reviewed and hemovigilance database data on transfusion reactions, we show the same to be true for SD plasmas in general. Though other studies have shown a statistically significant reduction in TRALI incidence with SD plasma, extreme heterogeneity in analyzed hemovigilance data decreases the accuracy of outcome measure estimates, likely preventing us from likewise showing this result. SD plasma fraction is positively associated with hyperfibrinolysis, but this can be controlled with concurrent administration of anti-fibrinolytics.

Analysis of hemovigilance datasets:

Hemovigilance datasets tend to be sparse with high heterogeneity and many non-comparative studies. Methods must be adopted to deal with these characteristics**.** Binomial distribution-based methods can accurately model sparse data, and modeling a random effect increases the accuracy of the outcome estimate. Bivariate methods allow inclusion of non-comparative studies, though whether or not their inclusion ultimately improves accuracy will ultimately depend on the data characteristics.

Conclusion
The Netherlands' switch from FFP to SD plasma did not result in any notable changes in clinical use. SD plasma leads to fewer allergic reactions than FFP; SD plasma fraction is associated with fibrinolysis. The characteristics of hemovigilance data - sparse data with high heterogeneity and many non-comparative studies – make pooling of data for safety comparisons challenging. Many of these challenges can be addressed with proper methodology, though heterogeneity in reported outcomes from current hemovigilance systems needs to decrease to allow more efficient research.

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“You just pull the little handle...”

Closing

Nederlandse samenvatting
Curriculum Vitae
Acknowledgments

Nederlandse samenvatting

Omniplasma vs. FFP:
In 2014 is Nederland overgestapt van vers bevroren plasma (FFP) naar Omniplasma, een solvent/detergent gepoolde plasma, met als verwachting dat de risico's op allergische reacties en TRALI omlaag zouden gaan terwijl het aantal eenheden plasma ongeveer gelijk zou blijven. We zijn een post-marketing onderzoek begonnen om deze twee producten te vergelijken op basis van effectiviteit en risico op transfusiereacties. Onze resultaten laten zien dat het plasmagebruik niet veranderd is sinds de overstap naar Omniplasma – in zowel het absolute aantal als de verhouding tussen plasma en erytrocytenconcentraten eenheden die tijdens dezelfde episode getransfundeerd zijn. Het gebruik van Omniplasma is geassocieerd met een verlaagd risico op allergische reacties. We zagen te weinig gevallen van TRALI om daar een zinvolle uitspraak over te kunnen doen. Door het analyseren van zowel peer-reviewed data op transfusiereacties als data van hemovigilantiedatabanken, laten we zien dat hetzelfde geldt voor SD plasma's in het algemeen. Hoewel andere studies een statistisch significante reductie van de incidentie van TRALI met SD plasma hebben kunnen laten zien, heeft de heterogeniteit van geanalyseerde data de nauwkeurigheid van de uitkomstschattingen in onze studie beperkt met als gevolg dat wij dit resultaat niet konden bevestigen. Onze resultaten laten ook zien dat SD-plasma is geassocieerd met hyperfibrinolyse, en dat dit gecorrigeerd kan worden door het gelijktijdig toedienen van antifibrinolytica's.

Analyseren van hemovigilantiedatabanken
Hemovigilantiedatabanken bevatten meldingen van zeldzame gebeurtenissen en de incidentieschattingen van deze gebeurtenissen verschillen sterk; er is een grote heterogeniteit in de incidentieschattingen. De statistische methoden die we gebruiken om te komen tot betrouwbare schattingen en vergelijkingen van risico's moeten met deze eigenschappen rekening houden. Methoden gebaseerd op de binomiale verdeling zijn geschikt voor het analyseren van laagfrequente gebeurtenissen, en het toevoegen van een random-effect kan de nauwkeurigheid van de uitkomstschattingen verbeteren. Bivariate methoden kunnen gebruikt worden om resultaten van niet-vergelijkende studies te meta-analyseren, maar of deze methoden de nauwkeurigheid juist schatten is afhankelijk van de dataeigenschappen.

Conclusie
Het overstappen van FFP naar Omniplasma in Nederland heeft niet geleid tot opvallende veranderingen in klinisch gebruik. SD plasma is geassocieerd met minder allergische reacties dan FFP; SD-plasma is geassocieerd met verhoogde fibrinolyse. De eigenschappen van hemovigilantiedata – spaarzame data met hoge mate van heterogeniteit en veelal niet-vergelijkende studies – maken

het poolen van data voor het vergelijken van de incidentie van bijwerkingen uitdagend. Speciaal hierop aangepaste statische methodologie helpt om sommige van die uitdagingen het hoofd te bieden, maar de bewerkstelliging van een lagere heterogeniteit in meldfrequentie van transfusiereacties en incidenten in de transfusie keten dan in de huidige hemovigilantie-systemen gevonden wordt, is nodig voor efficiënter vergelijkend onderzoek.

Curriculum Vitae

Nicholas, the second of three sons of a Lebanese father and a Palestinian mother, was born and raised in Oklahoma, USA. He graduated from Casady high school and earned a bachelor's degree in aerospace engineering from Purdue University in West Lafayette, Indiana and a master's degree in the same field at Stanford University in Palo Alto, California. Having trained as a dancer since a young age, he was invited to spend a year studying classical ballet at the Conservatoire Darius Milhaud in Aix-en-Provence, France on a Rotary International scholarship and joined the adjoining summer's intensives at the Washington Ballet (Washington, D.C.) and Ballet Pacifica (Long Beach, California). He spent the next five years working as a civil servant aerospace engineer at NASA's Johnson Space Center in Houston, Texas by day and on contract as a principle dancer with Houston Repertoire Ballet, Houston Ballet Theatre, and City Ballet of Houston in the evenings. After resigning his ballet and NASA contracts, he earned his bachelor's degree in medicine at the American University of Beirut before coming to Leiden University on an ERASMUS Mundus scholarship to spend a year performing cell biology in the Ear, Nose, and Throat department of the Leiden University Medical Center (LUMC). He then joined the PhD program in the department of Clinical Epidemiology in 2013 as a researcher at the Jan van Rood Center for Clinical Transfusion Research, a partnership between Sanquin, the Netherlands' national blood bank, and the LUMC. Under the advisorship of Prof. J.G. (Anske) van der Bom (LUMC) and Dr. Martin Schipperus (Haga Teaching Hospital), he spent four years comparing plasma products with regard to their risk of rare adverse events and researching the epidemiological methodologies used to do so. In 2017, he spent six months as a guest researcher at Monash University's Transfusion Research Unit in Melbourne, Australia, under the advisorship of Prof. Erica Wood. He returned to the Netherlands at the end of 2017 to finish his PhD and prepare for his medical rotations.

Acknowledgements

A few months prior to the inaugural launch of the *Space Shuttle* in 1981, a NASA analysis showed that if the astronauts used the vehicle's ejection seats to parachute to safety in an emergency during launch, their trajectories would take them through the vehicle's rocket plume as they fell. What would happen next lay somewhere on a scale from 'nothing' to 'burned to a crisp', a full decade before we had the computing power to know for sure. At a pre-launch press conference, a reporter said to the crew '*It's still not completely clear to me whether or not you can abort with the ejection seats during the burn of the [rocket boosters].'* Astronaut John Young's reply – *'you just pull the little handle'.*

This manuscript is the product of a journey that has brought me together with a sea of talented people these past five years, unfortunately too numerous to name here. To all who have contributed, directly or indirectly, this work and my thanks belong to you. To my advisors, Anske and Martin, I am grateful to you for much that is to be expected from PhD advisors – for your support and mentoring, both educational and emotional, these past five years. More than this, I am thankful to you for teaching me to fight the fights that need fighting. That the imperfections of the peer review process are acceptable, but un-scientific influence decidedly not; that even when the results are disappointing to a powerful few, follow your scientific obligation, pull the little handle, and jump through fire.