Is O really the universal Donor?

IS O REALLY THE UNIVERSAL DONOR?

By REBECCA BARTY, MLT, BA

A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science

McMaster University MASTER OF SCIENCE (2015) Hamilton, Ontario (Health Research Methodology)

TITLE: IS O REALLY THE UNIVERSAL DONOR?

AUTHOR: Rebecca Barty, MLT, BA

SUPERVISOR: Professor Nancy M. Heddle

xiii, 120

PREFACE

We have recently reported on a retrospective analysis of transfused adult patients, which showed an association between in-hospital mortality and the transfusion of red blood cells (RBCs) that were ABO compatible but non-identical. This finding challenges the long-standing transfusion medicine practice of using group O blood as the universal donor. If this association is real, the biological mechanism which leads to poor patient outcomes is unknown; however, a review of the literature suggested that inflammation could play an important role. Hence, I hypothesized that: *The transfusion of RBCs that are ABO compatible but not identical can result in an increased inflammatory response, which will be detected by measuring biomarkers of inflammation.*

The objectives of this thesis were: 1) to identify a theoretical framework involving inflammation associated with blood transfusion; 2) to develop a research question using the PICOT format to explore whether there was evidence of inflammation following the transfusion of compatible but ABO non-identical blood; and 3) to design a study to address the research question, considering both methodological and ethical issues.

Chapters 1 and 2 provide background information and summarize a literature review relating to inflammation and transfusion, including the associated laboratory biomarkers used to assess an inflammatory response. The theoretical framework for the study is also provided. Chapters 3 and 4 describe work performed to select an appropriate patient population to study and to select the most appropriate study design. This work led to the development of the following research question:

Is it feasible to perform a randomized crossover trial in chronically transfused blood group A patients with myelodysplastic syndrome (MDS), that looks for laboratory evidence of inflammation (in vitro biomarkers measured at baseline and pre-specified times between 1 and 96 hours post transfusion), following the transfusion of group O RBCs (ABO non-identical) compared to transfusion of group A (ABO identical) RBCs?

In chapter 5, I present a pilot study design to address this research question and discuss ethical issues that had to be considered in chapter 6.

This proposed research will focus on laboratory evidence of an increased inflammatory response following the transfusion of ABO non-identical blood compared to ABO identical transfusions. A pilot design was selected as it became apparent that there were a number of feasibility issues that should be evaluated before proceeding to a larger adequately powered study. If this research using a stable population of patients with MDS detects laboratory evidence of an inflammatory response, future studies could involve assessing clinical outcomes in a population at higher risk of morbidities mediated by inflammation.

ABSTRACT

An association between compatible but ABO non-identical red blood cell (RBC) transfusions and increased in-hospital mortality was identified in an observational study. A review of the literature was performed to explore plausible biological mechanisms and inflammation was chosen. This thesis describes a body of work that was performed to develop a PICOT research question and design a pilot feasibility randomized crossover trial in patients with myelodysplastic syndrome (MDS) to determine whether there is evidence of an inflammatory response resulting from transfusion of ABO non-identical RBCs compared with the transfusion of ABO identical RBCs. The work undertaken as part of this thesis included: identifying a theoretical framework to guide the selection of outcome measures that would detect inflammation; identifying an appropriate and feasible population to study; designing the feasibility pilot study to answer the research question that was developed; and a discussion of ethical issues that were considered as the design of the pilot study was developed. The work that was done to develop the elements of PICOT resulted in the following research question: *Is it feasible to perform a randomized crossover trial in chronically transfused blood group A patients with myelodysplastic syndrome (MDS), that looks for laboratory evidence of inflammation (in vitro biomarkers measured at baseline and pre-specified times between 1 and 96 hours post transfusion), following the transfusion of group O RBCs (ABO non-identical) compared to transfusion of group A (ABO identical) RBCs?*

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Prof. Nancy Heddle for her support, encouragement, and mentorship. I was born asking questions, and she has taught me how to transform those questions into research. I have been fortunate to be able to blend my medical laboratory technologist training with my interests in research methodology and work in a discipline I love - Transfusion Medicine.

Thank you to my thesis committee members, Dr. Donald Arnold and Dr. Richard Cook, for their time, advice, and guidance. I would also like to thank Dr. Cyrus Hsai for his involvement as my external reviewer.

To my friends and coworkers at the McMaster Transfusion Research Program (MTRP) I am thankful for your support. A special thank you to Yang Liu, Dr. Menaka Pai, Aicha Traore, Grace Wang, Heather Patterson, Korinne Hamilton, and Shannon Lane.

I am grateful to my friends and family that have continuously supported me in my academic journey. I wish to dedicate this to my mother and father, Ingrid and Robert Barty, who instilled me with strength, courage, and my work ethic. To my sister, Lisa, thank you for reading my (many) drafts. And finally, to my loving husband, Aaron Toni, thank you for providing love and support when needed.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

AD	Anno Domini
AML	Acute Myeloid Leukemia
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BC	Before Christ
BPI	Bactericidal Permeability Increasing Protein
CBC	Complete Blood Count
CBS	Canadian Blood Services
CI	Confidence Interval
CIC	Circulating Immune Complexes
CRP	C-Reactive Protein
DSMB	Data and Safety Monitoring Board
ESAS	Edmonton Symptom Assessment System
GCP	Good Clinical Practice
HCPs	Health Care Professionals
HiREB	Hamilton Integrated Research Ethics Board
HHS	Hamilton Health Sciences
HRLMP	Hamilton Regional Laboratory Medicine Program
ICD-10	International Classification of Disease (10)
IL-1β	Interleukin-1beta
IL-6	Interleukin-6
IL-8	Interleukin-8
INFLAME-P	Inflammation evaluation of chronically transfused MDS patients with ABO non-identical red blood cell transfusions – Pilot Study
IPSS	International Prognostic Scoring System
IQR	Interquartile range
JHCC	Juravinski Hospital and Cancer Centre
Le	Lewis
NICU	Neonatal Intensive Care Unit
MDS	Myelodysplastic Syndromes
MTRP	McMaster Transfusion Research Program
NO	Nitric oxide
O_2	Oxygen
PMN	Polymorphonuclear leukocytes
RBC	Red blood cell
RBCs	Red blood cells
RCT	Randomized Controlled Trial
REB	Research Ethics Board
REDCap	Research Electronic Data Capture
RR	Relative Risk
SAS	Statistical Analysis System
SJHH	St. Joseph's Healthcare Hamilton

SC	Steering Committee
TCP-2	Tri-Council Policy Statement 2
TBD	To Be Determined
TNF-α	Tumor necrosis factor-alpha
TMS	Transfusion Medicine Services
TRALI	Transfusion Related Acute Lung Injury
TRUST	Transfusion Registry for Utilization Surveillance and Tracking

DECLARATION OF ACADEMIC ACHIEVEMENT

A systematic review of the literature was undertaken by R. Barty to identify an appropriate biological/theoretical framework and the outcome measures that would inform a well-designed PICOT research question.

The exploratory analysis plan for MDS patients using the TRUST database was developed by R. Barty and the analysis was conducted by Y. Liu (biostatistician) and reviewed and interpreted by R. Barty.

The statistical analysis plan for the Group O project was developed by R. Barty and reviewed by N.M. Heddle, Y. Liu and M. Pai. Data analysis was performed by Y. Liu. The results were interpreted by R. Barty and the manuscript was drafted by R. Barty.

A pilot feasibility study using a randomized crossover design was developed by R. Barty with valuable guidance from N.M Heddle.

Ethical considerations were explored and summarized by R. Barty.

Information and feedback on the study design challenges were provided by Dr. A. Shih, Dr. C. Hillis, and Dr. R.J. Cook.

CHAPTER 1: INTRODUCTION

Red blood cell (RBC) transfusions are aimed at increasing oxygen capacity, reversing the signs and symptoms of anemia, and alleviating bleeding (Napolitano et al., 2009). RBC transfusions can be lifesaving; however, they are not without risk (Cata, Wang, Gottumukkala, Reuben, & Sessler, 2013; Tuinman et al., 2011). The ABO blood group system has clinical importance related to transfusion, as ABO antibodies are developed by three to six months of age when the corresponding antigens are not expressed on the cell membrane; hence, the need to ensure that blood selected for transfusion is ABO compatible. ABO antigen expression is not limited to the red cell surface, but is also found on most tissues, and is also present as soluble antigen in the secretions of 80% of the population.

COMPATIBILITY OF ABO BLOOD

It is standard practice to provide ABO compatible RBCs for transfusion, and in most cases the transfused RBCs will be of the same ABO group as the patient's RBCs (ABO identical). However, group O RBCs are recognized as the universal donor as they lack expression of A and B antigens on their surface. Hence, group O RBCs can be transfused to individuals whose blood group is A, B, or AB. This type of transfusion is termed ABO compatible though non-identical. Although there will be small concentrations of anti-A and anti-B passively infused when ABO non-identical RBCs are given, the concentration of these antibodies is low so adverse events rarely occur. Thus, ABO non-identical RBC transfusions are used for transfusion therapy in situations involving traumas, blood

shortages or when a specific red cell phenotype is required. In Table 1, the four ABO group phenotypes (O, A, B, AB) are listed along with the antigen expression on the RBC, the antibody(ies) found in the plasma, other ABO groups that are compatible for RBC transfusion, and, the terminology used in this thesis for ABO identical and non-identical (but compatible) RBC transfusions.

Table 1: Summary of RBC and serum/plasma characteristics for the four ABO groups and RBCs that are compatible.

ABO Group	Antigen on Red Cells	Antibody in Serum/Plasma	Compatible RBCs	Terminology
O	No A or B	anti-A, anti-B	O	ABO identical
A	A	anti-B	A	ABO identical
			O	ABO non-identical
B	B	anti-A	B	ABO identical
			O	ABO non-identical
AB	A and B	none	AB	ABO identical
			A	ABO non-identical
			B	ABO non-identical
			O	ABO non-identical

THE EFFECT OF RECEIVING ABO NON-IDENTICAL RBCS

A recent retrospective analysis conducted by researchers at the McMaster Transfusion Research Program (MTRP) has raised the hypothesis that transfusing group O RBCs to group A individuals could be harmful. Pai et al., (2015) showed an association between transfusion of RBCs that were compatible but ABO non-identical, and in-hospital mortality. Group A patients receiving group O RBCs were found to have a significant increased risk of in-hospital mortality compared to patients receiving ABO identical blood (Relative Risk (RR)=1.79, 95% Confidence Interval (CI): 1.20, 2.67; p=0.0047; Table 2) (Pai et al., 2015). The results were similar when trauma patients (≥ 6 RBCs

transfused within 24 hours) were excluded from the analysis, suggesting that the observed association was not biased by trauma patients who have a higher likelihood of mortality and often receive uncrossmatched group O RBCs (Pai et al., 2015).

Table 2: Effect of exposure to non-identical blood on risk of in-hospital death in all patients (n=18,843)

	RR	95% CI	p
Covariates			
MODEL 1[*,†]			
Patient's ABO Group			
A versus AB	1.096	(0.824,1.457)	0.531
B versus AB	0.931	(0.687,1.262)	0.646
Global test, p			0.189
Time-dependent exposure to non-identical units			
Exposed to non-identical units vs. not exposed	1.221	(0.916,1.629)	0.173
MODEL 2[*,‡]			
Time-dependent exposure to non-identical units by Patient ABO Group			
A: Exposed to non-identical units vs. not exposed	1.787	(1.195,2.672)	0.005
B: Exposed to non-identical units vs. not exposed	0.636	(0.303,1.335)	0.232
AB: Exposed to non-identical units vs. not exposed	1.010	(0.620,1.647)	0.967
Interaction Test (2 df), p			0.034

[*]Cox regression models controlled for age (years), creatinine (µmol/L) and hemoglobin (g/dL) as continuous variables and were stratified by the cumulative number of units of blood transfused that were <7 days old, 7 to 28 days old, and >28 days old, year of admission and disease group.
[†]Model 1 does not include interaction terms.
[‡]Model 2 includes an interaction between patient's blood group and a time-dependent indicator of non-identical transfusion.

The association that was observed when group A patients received group O RBCs was not apparent in group B or group AB patients who received ABO non-identical RBCs. There was a trend towards a lower risk found in Group B patients although the 95% CI was wide. For AB patients receiving non-identical blood the RR was 1.010 but the CI included values consistent with both a reduction of risk and an increase in risk. These

data suggest significant heterogeneity in the effect of exposure to non-identical RBC transfusion according to a patient's ABO blood group. Although there are some known differences between ABO groups (i.e., antigen concentrations, H antigen expression and von Willebrand's concentrations), it is unclear how these differences could contribute to a biological process that results in harm (Gill, Endres-Brooks, Bauer, Marks Jr, & Montgomery, 1987; Murphy & Pamphilion, 2005). The findings from this study were contrary to routine practice as group O RBCs have been given to group A individuals for over a 100 years and this practice is considered to be safe. If the above finding is true, the causes may be multifaceted, possibly attributable to donor, product, or recipient factors, or a combination of all three. Identifying the causal pathway that explains the finding reported by Pai et al., (2015) will be complex and challenging. As a starting point we searched the literature to identify possible biological reasons for the observed increase in mortality.

CHAPTER 2: IF ABO GROUP COMPATIBLE BUT NON-IDENTICAL BLOOD CAUSES HARM – WHAT IS THE MECHANISM?

A literature review was undertaken to explore the plausible mechanisms that have been associated with patient morbidity and/or mortality related to RBC transfusions. Most of this literature related to possible mechanisms that could affect patient outcome if they were transfused with stored blood as this has been a controversial topic for over 15 years. From this literature a number of biological mechanisms and some interrelated pathways that could cause patient harm were identified including: iron, infection, oxygen delivery, increased consumption of nitric oxide, deformability, and inflammation (Doctor & Spinella, 2012). Some of these mechanisms are hypothetical; however, inflammation is well documented in the transfusion literature as a cause of adverse events in some patients; hence, it was selected as the mechanism to be explored in this thesis (Bilgin & Brand, 2008; Yavuz M Bilgin, van de Watering, Versteegh, van Oers, & Brand, 2010; Brand, 2002; Cata et al., 2013; Dong et al., 2012; Escobar et al., 2007; Fransen, Maessen, Dentener, Senden, & Buurman, 1999; Grimshaw, Sahler, Spinelli, Phipps, & Blumberg, 2011; Hamada et al., 2001; Johnson et al., 2003; Jy et al., 2011; Keir, McPhee, Andersen, & Stark, 2012; Kim-Shapiro, Lee, & Gladwin, 2011; Kristiansson, Soop, Saraste, & Sundqvist, 1996; Mangalmurti et al., 2009; Miyaji et al., 2009, 2010; Napolitano, 2006; Sakagawa et al., 2007; Senay et al., 2009; Tinmouth et al., 2006; Tuinman et al., 2011; Walter et al., 2006; Ydy, Slhessarenko, & de Aguilar-Nascimento, 2007).

INFLAMMATION

Classic features of the inflammatory response were described as early as 1600 BC in Egyptian papyrus writings. In the 1st Century (AD) the Roman physician Celsus described the four

cardinal signs of inflammation: rubor (redness), tumor (swelling), calor (heat), and dolar (pain). In the 2nd Century (AD) the famed Greek physician Galen added the fifth sign of inflammation: function lasea (loss of function) (Rather, 1971). These five signs reflect the three major events of inflammation: (1) vasodilation, (2) edema, and (3) influx of phagocytes.

VASODILATATION

The alteration of vascular permeability occurs when blood is carried away from the affected area and dilation of the blood vessels results in engorgement as protein-rich fluid enters the extravascular tissue. The increase in fluid into the tissue is responsible for tissue redness (erythema) and an increase in temperature.

EDEMA

Edema is responsible for tissue swelling; edema results from an increase in capillary permeability, which causes fluid to accumulate (exudate) with higher protein content than normal.

INFLUX OF PHAGOCYTES

The influx of phagocytes from the capillaries into the tissues involves a complex sequence of processes. Adherence of the cells to the endothelial wall of blood vessels (margination) is followed by their emigration between the capillary-endothelial cells into the tissue (diapedesis or extravasation) and finally their migration through the tissue to the site of inflammatory response (chemotaxis).

SEARCH STRATEGY

The objective of the literature search was to identify: a theoretical framework that could be used to understand the relationship between inflammation and transfusion; laboratory biomarkers that

have been used to detect inflammation and would be appropriate to use in this study; and, the optimal timing of sample collections for the biomarker testing. A search was performed using the electronic database Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) (1946 to present), applicable citations within the reference lists of articles identified were scanned. Language was restricted to English. Narrative reviews and letters to the editor were reviewed. The details of the search strategy are included as Appendix I and the flow chart for selection of articles is Appendix II.

TRANSFUSIONS AND INFLAMMATION

The literature search identified numerous reports related to transfusion that assessed the inflammation in humans (patients or healthy volunteers) (Avall, Hyllner, Bengtson, Carlsson, & Bengtsson, 1999; Yavuz M Bilgin et al., 2010; Bordin et al., 1999; Brand, 2002; Cholette et al., 2012; Fransen et al., 1999; Gafter, Kalechman, & Sredni, 1996; Hampton et al., 2014; Hod et al., 2010; Hult, Malm, & Oldenborg, 2013; Jiwaji et al., 2014; Johnson et al., 2003; Keir et al., 2012; Lee et al., 2012; Liem, O'Gorman, & Brown, 2004; Locke et al., 2005; Milasiene, Stratilatovas, Characiejus, Kazbariene, & Norkiene, 2007; Miyaji et al., 2009, 2010; Perttila et al., 1994; Senay et al., 2009; Stark, Keir, & Andersen, 2013; Subramanian et al., 2014; Surinenaite, Prasmickiene, Milasiene, Stratilatovas, & Didziapetriene, 2009; Theodoraki, Markatou, Rizos, & Fassoulaki, 2014; Vlaar et al., 2012; Ydy et al., 2007), using animal models (Belizaire et al., 2012; Callan et al., 2013; Hendrickson, Hod, Hudson, Spitalnik, & Zimring, 2011; Hendrickson, Hod, Cadwell, et al., 2011; Hod et al., 2010; McMichael, Smith, Galligan, Swanson, & Fan, 2010; Stowell et al., 2013), and in the context of different blood components (Delclos, Yeh, & Blumberg, 1983; Fransen et al., 2002; Keir et al., 2012; Kristiansson, Soop, Saraste, et al., 1996; Kristiansson,

Soop, Shanwell, & Sundqvist, 1996; Mynster, 2001; Nagura, Tsuno, Tanaka, Matsuhashi, & Takahashi, 2013; Seghatchian, Krailadsiri, Dilger, Thorpe, & Wadhwa, 2002; Stark et al., 2013; Wadhwa, Seghatchian, Dilger, Contreras, & Thorpe, 2000).

Various populations were studied (healthy volunteers; medical patients; surgical populations; pediatric and neonatal patients; adults; and small or large animal); different blood components were assessed (plasma, platelets, RBCs, exchange transfusion, autologous and allogenic RBCs; and effect of processing (cryopreservation, non-leukoreduced, leukoreduced, buffy coat removed, washed, and unwashed products).

Many laboratory markers were measured at variable time points and in different combinations. The laboratory testing across studies varied from measuring a limited selection of cytokines and other biomarkers to the utilization of multi-test kits that measured several biomarkers at once. The search identified a range of biomarkers that include cytokines, bioactive substances and routine laboratory tests to assess inflammation and the interrelated pathways of inflammation related to transfusion or blood products (transfusion and/or storage).

The biomarkers most frequently used to detect inflammation included: interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), interleukin-8 (IL-8) and (interleukin-1beta (IL-1β) (Avall et al., 1999; Bordin et al., 1999; Cholette et al., 2012; Fransen et al., 1999; Gafter et al., 1996; Hampton et al., 2014; Hod et al., 2010; Hult et al., 2013; Jiwaji et al., 2014; Johnson et al., 2003; Keir et al., 2012; Lee et al., 2012; Liem et al., 2004; Locke et al., 2005; Milasiene et al., 2007; Perttila et al., 1994; Senay et al., 2009; Stark et al., 2013; Subramanian et al., 2014; Surinenaite et al., 2009; Theodoraki et al., 2014; Vlaar et al., 2012; Ydy et al., 2007). Several of these studies included C-reactive protein (CRP) as a measurement of inflammation (Ades, Itzykson, & Fenaux, 2014; Cholette et al., 2012; Fransen et al.,1999,

2002; Hod et al., 2010; Jiwaji et al., 2014; Miyaji et al., 2009, 2010; Senay et al., 2009; Ydy et al., 2007). In a study of transfusion in cardiac surgery assessing the inflammatory mediators that were released into the plasma included testing of bactericidal permeability increasing protein (BPI) as a measure of polymorphonuclear leukocytes (PMN) activation (Fransen et al., 1999, 2002).

CYTOKINES, TRANSFUSION REACTIONS AND LEUKOREDUCTION

The inflammatory response as assessed in stored blood using in vitro and animal studies was attributed to delayed PMN apoptosis and enhanced PMN priming (Biffl et al., 2001; Karamlou et al., 2005; Miyaji et al., 2010; Sparrow & Patton, 2004). Bioactive substances accumulate with blood storage, including cytokines and pro-inflammatory lipids (Escobar et al., 2007; Fransen et al., 2002; Hod et al., 2010; Muylle, 1995; Nielsen et al., 1997; Rao, Howard, & Teague, 2006; Shanwell, Kristiansson, Remberger, & Ringdén, 1997; Silliman, Clay, Thurman, Johnson, & Ambruso, 1994; Wadhwa et al., 2000). These biological response modifiers are known to play a role in transfusion reactions. The pro-inflammatory cytokines TNFα, IL-1β and IL-6 accumulate in the plasma of platelet products when they are not leukoreduced and have been associated with adverse events when the platelet concentrate is transfused (Heddle et al., 1994, 1999; Lin et al., 2002; Muylle, Wouters, & Peetermans, 1996; Muylle, 1995; Stack & Snyder, 1994). Leukoreduction of the platelet product prevents accumulation of cytokines derived from leukocytes (Heddle et al., 1993). Other reports have also shown that leukoreduction is associated with a reduced inflammatory response, or reduced detection of inflammatory biomarkers in vitro and in vivo (Locke et al., 2005; Mynster, 2001), and better patient outcomes (Jiwaji et al., 2014; Miyaji et al., 2010; Wadhwa et al., 2000). However, other bioactive substances which are not

leukocyte derived can still accumulate in leukoreduced products. Examples include: platelet-derived factors (CD40L) (Glenister & Sparrow, 2010; Khan et al., 2006); lipopolysaccharide-binding protein; and bactericidal permeability-increasing protein (neutrophil activation) (Fransen et al., 1999; Kristiansson, Soop, Saraste, et al., 1996; Locke et al., 2005).

TIMING OF SAMPLE COLLECTION FOR TESTING

Variability in the timing of sample collection when testing for biomarkers was identified. The minimum sampling times reported were a baseline sample within six hours prior to the transfusion and a post transfusion sample collected within one hour of completing the transfusion (Locke et al., 2005). In studies where the follow-up was within 24 hours limitations were found including the inability to determine if the results were sustained or transient (Keir et al., 2012; Stark et al., 2013), and, a lack of detailed profiling beyond 24 hours (Jiwaji et al., 2014). A healthy volunteer study collected samples two hours prior and two and 48 hours post transfusion. Serial testing post transfusion was reported up to 72 hours (Hod & Spitalnik, 2011; Miyaji et al., 2009) and, in the surgical setting sample collection varied from after the surgery was complete (Senay et al., 2009); to four days after the surgery (Bordin et al., 1999; Ydy et al., 2007); to 14 days after the surgery (Gafter et al., 1996; Milasiene et al., 2007; Surinenaite et al., 2009), and up to 21 days after the surgery (Perttila et al., 1994).

THEORETICAL FRAMEWORK

The theoretical framework selected for this study (Figure 1) was proposed by Twomley et al., in 2006. This framework suggests that the response of RBC transfusions to attenuate anemia is dependent on the recipient's humoral immunity and the RBC blood product attributes. The

recipient's humoral immunity is dependent on other coexisting conditions and the inflammatory state. The RBC transfusion content and product quality may vary depending on the blood processing method, storage, and blood donor attributes. It is reasonable that inflammation can be attributed to, and is interrelated with a multitude of clinical pathways when treating anemic patients with RBC transfusions (Twomley, Rao, & Becker, 2006).

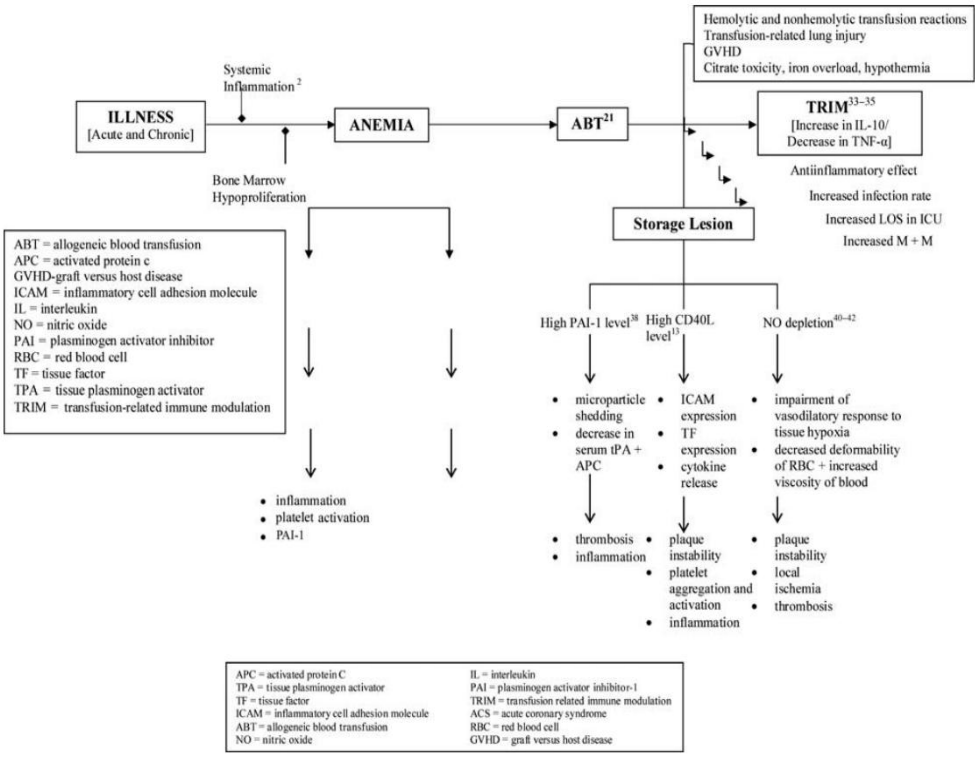


Figure 1: Red blood cell transfusion for the treatment of anemia: potential mechanisms of morbidity and mortality
[copyright license obtained]

In summary the selection of inflammation as the biological framework to be used in this pilot study stems from the transfusion literature indicating that inflammation is a documented phenomenon that can be associated with transfusion; however, these reports lack details of the recipient and the donor ABO compatibility. Passive transfer of ABO antibody occurs when ABO non-identical RBCs are transfused and it is plausible that this antigen-antibody

incompatibility could trigger an inflammatory response. Detection of, or changes in markers of inflammation could indicate that there is an associated biological response which signals a physiological change when a patient is exposed to ABO non-identical RBCs, which, under certain conditions, could cause harm. The literature identifies numerous biomarkers which have been used to detect inflammation.

PROPOSED BIOMARKERS FOR INFLAME-P

The biomarkers/cytokines that will be measured in this study are described below and summarized in Table 3. Their selection was guided by the literature describing inflammation following transfusion of blood components.

1. C-REACTIVE PROTEIN (CRP)

C-reactive protein (CRP) is an acute-phase reactant which increases in concentration in response to cytokines when infection, inflammation, or tissue damage occurs (Aguilar-Nascimento, Marra, Slhessarenko, & Fontes, 2007; Ballantyne & Nambi, 2005; Epstein, Gabay, & Kushner, 1999; Lobo, 2012). CRP can also activate the classic pathway of complement (Fransen et al., 1999; Steel & Whitehead, 1994). CRP was selected as a marker to detect the presence of an inflammatory response in this pilot study as it is an accepted marker of inflammation with broad clinical application, has good sensitivity, has low cost compared to other markers, and it is a readily available test (Aguilar-Nascimento et al., 2007). CRP levels rise in response to IL-6; therefore if testing of cytokines is problematic CRP could be an important biomarker (Epstein et al., 1999). Testing requires a four millilitre green heparinized tube of blood and will be

conducted by the Hamilton Regional Laboratory Medicine Program (HRLMP) (Hamilton, ON). The reference range for a normal CRP in the adult (> 18 years of age) population would be less than or equal to 5.1 mg/L.

2. CYTOKINES

Cytokines are a diverse group of small (8 to 40,000D) secreted soluble proteins that can be synthesized from nearly every cell (Dinarello, 2000). Some cytokines are acute-phase proteins that permit communication between immune cells in the coordination of the inflammatory response (Zhou, Fragala, McElhaney, & Kuchel, 2010). The following cytokines will be measured in this study:

INTERLEUKIN-6 (IL-6)

Interleukin-6 (IL-6) is a helical glycoprotein ranging in size from 20 to 30 kD (Maggio, Guralnik, Longo, & Ferrucci, 2006). Neutrophil and mononuclear phagocyte derived IL-6 is a pleiotropic cytokine that stimulates the adhesive interaction between neutrophils and cardiac myocytes and induces an acute-phase response, and therefore is a sensitive marker of the inflammatory response (Fransen et al., 1999; Maggio et al., 2006). Further, IL-6 is a known pro-inflammatory cytokine with pyrogenic activity (Taniguchi et al., 1999).

TUMOR NECROSIS FACTOR-ALPHA (TNF-A)

This proinflammatory cytokine's role in the systemic inflammatory response is its ability to recruit leukocytes and its function in apoptosis (Zhou et al., 2010).

INTERLEUKIN-1BETA (IL-1B)

IL-1β is a member of the interleukin-1 family of cytokines. Due to its involvement in a variety of cellular activities including: cell proliferation; differentiation; and apoptosis, it is considered an important mediator of inflammation.

INTERLEUKIN-8 (IL-8)

IL-8 has chemotactic properties and specifically attracts neutrophils to the site of inflammation (Davenport, Strieter, Standiford, & Kunkel, 1990; Liem et al., 2004).

When collecting samples for cytokine testing there are a number of considerations:

1. Stability of samples has been reported to rapidly fluctuate. For example, it has been reported that IL-6 left at room temperature degrades and at 4 hours the reduction was significant; conversely, TNF-α levels increase during sample storage (Flower, Ahuja, Humphries, & Mohamed-Ali, 2000; Zhou et al., 2010).
2. Measurement can be done in single assays or using highly sensitive multiplex technologies where many cytokines can be measured from a single small sample.
3. Cytokine levels have been reported to be impacted by diurnal effects (due to circadian rhythms), the feeding state, stress level of the patient, the anticoagulant used in the specimen and, the method and duration of sample storage. For example, the literature has reported that TNF-α values are inversely related to plasma cortisol levels and crest early in the morning (Petrovsky, McNair, & Harrison, 1998; Zhou et al., 2010). Elevations of CRP and IL-6 have been reported after the consumption of a high fat meal, whereas TNF-α level showed a decrease (Blackburn et al., 2003; Esposito et al., 2002; Zhou et al.,

2010). Vitamins can also cause an elevated level of plasma cytokines (Carroll & Schade, 2003; Esposito et al., 2002; Zhou et al., 2010).

4. Standardized procedures often recommend that when collecting plasma samples, whole blood be collected with an anticoagulant, put on ice immediately, and centrifuged within 30 minutes. Serum samples can also be collected and allowed to clot at room temperature for 30 minutes before centrifuging for 15 minutes. Many cytokines have a short half-life and can degrade once the sample is drawn. Hence, many manufacturers of testing kits recommend samples are stored frozen at -20°C within 30 minutes of being drawn. If samples are stored frozen it is suggested they be stored in small aliquots so that freeze-thawing is avoided.

   It is important to document sample collection times. The sample collection schedule for this pilot study will adhere to the specified serial testing times as guided by the literature and reflected in the study design. The purpose of the serial testing is to provide kinetic information of the inflammation markers; provided an inflammatory response is detected. This will inform the frequency of further testing in a larger scale trial.

To avoid some of these issues patients will be asked not to change their dietary habits other than refraining from consuming high fat foods while on the study. Discontinuing vitamin supplements will also be recommended with physician approval.

Collection, centrifugation and freezing of the post-transfusion serial timed samples will be contracted to mobile services (LifeLabs, Toronto, ON). This study will assess the feasibility of collecting all of the timed samples with appropriate documentation, freezing of samples within a pre-stated time frame, and transporting samples to the coordinating centre using appropriate pre-specified shipping containers (refer to chapter 5). All cytokine testing will be carried out

according to the manufacturers' methods by a qualified research assistant at McMaster University, Hamilton, ON.

3. CIRCULATING IMMUNE COMPLEXES (CIC)

Circulating immune complexes (CIC) may be formed between the patient's soluble ABH antigens and the passively transfused A and B antibodies (Heal, Masel, Rowe, & Blumberg, 1993). The process of phagocytosis clears CIC; however, if phagocytosis does not happen, the CIC can be deposited in the endothelial or vascular structures, initiating an inflammatory response. Serum or plasma will be collected and stored frozen (-20°C) and CIC will be measured utilizing a commercially purchased ELISA kit (Immunomedics, Newark, NJ, USA).

4. CD40 LIGAND

CD40 ligand is a 48-kDA transmembrane glycoprotein of the TNF-α receptor family, expressed on endothelial and epithelial cells, monocytes, and macrophages (Ballantyne & Nambi, 2005; Khan et al., 2006). It is primarily a platelet-derived proinflammatory mediator found in soluble and cell-associated forms in transfused RBCs. Soluble CD40L activates macrophages and elicits the production and release of multiple proinflammatory cytokines. CD40 ligand has been identified as a potential active mediator in stored blood products (Urner et al., 2012). Serum or plasma will be collected and stored frozen (-20°C) and will be measured utilizing a commercially purchased ELISA kit (R&D Systems, a Bio-Techne brand, Minneapolis, MN).

5. BACTERICIDAL PERMEABILITY INCREASING PROTEIN (BPI)

Bactericidal permeability increasing protein (BPI) is a cationic protein of 456 amino acids known to bind lipopolysaccharides, exert a bactericidal effect on gram-negative bacteria, and neutralize the activities of lipopolysaccharides (Dentener et al., 1997; Elsbach, 1994; Fransen et al., 1999). BPI is a surrogate marker of activation of PMN (Fransen et al., 1999; Urner et al., 2012). In RBC products BPI is released from azurophilic granules following PMN activation by the plastic blood bag and PMN disintegration from cold (4°C) storage (Fransen et al., 1999). BPI can be detected within 10-30 minutes after stimulation and may continue to increase for up to 4 hours, decreasing 24 hours after stimulation (Dentener et al., 1997). Commercially available ELISA kits will be utilized for measuring BPI levels (R&D Systems, a Bio-Techne brand, Minneapolis, MN). Serum sample will be collected and frozen at -20 °C until testing, as indicated by the manufacturers' recommendations.

Table 3: Biomarkers/Cytokines to be measured in INFLAME-P

Test	Description	Expected Response if inflammation occurs	Half life
C-Reactive Protein	Found in blood Acute phase protein	Rises in response to inflammation Rises above normal within 6 hours	Constant – level mainly determined by the rate of production (severity of precipitating cause)
Cytokines (Proinflammatory)			
IL-6	May have pro coagulant effects	Increase	Short half life 2-6 hours
TNF-α	Cytokine involved in systemic inflammation	Increase	Peaks at 2 hours; half life 18.2 minutes
IL-1β	Mediator of inflammation	Increase	2.5 hours
IL-8	Mediators of inflammation	Increase	9-10 hours
Circulating Immune Complexes	Antibody to a soluble antigen	Increase if inflammation is antibody mediated	Several hours to several days post transfusion
Bactericidal permeability increasing protein	Identified in neutrophils and other tissues Binds to lipopolysaccharides (potent activator of the immune system)	Increase	Short half life

CHAPTER 3: CONSIDERATIONS FOR THE STUDY DESIGN

OPTIONS FOR THE STUDY DESIGN

Developing a good research question using the PICOT format also requires the various study design options to be carefully considered (Heddle, 2007). There were two experimental designs that were considered for this study: the parallel arm randomized controlled trial (RCT); and a randomized crossover design. A description of each design and the advantages and disadvantages of each of these two designs are summarized below.

The RCT parallel arm trial is the most frequently used experimental study design. Each study participant is randomly assigned to either the study treatment arm or the control arm and receives the allocated intervention for the duration of the treatment period. Ideally the outcome should be clinically relevant; however, surrogate outcomes (i.e., a laboratory test, vital signs) are also used. The outcome is measured over a defined period of follow-up. The sample size calculation for a parallel arm design depends on a number of factors, including: type I error (α), type II error (β), power ($1-\beta$), minimal clinically relevant difference (if a superiority study), and variance (Noordzij, Dekker, Zoccali, & Jager, 2011). It is also important to identify if the trial will test a hypothesis of non-inferiority, equivalence, or superiority as this will also affect the analysis and the sample size calculation. Because there are two sources of variability (within subject and between subject), the sample size for a parallel arm RCT design is typically larger than the sample size required for a crossover design.

In a crossover RCT design, the study participant will receive each of the study interventions in a randomized order. This design is most useful when investigating interventions in patients with a chronic and incurable disease and when the intervention's effect is rapid and of short duration. The stability of the patient over time is essential; any outcome effect noted must be attributable

to the treatment intervention provided and not have occurred without the treatment as an effect of disease progression.

The advantages of the crossover design are smaller sample sizes than a parallel arm RCT because the paired analysis increases statistical power, and the within-subject comparisons ensure that all baseline variables are equally distributed (i.e., gender, age); thus removing between-patient variability (Hulley, Cummings, Browner, Grady, & Newman, 2013). Disadvantages of this design include: doubling the duration of the study for individual patients; the additional expense required to measure the outcome at the beginning and end of each crossover period (although this may be offset by the smaller sample size requirement); the potential complexity of the analysis; and discerning the possible carryover and period effects when interpreting the results (Hulley et al., 2013). Table 4 summarizes the advantages and disadvantages of the two study designs.

Table 4: Advantages and disadvantages of parallel arm versus crossover RCT

	Parallel Arm RCT		**Crossover RCT**	
	Advantages	**Disadvantages**	**Advantages**	**Disadvantages**
Sample size		-larger sample size	-smaller sample size; paired analysis provides increased powers	
Drop out and loss to follow up		-high drop out if intervention has undesirable side effect little incentive to stay on study		-loss of paired data
Outcome	-rapid onset -disease could be curable		-rapid onset -useful for non-curable disease	
Stable patient	-not required			-requires stable patient; act as own control
Rare diseases/ conditions		-not appropriate	-appropriate	

Within-patient variability	-not applicable		-self-control baseline variables are equally distributed (i.e., gender, age)	
Between patient variability		-difference between groups	-overcomes difference between groups	
Duration	-shorter compared to crossover			-increased duration (depending on # of crossover episodes)
Analysis	-causal inferences			-complex; consider carryover and assess for period effects
Ethical		-not always ethical to randomize participant to an intervention/control arm		-length of washout period, time withholding treatment (washout period)
External validity		-may not be generalizable if too controlled		-may not be generalizable if very select patient group
Cost		-may be expensive		-may be expensive

As the research question for INFLAME-P developed, the crossover design was selected as the most suitable study design. The reasons for this decision are summarized in Table 5.

Table 5: Advantages and disadvantages of parallel arm versus crossover RCT related to INFLAME-P

Issue	Considerations/Rationale	Design Decision Favours	
		RCT	CO*
Ethical	Patients occasionally receive ABO non-identical blood typically if there is a shortage, if they are alloimmunized and need a specific phenotype, or if blood is close to outdating; however, it is not standard practice (other than neonates) to give only group O blood to a non-group O patient. As only one group O transfusion episode is planned for each patient there would be no ethical concerns with either design.	x	x
Patients with MDS as the study population	Patients with MDS are chronically transfused; have period during their illness where their disease is stable (clinical stability), and the effect of transfusion is temporary (weeks); hence, this population meets the requirement for a crossover study.		x
Ability to recruit	MDS is a rare disease so the pool of patients to recruit is limited. The crossover design typically requires fewer patients.		x
Intervention and Comparison	Since many MDS patients require chronic transfusion therapy a crossover design would allow both the ABO identical RBC and the ABO non-identical RBC to be given to the same patient and the results compared within that patient. Hence patients can serve as their own control, which ensure that baseline covariates are balanced.		x
Outcome/ Response	The effect of the transfusion in the MDS population is temporary; hence patients will return to their baseline anemia state and require another transfusion. The half-life of the biomarkers that will be measured in the study is also relatively short so there will be no concern related to a carryover effect.		x
Duration of study	The follow-up period for patients is doubled with a crossover design. This is a concern for the MDS population as these patients are elderly and experience fatigue; hence, from that perspective a RCT would potentially be a more appropriate design.	x	
Analysis	The analysis of a crossover design is more complicated than the analysis using a parallel arm RCT; however, access to biostatisticians for doing the analyses is not an issue with our research group.	x	x
Cost	Both study designs could be expensive; however, the crossover design will have less cost associated with assessing eligibility and recruitment as one patient will get both transfusion types.		x

*CO=crossover

CHAPTER 4: RATIONALE FOR THE SELECTION OF THE MDS POPULATION

MYELODYSPLASTIC SYNDROMES (MDS)

Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders since there is a vast spectrum of disease severity (Ades et al., 2014; Cazzola & Malcovati, 2005, 2010; Pereira et al., 2010). MDS is most often diagnosed in the elderly population, with a median age at diagnosis of 65 to 70 years, less than 10% of MDS patients are younger than 50 years (Ades et al., 2014; Tefferi & Vardiman, 2009). With the exception of the subtype of MDS with isolated 5q deletion, these heterogeneous disorders have a slight male predominance (Ades et al., 2014; Garcia-Manero, 2014).

MDS describes a myeloid clonal disorder causing clinical symptoms resulting from cytopenias and hypercellular bone marrow, with the potential for patients with MDS to progress into acute myeloid leukemia (AML) (Szende et al., 2009). Symptoms usually exhibited in this population include fatigue, pallor, infection, and bleeding, often associated with laboratory evidence of anemia, neutropenia, and thrombocytopenia (Faderl & Kantarjian, 2004; Szende et al., 2009). Available treatment options are dependent on the diagnosis and risk assessment, and include growth factor support, lenalidomide, hypomethylating agents, intensive chemotherapy, and allogeneic stem cell transplant (Garcia-Manero, 2014).

INCIDENCE & PREVALENCE

The annual incidence of MDS in the general population varies in the literature, from approximately three to five new cases per 100,000 people per year (Aul, Giagounidis, & Germing, 2001; Malcovati et al., 2013; Moskovtchenkol, 1996; Neukirchen et al., 2011; Radlund, Thiede, Hansen, Carlsson, & Engquist, 1995; Rollison et al., 2008). However, this rate

escalates to 15-50 per 100,000 per year in those over 70 years of age (Ades et al., 2014; Garcia-Manero, 2014; Neukirchen et al., 2011; Tefferi & Vardiman, 2009). Generally, these rates are not attributable to ethnicity; with the exception of the Asian cohort of MDS patients presenting at a younger age with hypocellular marrow, and less commonly with the isolated 5q deletion compared to the Western populations (Ades et al., 2014; Matsuda et al., 2005).

RATIONALE FOR SELECTION OF MDS PATIENTS FOR THIS STUDY

The MDS population has been selected for this study as many of these patients categorized with International Prognostic Scoring System (IPSS) low risk/intermediate-1 disease are known to require chronic RBC transfusions which would allow for a crossover design to be used. However, for a crossover design to be valid the patient's disease status would have to be stable so that their baseline state prior to each transfusion was similar. Hence, the challenge is to identify transfusion dependent MDS patients who will be clinically stable over the duration of the study.

MDS STABILITY ASSESSMENT

The Stability Assessment Algorithm developed by Sholapur (2015) will be used to identify stable patients with MDS (Appendix III). This tool was developed using retrospective data collected and analyzed on a cohort of 21 MDS patients who received greater than three RBC transfusions over a six-month observation period at two Hamilton hospitals (St. Joseph's Healthcare Hamilton (SJHH) and Juravinski Hospital and Cancer Centre (JHCC)). Patients were considered stable if the first and third quartile around the median number of days between transfusion episodes did not exceed 14 days; and the interquartile range (IQR) around their

median pre-transfusion hemoglobin count was less than 10 g/L. Hospitalization episodes had to be less than three times within the six-month observational period and be unrelated to MDS or another chronic illness. Stability also required no severe infections during the observation period. Severe infection for the MDS stability assessment was defined as: persisting for ≥14 days; requiring IV antibiotics to treat; or admission to hospital; or requiring oxygen, fluids to support blood pressure, requiring intubation; or clinically documented septic shock (Sholapur, 2015). The final algorithm was reviewed by physicians who care for MDS patients and by a biostatistician and was felt to have face validity; however, this tool has not yet been validated. The use of this algorithm for the INFLAME-P study will provide validation information.

A RETROSPECTIVE ANALYSIS TO INFORM RECRUITMENT

To determine how many potentially eligible patients with MDS existed in Hamilton, we queried the Transfusion Registry for Utilization Surveillance and Tracking (TRUST) database from January 1st, 2003 to December 31st, 2012. In TRUST, the diagnosis information is available for any patient admitted to Hamilton Health Sciences (HHS). Patients with MDS were identified using the International Classification of Disease (10) (ICD-10) code D46 (Appendix IV, ICD-10 codes) ("International Statistical Classification of Diseases and Related Health Problems 10th Revision," n.d.). Patients were excluded from the analysis when they had an ICD-10 code (C90-95) of leukemia (Appendix V, ICD-10 codes for leukemia) ("International Statistical Classification of Diseases and Related Health Problems 10th Revision," n.d.). For each eligible patient the database was searched for a history of RBC transfusions and the patient's ABO group. When RBCs were transfused the number and ABO group of the RBCs were also captured.

We identified 503 MDS patients; of these 88 patients were excluded because a leukemia diagnosis was coded prior to the MDS code. There were 80.5% (334/415) of patients with MDS who received RBC transfusions. Of the transfused patients, 37.8% (126/334) were group A, the patient blood group of interest for this study (Figure 2). This represents on average 10 new transfused patients with MDS at HHS per year.

There are limitations to this exploratory retrospective analysis. The TRUST database contains diagnosis information only for inpatient admissions; hence, it is likely that there are some patients with MDS that would have been missed because they had never been admitted to a Hamilton hospital. The 10-year period analyzed did not include data from SJHH; hence, the information on patient numbers to inform recruitment for INFLAME-P is underestimated. Since HHS is an academic tertiary centre with referrals for specialized blood disorders there is a chance patients seen at the HHS may have returned to their general practitioners or community hospitals for subsequent care including transfusions. Progression to leukemia or death may have occurred outside HHS and this information would not be captured and the patients would be lost to follow-up. From this exploratory analysis we can anticipate that there would be a minimum of 10 patients/year that could be assessed for eligibility; and although we do not know the exact number of patients treated at SJHH we can assume that the number of potentially eligible patients will be even higher.

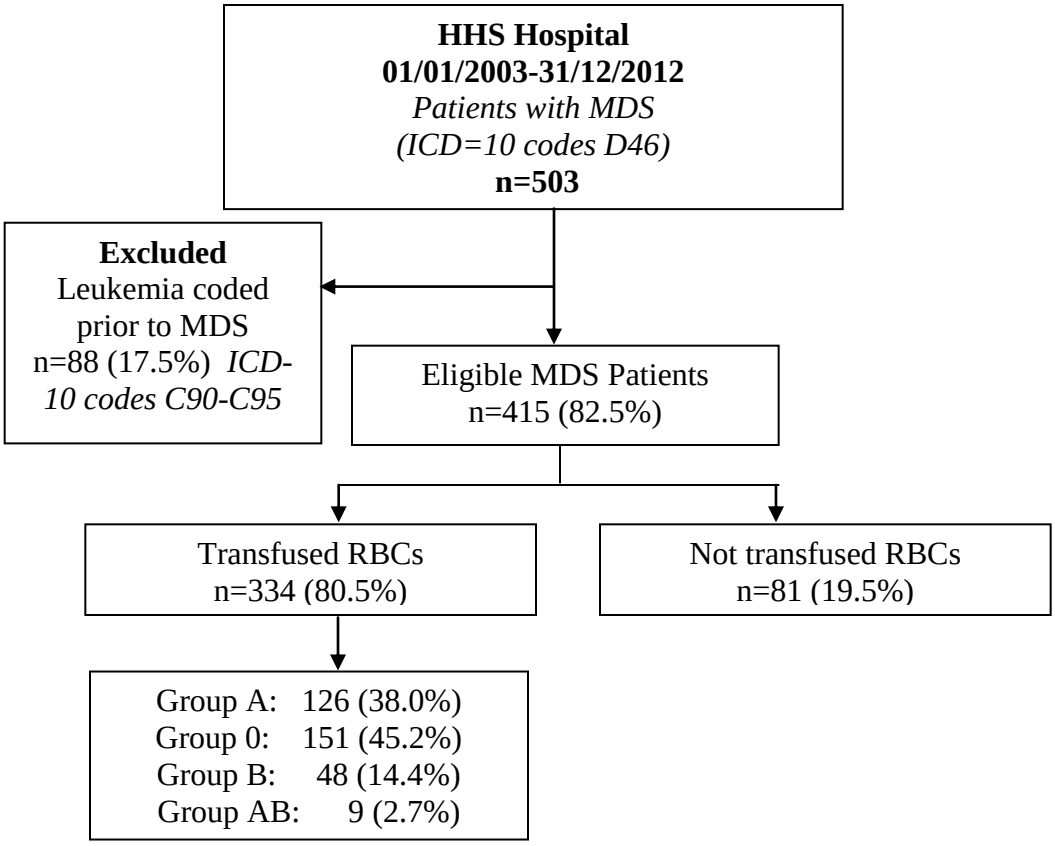


Figure 2: Summary of the number of patients with MDS who were treated at HHS between 2003 and 2012 and the percentage who required transfusion

DO GROUP A PATIENTS WITH MDS RECEIVE GROUP O BLOOD?

If non-identical ABO RBCs are being transfused to patients especially patients with MDS, then this would be considered standard practice and there would be no ethical concerns using ABO non-identical RBCs as the intervention for this study.

While working on this thesis I was the lead investigator for a retrospective study that used TRUST data from a 12-year period to determine the utilization patterns of group O RBC utilization by ABO non-identical recipients and to explore the reasons why ABO non-identical RBCs were given (Appendix VI). Although this study was designed to understand the reasons that could contribute to shortages of group O RBCs, the results confirm that ABO non-identical

RBCs are being transfused. Almost 9% of the group O RBCs transfused were given to patients that were not group O (13,509/ 151,645). This practice of transfusing ABO non-identical RBCs appeared to be long standing as it occurred during all 12 years studied. The percentage of group O RBC transfusions given to non-identical recipients by fiscal year varied from 7.8% to 11.1% with a steady increase in frequency from 2011 to 2013.

Using the data set of MDS patients described earlier to assess recruitment I was also able to confirm whether ABO non-identical blood was given to this patient population. Of the 334 MDS patients who were transfused, 48 patients (14.4%) received one or more ABO non-identical RBC during their transfusion therapy and 20/48 patients (41.7%) were group A. During 2012, the transfused patients received a total of 808 RBCs and 106 (13.1%) were compatible but ABO non-identical (Table 6). The transfusion of ABO non-identical RBCs are part of standard transfusion therapy in Hamilton hospitals and are given to patients with MDS; therefore, transfusing ABO non-identical red cells as the study intervention is not an ethical concern.

Table 6: Number of RBC units transfused to MDS patients in 2012 classified by ABO identical versus ABO non-identical

	Number (%) of RBCs Transfused		
ABO Group of patients with MDS	**ABO Identical**	**ABO Non-Identical**	**Total**
A	239 (84.5)	44 (15.6)	283
B	153 (71.2)	62 (28.8)	215
O	310 (100)	0 (0)	310
Total	702	106	808

CHAPTER 5: INFLAME-P: INFLAMMATION EVALUATION OF CHRONICALLY TRANSFUSED MDS PATIENTS WITH ABO NON-IDENTICAL RBCS: A PILOT STUDY DESIGN

In this chapter the study design for the feasibility pilot study INFLAME-P is presented. When performing clinical experimental research a pilot study can be useful to demonstrate the feasibility of performing the study as designed and to inform design changes that may be needed to move forward with a larger adequately powered study. INFLAME-P is designed as a pilot feasibility study as there were a number of study features where feasibility needs to be demonstrated. However, INFLAME-P is also what I have termed an exploratory pilot study as it is important to demonstrate whether or not there is any evidence from laboratory biomarker test results that supports the hypothesis of an inflammatory response following transfusion of ABO non-identical blood. The results from the biomarker testing in INFLAME-P will need to be considered in the decisions around moving forward with this research. The framework around this decision making process has also been addressed in this design chapter.

RESEARCH QUESTION

Is it feasible to perform a randomized crossover trial in chronically transfused blood group A patients with myelodysplastic syndrome (MDS), that looks for laboratory evidence of inflammation (in vitro biomarkers measured at baseline and pre-specified times between 1 and 96 hours post transfusion), following the transfusion of group O RBCs (ABO non-identical) compared to transfusion of group A (ABO identical) RBCs?

The feasibility outcomes for the pilot study include: the identification and recruitment of eligible patients (consent rate); ability to blind the patient to the treatment allocation; evidence that the eligibility criteria identify patients with stable disease; feasibility of obtaining blood samples for

biomarker testing (collection, freezing and shipping); evidence of a signal of increased inflammation based on biomarker test results; and assessment of drop-out rates and losses to follow-up. .

STUDY HYPOTHESIS

The transfusion of RBCs that are ABO compatible but not ABO group specific can result in an increased inflammatory response, which will be detected by an increase in the concentrations of the various biomarkers of inflammation that will be tested in this study. Biomarker evidence of inflammation will not be detected when ABO identical blood is transfused. It is possible that inflammation may contribute to poor patient outcomes.

TRIAL DESIGN

The pilot randomized crossover trial will be conducted in two Hamilton hospitals (JHCC and SJHH), where treatment for MDS is provided (Figure 3). The trial will be registered on a trial registry website such as, https://clinicaltrials.gov (National Library of Medicine, Bethesda MD, USA) or http://www.isrctn.com (BioMed Central, London, UK). The study will be submitted to the Hamilton Integrated Research Ethics Board (HiREB) for approval prior to patient recruitment.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA

Patients will be considered eligible for the study if they meet all of the following criteria:

1. Adult (age ≥18 years of age);

2. Diagnosis of MDS without leukemia (IPSS classified or physician indicated as either low- risk or intermediate-1);
3. Stable disease (assessed using the MDS Stability Assessment tool; chapter 4, Appendix III) (Sholapur, 2015);
4. ABO blood group A;
5. Expected to receive at least one RBC transfusion on two separate visits no more than 6 weeks apart;
6. Receiving RBC transfusions in an outpatient setting; and,
7. Able to provide informed consent.

EXCLUSION CRITERIA

Patients meeting the inclusion criteria will be excluded if:

1. WHO Bleeding score > Grade 2 at time of screening. WHO bleeding severity scale; Appendix VII (Heddle et al., 2009, 2011).

PATIENT RECRUITMENT

A member of the patient's circle of care will identify MDS patients who come to the out-patient clinics at the two participating hospitals and notify the study research assistant. The research assistant will obtain permission from the treating physician to assess patient eligibility and if eligible, whether the patient could be approached to participate in the study. Assessment of eligibility will be performed through a chart review and querying the TRUST database for transfusion history, hemoglobin values, interval between transfusions, and hospitalizations. When a patient is eligible, the research assistant will provide study information to the patient and answer any questions related to the study. Written informed consent will be obtained. This

process protects potential study participants from feeling coerced into participation, as the patient physician and other caregivers are not involved in the consent process. Once a patient has consented, the first study transfusion will occur at the next clinic visit requiring a transfusion. This will allow clinic appointments for study patients to be scheduled in the morning, making the time of blood sample collection post transfusion more convenient.

RANDOMIZATION

STEP 1: DEVELOPMENT OF THE RANDOM ALLOCATION SCHEDULE

A statistician not directly involved in the study will develop a computer-generated randomization allocation schedule. Patients will be randomly allocated to receive one of two possible RBC treatment types: group A RBCs for the first transfusion episode followed by transfusion of group O RBCs for the next transfusion episode; or group O RBCs followed by group A RBCs. There will be an equal number of treatment types with the order randomly allocated in blocks of two.

STRATIFICATION BY RISK GROUP AND BLOCKING

There may be some relevant differences between MDS risk categorization that might influence inflammation outcome measures. Patients categorized as IPSS low-risk or intermediate-1 are both eligible for the study. However, patients in the IPSS intermediate-1 category may have more cytopenias when compared to patients classified as low-risk. This may be relevant as a patient with a neutropenia could have a different inflammatory response compared to patients without neutropenia. Although the crossover design should negate this influence because each patient will receive both ABO identical and non-identical RBCs in random order, it is possible that the treatment order, by chance, could always result in intermediate-1 or low-risk patients

always getting RBC product types in the same order. To avoid this scenario, the treatment allocation schedule will be stratified by risk group and within each stratum there will be both treatment orders represented in equal numbers. Stratification by participating centre will also occur.

STEP 2: ASSIGNMENT OF PATIENTS TO THE ALLOCATION SCHEDULE

A centralized, internet-based randomization website will be used to assign patients to the allocation schedule. The website will be password protected and will be housed on a secure, encrypted server managed by the Computer Services Unit, McMaster University. The automated randomization system will include verification that a patient meets all of the eligibility criteria before randomization is allowed. Ideally, the individual accessing the web-based allocation schedule should not know the RBC order of the next treatment to be assigned; however, in this pilot study there is a tradeoff to maintain allocation concealment. With blocks of two, once the first treatment order is assigned (i.e., A RBCs followed by O RBCs), it will be apparent that the next patient randomized will be assigned to the opposite order (O RBCs followed by A RBCs). To avoid this situation larger blocks of variable sizes could be used; however, with the small sample size and two strata this would have a high likelihood of imbalance in treatment order within each stratum.

Once a patient has been allocated to a treatment schedule the Transfusion Medicine staff will be notified of the order of the group O and group A RBC transfusions. A comment will be placed in the patient's file in Meditech indicating the ABO group of the RBCs to be transfused.

TIMING OF RANDOMIZATION

Randomization will occur as close to the time of transfusion as possible. Typically this will occur when the consented patient arrives at the clinic for a scheduled transfusion. This is to prevent patients being randomized then not receiving a transfusion for some clinical or logistical reason. As patients are pre-booked for their clinic visits the research assistant will know when the patient is scheduled to be transfused. Communication will occur with the Transfusion Laboratory to ensure that both A and O RBCs are available for transfusion based on the order to which the patient is assigned.

INTERVENTION

The intervention exposure being assessed is the transfusion of ABO non-identical RBCs (group O). The comparator treatment (control) is the transfusion of ABO identical RBCs (group A). A patient will receive both the control and intervention RBCs in this crossover design. It is important that there is a washout period between the two treatment periods wherein the patient can return to a baseline clinical state; however, in this study the transfusion schedule, which is part of the patient's clinical therapy, provides a "natural" washout period. All RBCs transfused in the study will be supplied by Canadian Blood Services. Standard practice is to issue the oldest unit of ABO compatible blood in the available inventory. Although there is currently no clinical evidence that stored blood causes harm, every attempt will be made to provide group A and group O RBCs of similar storage duration (± 5 days). All RBCs selected for transfusion will undergo standard serological testing to ensure compatibility.

Participation in this study will have no impact on the decision to transfuse RBCs. The decision to transfuse in this patient population is typically based on the patient's hemoglobin falling to or

below 80 g/L. The number of RBCs given for each transfusion episode will be identical (typically 2) as this will control for inflammatory markers that could be susceptible to a dose response.

BLINDING

Ideally the patient, the physician and other health care professionals (HCPs) should not know which treatment the patient is receiving (A RBCs or O RBCs). Blinding will not be achieved in this study for a variety of reasons: 1) the laboratory staff have to issue the specified group of blood so they cannot be blinded to the treatment the patient is receiving; the Laboratory Information System requires entry of the ABO group of blood as a safety check; hence, the ABO group of the RBCs will be visible as part of the patient's medical record; using a block size of 2 will allow HCPs to figure out which ABO group will be given for the second transfusion as described above; and, regulatory standards require the ABO group on the RBC product, and an ABO check at the bedside to ensure that the right blood is being transfused to the right patient. It may be possible to blind the patient from knowing the ABO group of the blood being transfused by enclosing the RBC in an opaque bag when transfused (after the appropriate identity check has been done). This would be useful as any patient anxiety from knowing the ABO group of the RBC transfusion could impact some of the biomarker test results. This procedure will be implemented and assessed as one of the feasibility outcomes. The individuals conducting the biomarker testing will be blinded to the treatment that the patient has received by coding the samples to remove any reference to the ABO group of the blood transfused; however; biomarker testing is an objective measurement; hence, the lack of blinding would not be expected to affect the test results.

CROSSOVER DESIGN SCHEMATIC

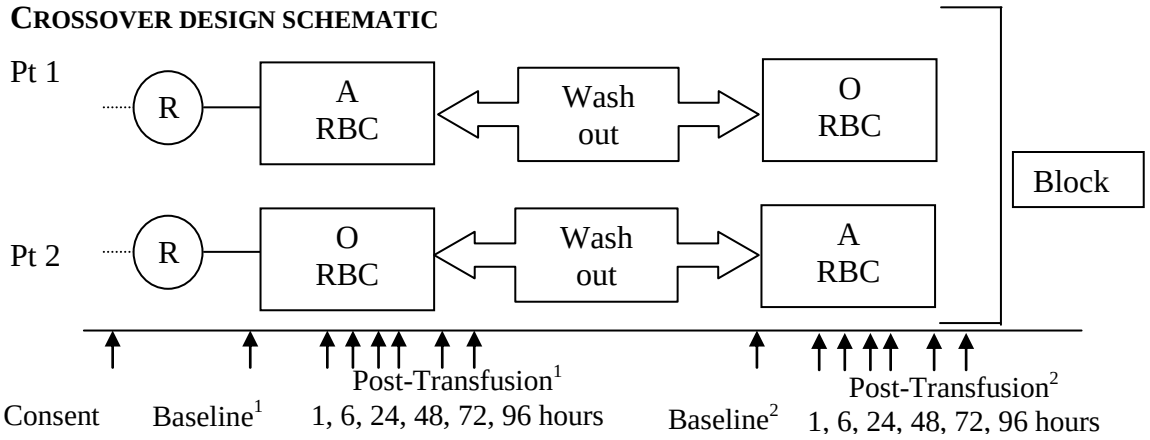


Figure 3: Crossover design schematic
R: Randomization
[1]: Sample collection during transfusion episode 1
[2]: Sample collection during transfusion episode 2

LABORATORY TESTING AND BIOMARKERS OF INFLAMMATION

In chapter 2 the biomarkers of inflammation that will be measured in this study have been summarized and the rationale for the tests selected have been provided. These biomarkers include:

- C-reactive protein
- Cytokines (Interleukin-6, Tumor necrosis factor-alpha, Interleukin-1beta, Interleukin-8)
- Circulating Immune Complexes
- Bactericidal permeability increasing protein

Each study participant will have blood drawn within an hour prior to the transfusion and at 1, 6, 24, 48, 72 and 96 hours post-transfusion for biomarker analysis as described in chapter 2. Two examples (Tables 7 (a and b) are provided to show the timing of the sample collection in relation to the time and the duration (2 hours, or 4 hours) of the RBC transfusion. Ideally, a 12-hour post-transfusion sample would also be collected; however, the time of evening/night that the collection would have to take place is unreasonable; hence, this time point is not included. The simulations provided in these two scenarios also suggest that it would be optimal for a

participant's clinic appointment and transfusion be scheduled as early as possible in the morning to ensure the timing of the 6-hour sample does not occur too late into the evening.

Table 7a: Summary of the timing of the blood draws post-transfusion assuming that the RBCs are transfused over a 2-hour period from 0800 to 1000 hours. The 6-hour sample would be drawn at 1600 hours and the samples over the next 4 days would be drawn at 1000 hours. If a 12-hour sample was taken it would have to be collected at 2200 hours on the day of the transfusion.

Time	Sample Draw	Day 1 Time	Sample Draw	Day 2 Time	Sample Draw	Day 3 Time	Sample Draw	Day 4 time	Sample Draw
0:00		0:00	14	0:00	38	0:00	62	0:00	86
1:00		1:00	15	1:00	39	1:00	63	1:00	87
2:00		2:00	16	2:00	40	2:00	64	2:00	88
3:00		3:00	17	3:00	41	3:00	65	3:00	89
4:00		4:00	18	4:00	42	4:00	66	4:00	90
5:00		5:00	19	5:00	43	5:00	67	5:00	91
6:00		6:00	20	6:00	44	6:00	68	6:00	92
7:00		7:00	21	7:00	45	7:00	69	7:00	93
8:00	TX start	8:00	22	8:00	46	8:00	70	8:00	94
9:00		9:00	23	9:00	47	9:00	71	9:00	95
10:00	Tx stop	10:00	24	10:00	48	10:00	72	10:00	96
11:00	1	11:00	25	11:00	49	11:00	73	11:00	
12:00	2	12:00	26	12:00	50	12:00	74	12:00	
13:00	3	13:00	27	13:00	51	13:00	75	13:00	
14:00	4	14:00	28	14:00	52	14:00	76	14:00	
15:00	5	15:00	29	15:00	53	15:00	77	15:00	
16:00	6	16:00	30	16:00	54	16:00	78	16:00	
17:00	7	17:00	31	17:00	55	17:00	79	17:00	
18:00	8	18:00	32	18:00	56	18:00	80	18:00	
19:00	9	19:00	33	19:00	57	19:00	81	19:00	
20:00	10	20:00	34	20:00	58	20:00	82	20:00	
21:00	11	21:00	35	21:00	59	21:00	83	21:00	
22:00	12	22:00	36	22:00	60	22:00	84	22:00	
23:00	13	23:00	37	23:00	61	23:00	85	23:00	

Table 7b: Summary of the timing of the blood draws post-transfusion assuming that the RBCs are transfused over a 4-hour period from 1200 to 1600 hours. The 6-hour post transfusion sample would be drawn at 2200 hours and the samples over the next 4 days would be drawn at 1600 hours. If a 12-hour sample was taken it would have to be collected at 0400 hours on the day after the transfusion.

Time	Sample Draw	Day 1 Time	Sample Draw	Day 2 Time	Sample Draw	Day 3 Time	Sample Draw	Day 4 time	Sample Draw
0:00		0:00	8	0:00	32	0:00	56	0:00	80
1:00		1:00	9	1:00	33	1:00	57	1:00	81
2:00		2:00	10	2:00	34	2:00	58	2:00	82
3:00		3:00	11	3:00	35	3:00	59	3:00	83
4:00		4:00	12	4:00	36	4:00	60	4:00	84
5:00		5:00	13	5:00	37	5:00	61	5:00	85
6:00		6:00	14	6:00	38	6:00	62	6:00	86
7:00		7:00	15	7:00	39	7:00	63	7:00	87
8:00		8:00	16	8:00	40	8:00	64	8:00	88
9:00		9:00	17	9:00	41	9:00	65	9:00	89
10:00		10:00	18	10:00	42	10:00	66	10:00	90
11:00		11:00	19	11:00	43	11:00	67	11:00	91
12:00	TX start	12:00	20	12:00	44	12:00	68	12:00	92
13:00		13:00	21	13:00	45	13:00	69	13:00	93
14:00		14:00	22	14:00	46	14:00	70	14:00	94
15:00		15:00	23	15:00	47	15:00	71	15:00	95
16:00	Tx stop	16:00	24	16:00	48	16:00	72	16:00	96
17:00	1	17:00	25	17:00	49	17:00	73	17:00	
18:00	2	18:00	26	18:00	50	18:00	74	18:00	
19:00	3	19:00	27	19:00	51	19:00	75	19:00	
20:00	4	20:00	28	20:00	52	20:00	76	20:00	
21:00	5	21:00	29	21:00	53	21:00	77	21:00	
22:00	6	22:00	30	22:00	54	22:00	78	22:00	
23:00	7	23:00	31	23:00	55	23:00	79	23:00	

COLLECTION OF SAMPLES

The Clinical Trials Laboratory at HHS will coordinate the specimen collection send-out kits; specimen storage; and batching of the specimens for analysis. Blood sample collection will be contracted out to LifeLabs Medical Laboratory Services (Toronto, ON) who will collect the timed samples at the study participant's home. If LifeLabs, cannot collect samples after 5 p.m. a research assistant will be hired to collect the timed samples that are scheduled for collection during the evening hours. Samples requiring freezing will be centrifuged and the plasma/serum allocated into four labelled aliquot cryotubes, which will be frozen and stored at -20°C. Samples will be couriered using validated standardized shipping protocols to ensure that the samples

arrive frozen at McMaster University. Batches of samples packed on dry ice will be sent every 6 months.

OTHER LABORATORY TESTS

SUBGROUP OF A

Approximately 80% of group A individuals phenotype as A_1 and 20% type as A_2. It is possible that a patient's A subgroup could contribute to adverse effects when an A individual is transfused with group O RBCs, as the amount of A antigen differs between the subgroups. A_1 has more A antigen and less H antigen, and A_2 has less A antigen and more H antigen. Thus, a recipient's A subtype may affect the amount of passive anti-A that binds to their red cells when O RBCs are transfused. As A_1 RBCs have more antibody binding potential, patients with the A_1 phenotype could have an increased inflammatory response when transfused with ABO non-identical RBCs compared to A_2 individuals. RBC phenotyping for A_1 will be performed by the HRLMP using standard phenotyping procedures.

ABH ANTIGENS, THE LEWIS SYSTEM AND SECRETOR STATUS

Group A secretors (80% of individuals) have soluble A and H antigen substances in their body fluids. Non-secretors do not have ABH substances in their secretions. It has been reported that non-secretors' inflammatory response is impaired (Lomberg, Jodal, Leffler, De Man, & Svanborg, 1992); hence, secretor status of the study patients will be determined as evidence of inflammation may be reduced in non-secretors. Secretor status can be determined in 94% of individuals by performing a Lewis phenotype. This testing will be done by the Transfusion Medicine Service within HRLMP.

Subgroup phenotyping and testing for secretor status will be performed only once on each patient. This will be done at the time of the first transfusion using the sample sent to the Transfusion Medicine Laboratory for compatibility testing. It is possible that the A_1 phenotyping will give mixed field reactions due to circulating RBCs from a previous transfusion; hence, the interpretation of the test may not be possible. The Lewis phenotyping to determine secretor status will be valid even if the patient has been recently transfused, as transfused RBCs acquire the recipient's phenotype within 48-72 hours post-transfusion.

ABO ANTIBODY TITRES ON NON-IDENTICAL PRODUCTS

Although considered rare, hemolysis due to the ABO incompatible plasma containing high titre anti-A or anti-B can occur (Denomme, 2011). Passively acquired ABO antibodies resulting from RBC transfusions may destroy the recipient's own red cells (de França, Poli, Ramos, Borsoi, & Colella, 2011). Binding of anti-A with the A antigen could result in the formation of CICs and mount an inflammatory response through the release of inflammation mediators. Samples from all group O RBCs will be collected using a sterile connecting device. The Transfusion Medicine Laboratory, HRLMP will perform titrations for anti-A and anti-B according to their standard operating procedure for titrations.

PATIENT QUESTIONNAIRE TO ASSESS SUCCESS OF PATIENT BLINDING

After each transfusion study participants will be asked if they knew the ABO group of the blood they received. If they respond in the affirmative they will then be asked how they knew and, what ABO group they think they received. Responses will be documented in a case report form and compared to the transfusion record. This information will enable an assessment of the blinding technique used for patients.

REMOVAL OF STUDY PARTICIPANTS

When a participant exits the study either because they have completed testing in each of the two transfusion episodes or due to early termination (patient/physician withdrawal, lost to follow-up) the study completion form should be completed immediately. This will generate notification to the Transfusion Medicine Services (TMS) that the patient is no longer on the study and any future RBC transfusions will be standard issue.

FEASIBILITY OUTCOMES

The feasibility outcomes for this pilot study are: the identification and recruitment of eligible patients (consent rate); evidence that the eligibility criteria identify patients with stable disease; feasibility of getting blood samples for biomarker testing (collection, freezing and shipping); evidence of inflammation from biomarker testing; and drop-out rates and losses to follow-up. The definitions of success for each feasibility outcome are provided in Table 8.

Table 8: Feasibility outcomes and their definitions of success

Feasibility Outcome	Definition of Success
Recruitment	➢ 10 patients recruited in a period that does not exceed 16 months.
Consent Rate	➢ 50% of eligible patients consent.
Use of opaque bags to blind patient to treatment	➢ Specific threshold was not created for this measure. All participants will be asked to indicate if the blood type of the transfusion episode was known. If everyone indicates 'No' - blinding was successful. However, where 'Yes' is indicated, the reason will be captured providing valuable information for moving forward to a future study.
Evidence that the eligibility criteria identifies patients with stable disease	➢ 90% of patients in the study have a baseline hemoglobin value for each transfusion episode where the first and third quartile around the median is within ± 10g/L; first and third quartile around the median number of days between transfusion episodes is within ± 7 days; no admissions to hospital for MDS related complications between transfusion episodes; and, no severe infectious complications during the study period.
Blood sample collection utilizing a Mobile Services Agency and/or Research Assistant (if required for off hours) and sample freezing and shipping	➢ 80% of samples are collected within 1 hour of the pre-specified time. ➢ 80% of samples collected are frozen within 30 minutes of collection. ➢ 100% of collected samples arrive at McMaster frozen.
Evidence of Inflammation	➢ See section on; Potential Finding of INFLAME-P below and the decision making framework outlined in Figure 4.
Loss to Follow-up and Dropout Rate (not crossing over)	➢ Less than or equal to 20%.

DATA COLLECTION AND DATA MANAGEMENT

DATABASE DEVELOPMENT

Data abstraction forms will be developed either using the research data capture system REDCap (Harris et al., 2009) or a Microsoft Access database (Redmond, WA, USA). Database integrity checks will be created during the development of the study database and forms.

DATA COLLECTION

Case report forms will be developed and pre-tested prior to the start of the study to ensure functionality. Study data collected will document patient screening, consent rates, eligibility, transfusion history and episodes, routine testing, study biomarkers, and other parameters of interest. Data will be extracted from four sources: out-patient clinic charts, electronic medical records (Sovera and Provider Portal), the Laboratory Information System (Meditech), and, the research/testing laboratory reports. A list of the study variables is provided in Appendix VIII.

DATA VERIFICATION, VALIDATION, AND INTEGRITY CHECKS

The study will have monitoring for data integrity, quality and regulatory compliance. Completed data will undergo a review by the data management staff. If there are missing data or if erroneous information is identified a data discrepancy report will be generated and sent to the research personnel for resolution. Data entry for biomarker results will be performed in duplicate to ensure accuracy. All other data will be reviewed in duplicate.

CONFIDENTIALITY AND PRIVACY ISSUES

A participant's name will be available only on the signed consent and screening log which will be stored in a secure research office. Study participants will be assigned a unique study identifier. Any source data related to the data collection forms will be securely stored in the study file within a locked office. Study data monitoring for source checking may require review of identified data; however, this information will be available only within the research office.

RECORDS RETENTION

After study completion, the investigator is responsible for maintaining all study documentation in a safe and secure location for a minimum of 25 years. The site investigator is ultimately responsible for the tracking of this information and storage of the study files.

SAMPLE SIZE

A pilot study sample size estimate should be driven based on the specific feasibility objectives and not the clinical outcomes (Arnold, Webert, Adhikari, & Cook, 2009). There are no known studies evaluating inflammation markers in transfused patients where ABO non-identical RBC transfusions have been assessed. The pilot study will inform the design and sample size calculation of the subsequent larger trial and will not be powered to detect a difference between the two treatment groups.

POWER CALCULATION FOR FEASIBILITY OUTCOME MEASURE

Based on information from TRUST provided in chapter 4, the number of available patients meeting the inclusion criteria will be approximately 10. Therefore, this exploratory pilot will target a sample size of 10 patients and yield 140 laboratory samples (7 serial time points x 2 transfusion episodes). As specified in Table 8, success related to sample collections will be met if 80% or more of the samples are collected within 1 hour of the pre-determined time point and samples are frozen within 30 minutes of collection. To evaluate the appropriate sample size for this feasibility requirement, the clinical margin was set at 70%, so the lower confidence interval cannot cross 70% to be considered a success. The number of samples required to be collected out of 140 total samples with the corresponding power is provided in Table 9 (CI level=95%, one side test). If a minimum of 112 samples are collected (observed percentage=80%), then we have

adequate power (80% or more) to conclude success of these two feasibility outcomes.

Table 9: Power calculation for expected number of samples collected

		Expected number of samples collected										
	105	**108**	**110**	**112**	**113**	**115**	**118**	**120**	**125**	**130**	**135**	**140**
Actual Power	0.32	0.55	0.70	0.83	0.88	0.95	0.99	>0.99	>0.99	>0.99	>0.99	>0.99

DATA ANALYSIS

The evaluation of the feasibility outcomes are the aim of the pilot study (Table 8). [All statistical testing will be carried out with SAS 9.3 (SAS Institute, Cary, NC).] Simple statistical analysis will be used to summarize the data collected. The mean and standard deviation will be reported for each continuous variable as well as the median and interquartile range (IQR). Frequencies (percentages) will be reported for categorical data variables.

A table will be created for each biomarker for each sampling time point with the following information described: allocation sequence; the pre-transfusion value, post-transfusion value and the delta (Δ (difference) =post-pre). An example is provided in Table 10.

Table 10: Study data table to be generated for each maker at the specified time point

Name of the Biomarker:								
Sampling time-point*:								
Study Participant	Allocation Sequence (AO, OA)	Type A			Type O			Δ
		Pre	Post	Δ^A	Pre	Post	Δ^O	Δ^O-Δ^A
1 2 . . . 10								
Mean SD p-value								

*Post-transfusion sampling time points are 1 hour, 6 hours, 24 hours, 48 hours, 72 hours, and 96 hours.

Δ^A= post-pre for type A transfusion episode

Δ^O= post-pre for type O transfusion episode

$\Delta = \Delta^O - \Delta^A$ delta, difference between the two groups

To determine the kinetic profile of each of the biomarkers we will plot the calculated delta versus the time of the testing for each patient and blood type.

For descriptive purposes the percent (%) change will be calculated per patient between treatment groups over the time periods.

PAIRED T-TEST

For any given biomarker, post-transfusion time point and blood type, the observed mean difference in the sample is calculated by taking the average difference found when transfused with type A (identical) minus the average difference when transfused with type O (non-identical) RBCs ($\hat{\Delta}$) for all participants. The standard deviation of the $\hat{\Delta}$ is divided by the square root of the sample size. This will be used to construct 95% CI for the Δ parameter and is the standard error used in the t-test. Thus if

$\hat{\Delta}^A$ = average Δ for type A RBC transfusion episode at a particular post-transfusion time

$\hat{\Delta}^O$ = average Δ for type O RBC transfusion episode at a particular post-transfusion time

then

$$\hat{\Delta} = \hat{\Delta}^A - \hat{\Delta}^O$$

is the estimated mean difference of the differences. If Δ is the true value then we specify the null and alternative hypothesis as H_0: $\Delta=0$ vs. H_A: $\Delta\neq0$ and use the t-statistic.

$$t = \frac{\hat{\Delta} - 0}{SD(\hat{\Delta})/\sqrt{n}}$$

which is t distributed on n-1 degrees of freedom.

The t-test will be calculated for each true point. The p-value will be found using the t-test table for critical values for the sample degrees of freedom, an alpha equal to 0.05, using a two-tailed test.

ORDER EFFECT

To determine if the treatment order of transfusion of ABO non-identical RBCs and ABO identical RBCs has an effect, each paired pre- and post-test will calculate the difference (Δ) when:

$$\Delta = (\Delta_{\text{ABO non-identical first}}) - (\Delta_{\text{ABO non-identical second}})$$

If the 95% CI of the difference of the mean includes zero there is no evidence of an order effect. The direction (positive or negative) of any difference calculated will be indicated if receiving non-identical first or second had a positive or negative effect. This exploratory analysis will also inform whether the markers selected are appropriate and whether the sample collection times need to be adjusted.

SAMPLE SIZE CALCULATION FOR INFLAME TRIAL

If there is an increased inflammatory response when A patients are transfused with O RBCs the above analysis will serve to identify the biomarker and the time point where there was the greatest difference observed. Therefore, the sample size calculation for the larger study will be determined for one biomarker for one pre-specified time point.

$$n \geq \frac{2\,[SD(\Delta)]^2 (Z_{\alpha/2} + Z_{\beta})^2}{(\Delta_{MCID})^2}$$

where,

Δ_{MCID} = Effect size
$Z_{\alpha/2} = 1.96$; $\alpha = 0.05$
$Z_{\beta} = 0.84$; power = 80%
SD = Standard Deviation
$\Delta = \Delta^{O} - \Delta^{A}$: delta, difference between the two groups
MCID = Most Clinically Important Difference

The primary outcome measure analysis will be a paired t-test as described above.

POTENTIAL FINDINGS OF INFLAME-P

Depending on the evidence supporting an inflammatory response post-transfusion, there are a number of potential next steps for this research. The process for moving forward will involve a consensus decision of an expert working group that will be formed to guide the direction that the research will proceed.

NEGATIVE RESULTS

If no inflammatory response is detected then it is unlikely that the decision will be to proceed with a larger INFLAME study. As the biomarkers selected to measure in this study appear to be the most sensitive and frequently used markers of inflammation, negative results would make it difficult to proceed. However, negative studies still provide useful information to inform future research.

POSITIVE RESULTS

If there was evidence of inflammation, this could negate the need for further biomarker data and a move to a study with clinical outcomes (if results were statistically significant), or support moving forward with an adequately powered INFLAME study (if trends support the inflammation hypothesis but are not statistically significant).

VARIABILITY OF RESULTS

If the biomarker test results are variable showing no obvious pattern of results when non-identical versus identical blood is transfused this would suggest that any inflammatory response observed is unrelated to the ABO identical/non identical nature of the transfusion and would not support a decision to proceed with a larger biomarker study. Cytokine studies are difficult to perform and interpret. The need for transfusion could contribute to the observed variability and patient disease factors may also play a role.

ROLE OF THE EXPERT WORKING GROUP

An expert work group will be formed to interpret the biomarker results of the INFLAME-P and make recommendations for proceeding. This group will be comprised of an immunologist, a hematologist, a methodologist, a biochemist, and the PI of the study. This group will determine if there is sufficient evidence to warrant further study, and if so, the direction that research should take. Figure 4 provides a proposed framework for decision making by the expert working group. Figure 5 outlines the process for the research plan progression.

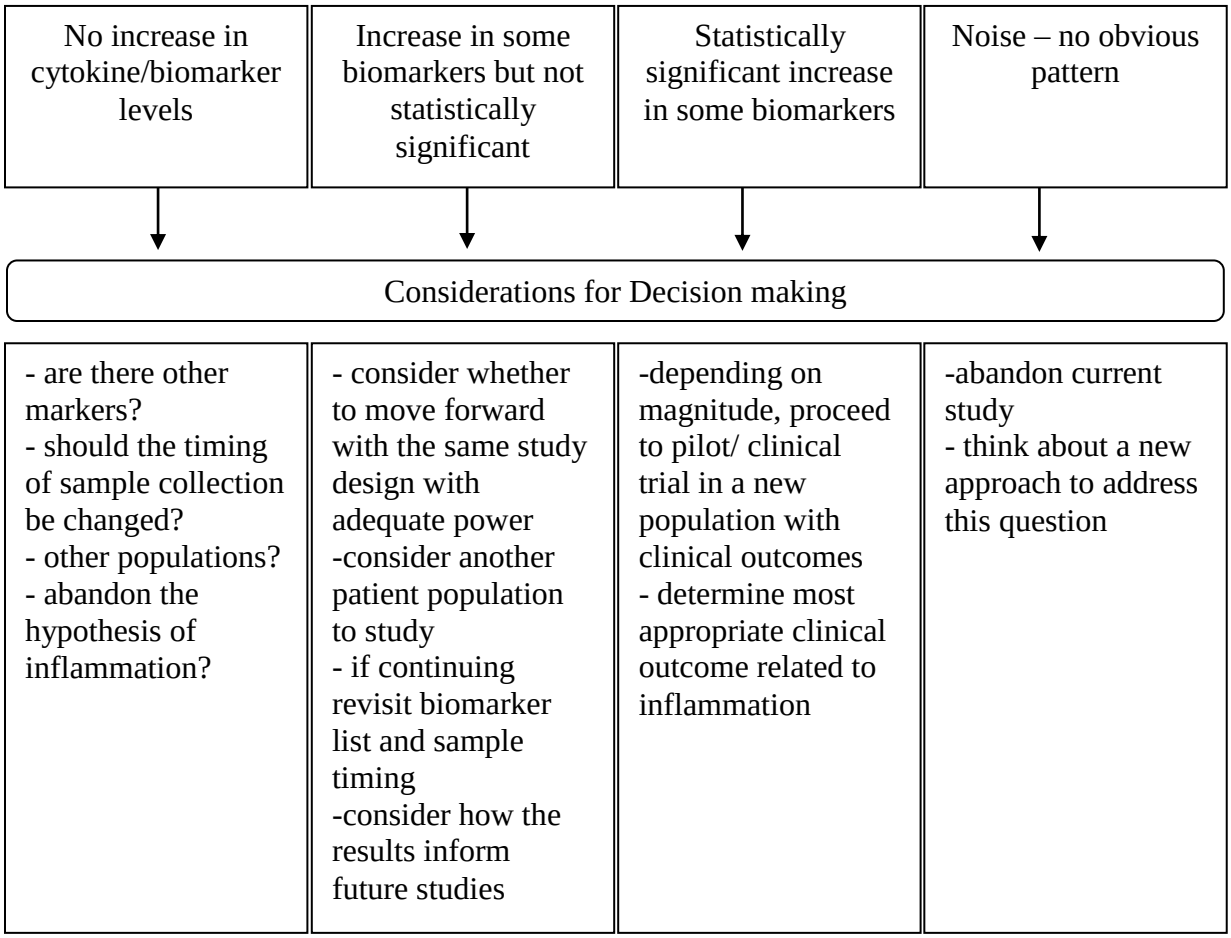


Figure 4: Interpretation of the INFLAME-P study results and framework for decision making around future steps

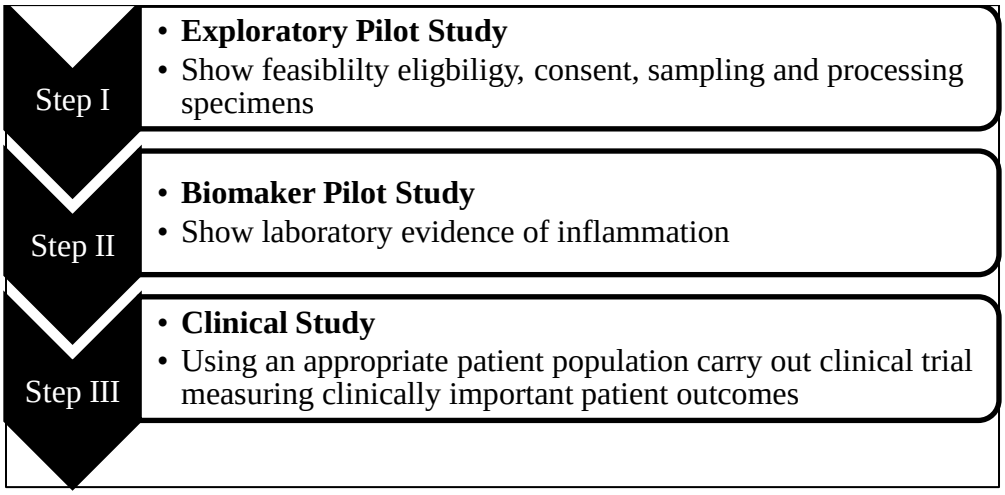


Figure 5: Process for research plan progression

TIMELINE

We anticipate that initial study start up tasks will take the up to four months and will include obtaining the required REB approval, development of the data collection forms and the study instruction and operations manuals. Study recruitment will occur over 16 months. The expected interval between transfusion episodes will be 6 weeks. The biomarker samples will be shipped to the testing site every 6 months. Data cleaning and data validation will be on-going and will commence after data collection from when the first study patient is completed. Statistical analysis, study reports, and the final manuscript will occur during the last four months of the study period. Figure 6 provides the scheduled study task timeline.

	Year 1												Year 2											
Task	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
REB Approvals																								
Implementation Planning																								
Recruitment/Randomization																								
Study Sample and Followup																								
Batched Samples Shipped																								
Sample Anaysis																								
Data Cleaning and Validation																								
Statistical Analysis																								
Report/Manuscript																								

Figure 6: Scheduled study task timeline

POTENTIAL SOURCES OF BIAS AND HOW THEY WILL BE MINIMIZED

Bias results from a systematic error when there is a one-directional deviation from the truth that is caused by some element of the study design or conduct of the study. It is important to identify any potential bias in advance to establish processes to minimize its impact. Appendix IX identifies and defines many possible biases that may introduce a potential risk in INFLAME-P, and the plan to minimize the risk (Delgado-Rodriguez & Llorca, 2004). The three potential

biases that would have the greatest threat for INFLAME-P include: allocation of intervention bias, referral bias and attrition bias. Each of these are described in more detail below.

Allocation of Intervention Bias: Once the first treatment allocation in each block of two is identified the subsequent treatment allocation will be known. As described previously it is not possible to blind laboratory and nursing staff; however, a strategy to blind patients to the ABO group of the RBCs they are receiving has been developed for this study. After the administrative ABO check by the nursing team the RBC unit will be covered with an opaque bag preventing the patient from seeing the ABO group on the label of the RBC. The person conducting the laboratory biomarker testing will also be blinded to the treatment allocation.

Referral Bias: A referral bias occurs when characteristics differ between settings from which patients are recruited. One of the two participating centres is a cancer referral centre and the other is not hence it is possible that the risk category of patients from the two centres may vary. MDS classification also differs between centres: one calculates IPSS score, whereas the other does not. Stratification (by centre and risk category) will be a strategy used to minimize the effects of referral bias.

Attrition Bias: Attrition bias could result from missing data due to loss to follow-up/drop-outs. This patient population is transfusion dependent and the patients are conditioned to frequent clinical visits and transfusions of RBCs. To avoid missing serial timed testing and risk of dropouts a mobile service agency and/or research assistant will be employed to collect samples at the patients' homes to lessen the burden of the frequency of testing. The transfusion and testing schedule will be reviewed prior to the study's first transfusion episode.

THE STUDY COMMITTEES

STEERING COMMITTEE (SC)

The Steering Committee (SC) provides oversight of the overall conduct of the trial. The SC, upon study initiation, will have scheduled meetings in person and/or by teleconference to update on study progress, troubleshoot problems, and ensure that accrual expectations are being satisfied and in an efficient manner. The committee will be comprised of the study principal investigator (Chair) site investigators, a biostatistician, and an immunologist.

DATA AND SAFETY MONITORING BOARD (DSMB)

The Data and Safety Monitoring Board (DSMB) will consist of three members, including the nominated chair. Members include a clinician not associated with the study with expertise in caring for patients with MDS, a methodologist, and a biostatistician. DSMBs have three roles: monitoring safety; advising on early termination of the study based on formal *a priori* stopping rules; and, monitoring the conduct of the study such as, recruitment. In this pilot study the last two roles are not applicable. There are no stopping rules since the pilot is assessing only feasibility measures (recruitment and other operational issues). Therefore, the DSMB for the INFLAME-P will be responsible to monitor safety by reviewing any adverse events associated with transfusion. There will be no formal stopping rules related to safety; however, if the committee has any concerns they will bring them to the attention of the steering committee.

KNOWLEDGE TRANSLATION

Dissemination of the studying findings will be carried out through the use of study reports, abstracts and manuscripts. Study findings are only valuable if they are used to inform best practices and if information from the research team is transferred to health care professionals and

decision makers without causing panic in the general population. In the context of a pilot study assessing the feasibility of a larger trial, publishing the results will provide valuable information to other researchers and may promote collaboration with respect to conducting a larger scale trial.

RELEVANCE OF STUDY FINDINGS

Every research project should consider the clinical impact of the findings and future implications for decision-making and policy changes. It is the goal of this pilot work to determine if it is feasible to conduct a study of blood group A MDS patients who will consent to serial timed sample collection for testing of laboratory biomarkers evaluating inflammatory response when randomized to receive ABO non-identical and ABO identical RBC in a crossover design. If the pilot work shows feasibility a larger RCT that is powered to make statistical inferences may be required. If the pilot work shows no evidence of increased inflammation, then other plausible reasons why O blood given to A patients causes harm should be explored. This work has the potential to impact transfusion policy and reduce the risk of serious complications that may result from transfusion.

CHAPTER 6: ETHICS AND EQUIPOISE

ETHICS IN CLINICAL RESEARCH

Research involving human subjects will always pose ethical concerns; this proposed research study works within all ethical regulations at the institutional level. The ethical considerations related to INFLAME-P are summarized below.

APPROVAL FROM THE INSTITUTIONAL REVIEW BOARD/RESEARCH ETHICS BOARD (REB)

Regulatory bodies require institutional REB review and approval before research involving humans is carried out. Ethics approval is contingent on the research study protocol having demonstrated: that participant risk is minimized; that any risks are reasonable in relation to anticipated benefits; the importance of the expected result and knowledge; that the selection of participants is equitable; that informed consent will be obtained from participants or their legally authorized representative; and that confidentiality will be maintained (Hulley et al., 2013). This study proposal (including consent form) will be submitted to the Hamilton Integrated Research Ethics Board (HiREB) for approval prior to commencing the study. Throughout the study period any study amendment(s), progress reports and study renewal(s), protocol deviation(s), local serious adverse event reporting, and a study completion form will be submitted to HiREB. This process of recruiting patients will conform to local REB requirements. All aspects of the study will operate under Good Clinical Practice (GCP) requirements to protect subjects' rights and welfare.

IS THERE EQUIPOISE?

Clinical equipoise exists when there is no evidence supporting if a treatment/intervention is known to be beneficial to the patient (Freedman, 1987). When equipoise exists it is appropriate and ethical to compare the two treatments in a randomized trial to resolve any uncertainty (Hulley et al., 2013). If we already know the answer to the research question the ethics of additional research is compromised. There are no studies that have explored the hypothesis that ABO non identical blood may cause harm; hence, equipoise does exist.

IS IT ETHICAL TO DO A PILOT?

Pilot studies are performed to determine the feasibility of conducting a trial and to gain insight into procedures that work and those that don't (Arnold, Webert, et al., 2009). There are advantages to undertaking a pilot study, as it informs the elements required to successfully implement a larger study through considering processes, resources, management, and other scientific and methodological aspects (Thabane et al., 2010). It is considered unethical to conduct a study that is known to be statistically underpowered (Halpern, Karlawish, & Berlin, 2002; Thabane et al., 2010). It would be unethical to implement a large randomized trial without demonstrating feasibility. In fact, several granting agencies require the inclusion of feasibility data as part of their evaluation of the scientific validity of a proposal when making funding decisions (Thabane et al., 2010). Disclosure to the participant of the purpose of the pilot study is essential so the intent is understood. The participant consent process should include being informed of the definition of a pilot study, the set feasibility outcomes, and the specified measures of success (Thabane et al., 2010).

There are some disadvantages of pilot studies. Attention to the interpretation of the feasibility objectives are required when the pilot study has been conducted in a small number of sites or only in a single centre (Loscalzo, 2009). The success of the pilot work might be attributed to enthusiastic and keen participating centres or when conducted by stakeholders in the research (Loscalzo, 2009). When pilot sites lack broad representation there may be some methodological and logistical challenges not identified or raised that could be detrimental and jeopardize the future success of a larger trial. The cost of a pilot study is another consideration. Some complex pilot work might be expensive to implement and yield little information (Loscalzo, 2009). Pilot studies seldom provide reliable estimates of sample size since they often are small in numbers (Arnold, Webert, et al., 2009; Loscalzo, 2009). A published report of a pilot study requires critical appraisal. Often pilot studies are not powered to detect harm or benefit; however, some results are presented in a way that might allow for misinterpretation and improper inferences might be implied or deduced (Loscalzo, 2009).

Although these limitations must be kept in mind, pilot studies allow us to test a hypothesis with a manageable patient size, assess the feasibility of the research and not squander resources (Arnold, Webert, et al., 2009; Arnold, Burns, et al., 2009). Since it is uncertain if inflammation is the underlying mechanism impacting post transfusion in-hospital mortality when ABO non-identical RBCs are transfused, this pilot study is an ethical first step before moving to a clinical trial.

IS IT ETHICAL TO GIVE GROUP O RBCS TO GROUP A PATIENTS WHEN YOU HYPOTHESIZE THAT IT CAUSES HARM?

The results of the exploratory analysis summarized in chapter 3 confirms that transfusion of group O RBCs to group A patients has become more prevalent during the last three years and is within the acceptable standard of care. Hence, there are no ethical concerns in giving group O blood to study patients; so as long as they have been adequately informed about the study and provide consent to participate.

If the transfusion of group O blood to group A patients is harmful, then this practice should be changed but it is unlikely that this practices will change without strong evidence showing that it is not safe (especially since it has been going on for decades). Changing this practice without evidence could also cause harm to patients. For example, if transfusion practice required that all patients receive ABO group identical blood then red cell transfusion in trauma would be delayed until the patient's blood group was determined and, transfusions for Group B and AB individuals could be compromised because of inventory shortages of these less common groups. On the other hand, if ABO non-identical transfusions are harmful, the impact is huge given that over 10% of group O RBCs are given to non-group O patients. Hence, to not investigate this hypothesis could be considered unethical. If INFLAME-P demonstrates laboratory evidence of inflammation when ABO non-identical blood is transfused, then moving to a RCT which evaluates clinical outcomes will raise important ethical issues. Such a future study would provide no obvious benefit to an individual patient: the benefit would be at a population level if ABO non-identical blood was inferior, as transfusion practice would change potentially avoiding many deaths. The ethics of such a study would have to be explored but is beyond the scope and intent of this thesis.

IS A CROSSOVER DESIGN ETHICAL AND VALID?

The consideration for the selection of the crossover design took into account the best suited patient population and the advantages and disadvantages of this particular study design. This question was dealt with in chapter 3.

CONCLUSIONS AND FUTURE DIRECTIONS

When designing a study it is critical to take time to develop a well composed research question that incorporates the essential elements of PICOT. In this iterative process several methodological challenges will arise. A literature search is important to determine if there is equipoise, to identify a theoretical/biological framework to support ones research, to inform the selection of the population, intervention, comparator, the outcome and when it should be measured (timing). The literature may also provide information to inform the sample size. However, sometimes there are gaps in information that require additional pilot work.

This thesis work developed the INFLAME-P study which will ascertain feasibility. Utilizing a randomized crossover study design, group A transfusion-dependent MDS patients will be studied to determine if inflammation is detected when group O RBCs are transfused. If the pilot study shows feasibility the major operational/design challenges will have been addressed, increasing the likelihood of success for a larger trial.

REFERENCES

Ades, L., Itzykson, R., & Fenaux, P. (2014). Myelodysplastic syndromes. *Lancet*, *383*(9936), 2239–2252. http://doi.org/10.1016/S0140-6736(13)61901-7

Aguilar-Nascimento, J. E. de, Marra, J. G., Slhessarenko, N., & Fontes, C. J. F. (2007). Efficacy of National Nosocomial Infection Surveillance score, acute-phase proteins, and interleukin-6 for predicting postoperative infections following major gastrointestinal surgery. *Sao Paulo Medical Journal*, *125*(1), 34–41.

Arnold, D. M., Burns, K. E., Adhikari, N. K., Kho, M. E., Meade, M. O., Cook, D. J., & McMaster Critical Care Interest, G. (2009). The design and interpretation of pilot trials in clinical research in critical care. *Crit Care Med*, *37*(1 Suppl), S69–74. http://doi.org/10.1097/CCM.0b013e3181920e33

Arnold, D. M., Webert, K. E., Adhikari, N. K., & Cook, D. J. (2009). Pilot trials in transfusion medicine. *Transfusion*, *49*(7), 1293–1295. http://doi.org/10.1111/j.1537-2995.2009.02154.x

Aul, C., Giagounidis, a, & Germing, U. (2001). Epidemiological features of myelodysplastic syndromes: results from regional cancer surveys and hospital-based statistics. *International Journal of Hematology*, *73*(4), 405–410. http://doi.org/10.1007/BF02994001

Avall, A., Hyllner, M., Bengtson, J. P., Carlsson, L., & Bengtsson, A. (1999). Greater increase in cytokine concentration after salvage with filtered whole blood than with washed red cells, but no difference in postoperative hemoglobin recovery. *Transfusion*, *39*(3), 271–276. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10204589

Ballantyne, C., & Nambi, V. (2005). Markers of inflammation and their clinical significance. *Atherosclerosis Supplements*, *6*(2), 21–29. http://doi.org/DOI 10.1016/j.atherosclerosissup.2005.02.005

Belizaire, R. M., Makley, A. T., Campion, E. M., Sonnier, D. I., Goodman, M. D., Dorlac, W. C., … Pritts, T. A. (2012). Resuscitation with washed aged packed red blood cell units decreases the proinflammatory response in mice after hemorrhage. *Journal of Trauma and Acute Care Surgery*, *73*(2), S128–S133.

Biffl, W. L., Moore, E. E., Offner, P. J., Ciesla, D. J., Gonzalez, R. J., & Silliman, C. C. (2001). Plasma from aged stored red blood cells delays neutrophil apoptosis and primes for cytotoxicity: abrogation by poststorage washing but not prestorage leukoreduction. *Journal of Trauma and Acute Care Surgery*, *50*(3), 426–432.

Bilgin, Y. M., & Brand, A. (2008). Transfusion-related immunomodulation: a second hit in an inflammatory cascade? *Vox Sanguinis*, *95*(4), 261–271.

Bilgin, Y. M., van de Watering, L. M. G., Versteegh, M. I. M., van Oers, M. H. J., & Brand, A. (2010). Effects of allogeneic leukocytes in blood transfusions during cardiac surgery on inflammatory mediators and postoperative complications*. *Crit Care Med*, *38*(2), 546–552.

Blackburn, P., Côté, M., Lamarche, B., Couillard, C., Pascot, A., Tremblay, A., … Després, J.-P. (2003). Impact of postprandial variation in triglyceridemia on low-density lipoprotein particle size. *Metabolism*, *52*(11), 1379–1386.

Bordin, J. O., Chiba, A. K., Carvalho, K. I. L., Takata, E. T., Falcao, R. P., Garcia, A. B., … Kerbauy, J. (1999). The effect of unmodified or prestorage white cell reduced allogeneic red cell transfusions on the immune responsiveness in orthopedic surgery patients. *Transfusion*, *39*(7), 718–723.

Brand, A. (2002). Immunological aspects of blood transfusions. *Transplant Immunology*, *10*(2), 183–190.

Callan, M. B., Patel, R. T., Rux, A. H., Bandyopadhyay, S., Sireci, A. N., O'Donnell, P. A., … Hod, E. A. (2013). Transfusion of 28-day-old leucoreduced or non-leucoreduced stored red blood cells induces an inflammatory response in healthy dogs. *Vox Sanguinis*, *105*(4), 319–327. http://doi.org/10.1111/vox.12058

Carroll, M. F., & Schade, D. S. (2003). Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation*, *108*(1), 24–31.

Cata, J. P., Wang, H., Gottumukkala, V., Reuben, J., & Sessler, D. I. (2013). Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions. *British Journal of Anaesthesia*, *110*(5), 690–701.

Cazzola, M., & Malcovati, L. (2005). Myelodysplastic syndromes--coping with ineffective hematopoiesis. *N Engl J Med*, *352*(6), 536–538. http://doi.org/10.1056/NEJMp048266

Cazzola, M., & Malcovati, L. (2010). Prognostic classification and risk assessment in myelodysplastic syndromes. *Hematol Oncol Clin North Am*, *24*(2), 459–468. http://doi.org/10.1016/j.hoc.2010.02.005

Cholette, J. M., Henrichs, K. F., Alfieris, G. M., Powers, K. S., Phipps, R., Spinelli, S. L., … Blumberg, N. (2012). Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: results of a prospective, randomized, controlled clinical trial. *Pediatr Crit Care Med*, *13*(3), 290–299. http://doi.org/10.1097/PCC.0b013e31822f173c

Davenport, R. D., Strieter, R. M., Standiford, T. J., & Kunkel, S. L. (1990). Interleukin-8 production in red blood cell incompatibility. *Blood*, *76*(12), 2439–2442.

De França, N. D. G., Poli, M. C. C., Ramos, P. G. de A., Borsoi, C. S. da R., & Colella, R. (2011). Titers of ABO antibodies in group O blood donors. *Revista Brasileira de Hematologia E Hemoterapia*, *33*(4), 259–262.

Delclos, K. B., Yeh, E., & Blumberg, P. M. (1983). Specific labeling of mouse brain membrane phospholipids with [20-3H]phorbol 12-p-azidobenzoate 13-benzoate, a photolabile phorbol ester. *Proc Natl Acad Sci U S A*, *80*(10), 3054–3058. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6304691

Delgado-Rodriguez, M., & Llorca, J. (2004). Bias. *Journal of Epidemiology and Community Health*, *58*(8), 635–641.

Denomme, G. (2011). Titers of ABO antibodies in group O blood donors: patient safety and blood product supply remain a challenge. *Revista Brasileira de Hematologia E Hemoterapia*, *33*(4), 250–251.

Dentener, M. A., Francot, G. J. M., Hiemstra, P. S., Tool, A. T. J., Verhoeven, A. J., Vandenabeele, P., & Buurman, W. A. (1997). Bactericidal/permeability-increasing protein release in whole blood ex vivo: strong induction by lipopolysaccharide and tumor necrosis factor-α. *Journal of Infectious Diseases*, *175*(1), 108–117.

Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest*, *118*(2), 503–508. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10936147

Doctor, A., & Spinella, P. (2012). Effect of processing and storage on red blood cell function in vivo. *Semin Perinatol*, *36*(4), 248–259. http://doi.org/10.1053/j.semperi.2012.04.005

Dong, A., Sunkara, M., Panchatcharam, M., Salous, A., Selim, S., Morris, A. J., & Smyth, S. S. (2012). Synergistic effect of anemia and red blood cells transfusion on inflammation and lung injury. *Advances in Hematology*, *2012*.

Elsbach, P. (1994). Bactericidal permeability-increasing protein in host defence against gram-negative bacteria and endotoxin. In *Antimicrobial Peptides, Ciba Foundation Symposium* (Vol. 186, pp. 176–187).

Epstein, F. H., Gabay, C., & Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, *340*(6), 448–454.

Escobar, G. A., Cheng, A. M., Moore, E. E., Johnson, J. L., Tannahill, C., Baker, H. V, … Banerjee, A. (2007). Stored packed red blood cell transfusion up-regulates inflammatory gene expression in circulating leukocytes. *Ann Surg*, *246*(1), 129.

Esposito, K., Nappo, F., Marfella, R., Giugliano, G., Giugliano, F., Ciotola, M., … Giugliano, D. (2002). Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans role of oxidative stress. *Circulation*, *106*(16), 2067–2072.

Faderl, S., & Kantarjian, H. M. (2004). Novel therapies for myelodysplastic syndromes. *Cancer*, *101*(2), 226–241. http://doi.org/10.1002/cncr.20381

Flower, L., Ahuja, R. H., Humphries, S. E., & Mohamed-Ali, V. (2000). Effects of sample handling on the stability of interleukin 6, tumour necrosis factor-α and leptin. *Cytokine*, *12*(11), 1712–1716.

Fransen, E. J., Rombout-Sestrienkova, E., Van Pampus, E. C. M., Buurman, W. A., Reutelingsperger, C. P. M., & Maessen, J. G. (2002). Prestorage leucocyte reduction of red cell components prevents release of bactericidal permeability increasing protein and defensins. *Vox Sanguinis*, *83*(2), 119–124.

Fransen, E., Maessen, J., Dentener, M., Senden, N., & Buurman, W. (1999). Impact of blood transfusions on inflammatory mediator release in patients undergoing cardiac surgery. *CHEST Journal*, *116*(5), 1233–1239.

Freedman, B. (1987). Equipoise and the Ethics of Clinical Research. *New England Journal of Medicine*, *317*(3), 141–145. http://doi.org/doi:10.1056/NEJM198707163170304

Gafter, U., Kalechman, Y., & Sredni, B. (1996). Blood transfusion enhances production of T-helper-2 cytokines and transforming growth factor in humans. *Clinical Science*, *91*(4), 519–524.

Garcia-Manero, G. (2014). Myelodysplastic syndromes : 2014 update. *American Journal of Hematology*, *89*(1), 97–108. http://doi.org/10.1002/ajh.00030

Gill, J. C., Endres-Brooks, J., Bauer, P. J., Marks Jr, W. J., & Montgomery, R. R. (1987). The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*, *69*(6), 1691–1695.

Glenister, K. M., & Sparrow, R. L. (2010). Level of platelet-derived cytokines in leukoreduced red blood cells is influenced by the processing method and type of leukoreduction filter. *Transfusion*, *50*(1), 185–189. http://doi.org/10.1111/j.1537-2995.2009.02353.x

Grimshaw, K., Sahler, J., Spinelli, S. L., Phipps, R. P., & Blumberg, N. (2011). New frontiers in transfusion biology: identification and significance of mediators of morbidity and mortality in stored red blood cells. *Transfusion*, *51*(4), 874–880. http://doi.org/10.1111/j.1537-2995.2011.03095.x

Halpern, S. D., Karlawish, J. H. T., & Berlin, J. A. (2002). The continuing unethical conduct of underpowered clinical trials. *JAMA*, *288*(3), 358–362.

Hamada, Y., Kohtani, T., Nakata, T., Takano, S., Tsunooka, N., Kawachi, K., & Kadota, M. (2001). [Blood transfusion under cardiopulmonary bypass is a possible inducer for inflammation?]. *Kyobu Geka. The Japanese Journal of Thoracic Surgery*, *54*(10), 835–838.

Hampton, D. A., Wiles, C., Fabricant, L. J., Kiraly, L., Differding, J., Underwood, S., … Schreiber, M. A. (2014). Cryopreserved red blood cells are superior to standard liquid red blood cells. *Journal of Trauma and Acute Care Surgery*, *77*(1), 20–27.

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., & Conde, J. G. (2009). Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, *42*(2), 377–381.

Heal, J. M., Masel, D., Rowe, J. M., & Blumberg, N. (1993). Circulating immune complexes involving the ABO system after platelet transfusion. *Br J Haematol*, *85*(3), 566–572. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8136280

Heddle, N. M. (2007). The research question. *Transfusion*, *47*(1), 15–17. http://doi.org/10.1111/j.1537-2995.2007.01084.x

Heddle, N. M., Cook, R. J., Tinmouth, A., Kouroukis, C. T., Hervig, T., Klapper, E., … Barty, R. L. (2009). A randomized controlled trial comparing standard-and low-dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. *Blood*, *113*(7), 1564–1573.

Heddle, N. M., Klama, L., Meyer, R., Walker, I., Boshkov, L., Roberts, R., … Levine, M. (1999). A randomized controlled trial comparing plasma removal with white cell reduction to prevent reactions to platelets. *Transfusion*, *39*(3), 231–238. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10204584

Heddle, N. M., Klama, L. N., Griffith, L., Roberts, R., Shukla, G., & Kelton, J. G. (1993). A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. *Transfusion*, *33*(10), 794–797.

Heddle, N. M., Klama, L., Singer, J., Richards, C., Fedak, P., Walker, I., & Kelton, J. G. (1994). The role of the plasma from platelet concentrates in transfusion reactions. *New England Journal of Medicine*, *331*(10), 625–628.

Heddle, N. M., Wu, C., Vassallo, R., Carey, P., Arnold, D., Lozano, M., … Liu, Y. (2011). Adjudicating bleeding events in a platelet dose study: impact on outcome results and challenges. *Transfusion*, *51*(11), 2304–2310.

Hendrickson, J. E., Hod, E. A., Cadwell, C. M., Eisenbarth, S. C., Spiegel, D. A., Tormey, C. A., … Zimring, J. C. (2011). Rapid clearance of transfused murine red blood cells is associated with recipient cytokine storm and enhanced alloimmunogenicity. *Transfusion*, *51*(11), 2445–2454.

Hendrickson, J. E., Hod, E. A., Hudson, K. E., Spitalnik, S. L., & Zimring, J. C. (2011). Transfusion of fresh murine red blood cells reverses adverse effects of older stored red blood cells. *Transfusion*, *51*(12), 2695–2702.

Hod, E. A., & Spitalnik, S. L. (2011). Harmful effects of transfusion of older stored red blood cells: iron and inflammation. *Transfusion*, *51*(4), 881–885. http://doi.org/10.1111/j.1537-2995.2011.03096.x

Hod, E. A., Zhang, N., Sokol, S. A., Wojczyk, B. S., Francis, R. O., Ansaldi, D., … Spitalnik, S. L. (2010). Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood*, *115*(21), 4284–4292. http://doi.org/10.1182/blood-2009-10-245001

Hulley, S. B., Cummings, S. R., Browner, W. S., Grady, D. G., & Newman, T. B. (2013). *Designing clinical research*. Lippincott Williams & Wilkins.

Hult, A., Malm, C., & Oldenborg, P. A. (2013). Transfusion of cryopreserved human red blood cells into healthy humans is associated with rapid extravascular hemolysis without a proinflammatory cytokine response. *Transfusion*, *53*(1), 28–33. http://doi.org/10.1111/j.1537-2995.2012.03710.x

International Statistical Classification of Diseases and Related Health Problems 10th Revision. (n.d.). Retrieved from http://apps.who.int/classifications/icd10/browse/2016/en

Jiwaji, Z., Nunn, K. P., Conway-Morris, A., Simpson, A. J., Wyncoll, D., Rossi, A. G., & Walsh, T. S. (2014). Leukoreduced blood transfusion does not increase circulating soluble markers of inflammation: a randomized controlled trial. *Transfusion*, *54*(10), 2404–2411.

Johnson, J. L., Moore, E. E., Gonzalez, R. J., Fedel, N., Partrick, D. A., & Silliman, C. C. (2003). Alteration of the postinjury hyperinflammatory response by means of resuscitation with a red cell substitute. *Journal of Trauma and Acute Care Surgery*, *54*(1), 133–140.

Jy, W., Ricci, M., Shariatmadar, S., Gomez-Marin, O., Horstman, L. H., & Ahn, Y. S. (2011). Microparticles in stored red blood cells as potential mediators of transfusion complications. *Transfusion*, *51*(4), 886–893. http://doi.org/10.1111/j.1537-2995.2011.03099.x

Karamlou, T., Schultz, J. M., Silliman, C., Sandquist, C., You, J., Shen, I., & Ungerleider, R. M. (2005). Using a miniaturized circuit and an asanguineous prime to reduce neutrophil-

mediated organ dysfunction following infant cardiopulmonary bypass. *Ann Thorac Surg*, *80*(1), 6–14.

Keir, A. K., McPhee, A. J., Andersen, C. C., & Stark, M. J. (2012). Plasma cytokines and markers of endothelial activation increase after packed red blood cell transfusion in the preterm infant. *Pediatr Res*, *73*(1), 75–79.

Khan, S. Y., Kelher, M. R., Heal, J. M., Blumberg, N., Boshkov, L. K., Phipps, R., … Silliman, C. C. (2006). Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood*, *108*(7), 2455–2462. http://doi.org/10.1182/blood-2006-04-017251

Kim-Shapiro, D. B., Lee, J., & Gladwin, M. T. (2011). Storage lesion: role of red blood cell breakdown. *Transfusion*, *51*(4), 844–851. http://doi.org/10.1111/j.1537-2995.2011.03100.x

Kristiansson, M., Soop, M., Saraste, L., & Sundqvist, K. G. (1996). Cytokines in stored red blood cell concentrates: promoters of systemic inflammation and simulators of acute transfusion reactions? *Acta Anaesthesiol Scand*, *40*(4), 496–501. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8738697

Kristiansson, M., Soop, M., Shanwell, A., & Sundqvist, K. G. (1996). Prestorage versus bedside white blood cell filtration of red blood cell concentrates: effects on the content of cytokines and soluble tumor necrosis factor receptors. *Journal of Trauma and Acute Care Surgery*, *40*(3), 379–383.

Lee, J. S., Sperry, J. L., Ochoa, J. B., Barclay, D., Namas, R., Vodovotz, Y., & Rosengart, M. R. (2012). Persistence of elevated plasma CXCL8 concentrations following red blood cell transfusion in a trauma cohort. *Shock, 37*(4), 373–377. http://doi.org/10.1097/SHK.0b013e31824bcb72

Liem, R. I., O'Gorman, M. R., & Brown, D. L. (2004). Effect of red cell exchange transfusion on plasma levels of inflammatory mediators in sickle cell patients with acute chest syndrome. *Am J Hematol, 76*(1), 19–25. http://doi.org/Doi 10.1002/Ajh.20054

Lin, J., Tzeng, C., Hao, T., Hu, H., Ho, Y., Lyou, J., … Yung, C. (2002). Cytokine release in febrile non-haemolytic red cell transfusion reactions. *Vox Sanguinis, 82*(3), 156–160.

Lobo, S. M. (2012). Sequential C-reactive protein measurements in patients with serious infections: does it help? *Crit Care, 16*(3), 130. http://doi.org/10.1186/CC11347

Locke, R., Paul, D., Touch, S., Mackley, A., Maduskuie, V., & Fawcett, P. (2005). Cytokine load in prestorage leukoreduced PRBC transfusions in premature infants. *J Perinatol, 25*(8), 526–530. http://doi.org/10.1038/sj.jp.7211340

Lomberg, H., Jodal, U., Leffler, H., De Man, P., & Svanborg, C. (1992). Blood group non-secretors have an increased inflammatory response to urinary tract infection. *Scand J Infect Dis, 24*(1), 77–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1589729

Loscalzo, J. (2009). Pilot Trials in Clinical Research Of What Value Are They? *Circulation, 119*(13), 1694–1696.

Maggio, M., Guralnik, J. M., Longo, D. L., & Ferrucci, L. (2006). Interleukin-6 in aging and chronic disease: a magnificent pathway. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *61*(6), 575.

Malcovati, L., Hellström-lindberg, E., Bowen, D., Adès, L., Cermak, J., Porta, M. G. Della, … Can, C. (2013). Diagnosis and treatment of primary myelodysplastic syndromes in adults : recommendations from the European LeukemiaNet Review Article Diagnosis and treatment of primary myelodysplastic syndromes in adults : recommendations from the European LeukemiaNet, *122*(17), 2943–2964. http://doi.org/10.1182/blood-2013-03-492884

Mangalmurti, N. S., Xiong, Z., Hulver, M., Ranganathan, M., Liu, X. H., Oriss, T., … Leel, J. S. (2009). Loss of red cell chemokine scavenging promotes transfusion-related lung inflammation. *Blood*, *113*(5), 1158–1166. http://doi.org/10.1182/blood-2008-07-166264

Matsuda, A., Germing, U., Jinnai, I., Misumi, M., Kuendgen, A., Knipp, S., … Tomonaga, M. (2005). Difference in clinical features between Japanese and German patients with refractory anemia in myelodysplastic syndromes. *Blood*, *106*(8), 2633–2640. http://doi.org/10.1182/blood-2005-01-0040

McMichael, M. A., Smith, S. A., Galligan, A., Swanson, K. S., & Fan, T. M. (2010). Effect of leukoreduction on transfusion-induced inflammation in dogs. *Journal of Veterinary Internal Medicine*, *24*(5), 1131–1137.

Milasiene, V., Stratilatovas, E., Characiejus, D., Kazbariene, B., & Norkiene, V. (2007). TGF-beta1 and TNF-alpha after red blood cell transfusion in colorectal cancer patients. *Exp Oncol*, *29*(1), 67–70. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17431392

Miyaji, K., Miyamoto, T., Kohira, S., Inoue, N., Itatani, K., Sato, H., … Takamoto, S. (2009). The influences of red blood cell transfusion on perioperative inflammatory responses using a miniaturized biocompatible bypass with an asanguineous prime. *International Heart Journal*, *50*(5), 581–589.

Miyaji, K., Miyamoto, T., Kohira, S., Itatani, K., Tomoyasu, T., Sato, H., & Ohara, K. (2010). The effectiveness of prestorage leukocyte-reduced red blood cell transfusion on perioperative inflammatory response with a miniaturized biocompatible bypass system. *J Thorac Cardiovasc Surg*, *139*(6), 1561–1567.

Moskovtchenkol, P. (1996). Epidemiological characteristics of myelodysplastic syndrome in defined French population I---. *British Journal of Cancer*, *60*, 288–290.

Murphy, M. F., & Pamphilion, D. (2005). *Practical Transfusion Medicine* (2nd ed.). Blackwell Publishing.

Muylle, L. (1995). The role of cytokines in blood transfusion reactions. *Blood Rev*, *9*(2), 77–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7580393

Muylle, L., Wouters, E., & Peetermans, M. E. (1996). Febrile reactions to platelet transfusion: the effect of increased interleukin 6 levels in concentrates prepared by the platelet-rich plasma method. *Transfusion*, *36*(10), 886–890.

Mynster, T. (2001). Effects of red cell storage and lysis on in vitro cytokine release. *Transfusion and Apheresis Science*, *25*(1), 17–23.

Nagura, Y., Tsuno, N. H., Tanaka, M., Matsuhashi, M., & Takahashi, K. (2013). The effect of pre-storage whole-blood leukocyte reduction on cytokines/chemokines levels in autologous CPDA-1 whole blood. *Transfusion and Apheresis Science*, *49*(2), 223–230.

Napolitano, L. (2006). Cumulative risks of early red blood cell transfusion. *The Journal of Trauma*, *60*(6 Suppl), S26–S34. http://doi.org/10.1097/01.ta.0000199979.95789.17

Napolitano, L. M., Kurek, S., Luchette, F. a, Corwin, H. L., Barie, P. S., Tisherman, S. a, … Yelon, J. a. (2009). Clinical practice guideline: red blood cell transfusion in adult trauma and critical care. *Critical Care Medicine*, *37*(12), 3124–3157. http://doi.org/10.1097/CCM.0b013e3181b39f1b

Neukirchen, J., Schoonen, W. M., Strupp, C., Gattermann, N., Aul, C., Haas, R., & Germing, U. (2011). Incidence and prevalence of myelodysplastic syndromes: Data from the D??sseldorf MDS-registry. *Leukemia Research*, *35*(12), 1591–1596. http://doi.org/10.1016/j.leukres.2011.06.001

Nielsen, H. J., Skov, F., Dybkjaer, E., Reimert, C. M., Pedersen, A. N., Brünner, N., & Skov, P. S. (1997). Leucocyte and platelet-derived bioactive substances in stored blood: effect of prestorage leucocyte filtration. *Eur J Haematol*, *58*(4), 273–278.

Noordzij, M., Dekker, F. W., Zoccali, C., & Jager, K. J. (2011). Sample size calculations. *Nephron Clinical Practice*, *118*(4), c319–c323.

Pai, M., Cook, R. J., Barty, R., Eikelboom, J., Lee, K., & Heddle, N. M. (2015). Exposure to ABO non-identical blood associated with increased in-hospital mortality in patients with group A blood (Manuscript submitted for publication.).

Pereira, A., Nomdedeu M Fau - Aguilar, J.-L., Aguilar Jl Fau - Belkaid, M., Belkaid M Fau - Carrio, A., Carrio A Fau - Cobo, F., Cobo F Fau - Costa, D., … Am, J. H. (2010). Transfusion intensity, not the cumulative red blood cell transfusion burden, determines the prognosis of patients with myelodysplastic syndrome on chronic transfusion support. *Am J Hematol*, (1096-8652 (Electronic)).

Perttila, J. T., Salo, M. S., Jalonen, J. R., Kuttila, K. T., Viinamaki, O., & Pulkki, K. J. (1994). Blood transfusion with autologous and leukocyte-depleted or standard allogeneic red blood cells and the immune response to open heart surgery. *Anesth Analg*, *79*(4), 654–660. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7943771

Petrovsky, N., McNair, P., & Harrison, L. C. (1998). Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. *Cytokine*, *10*(4), 307–312. http://doi.org/10.1006/cyto.1997.0289

Radlund, a, Thiede, T., Hansen, S., Carlsson, M., & Engquist, L. (1995). Incidence of myelodysplastic syndromes in a Swedish population. *European Journal of Haematology*, *54*(3), 153–156.

Rao, R. S., Howard, C. A., & Teague, T. K. (2006). Pulmonary endothelial permeability is increased by fluid from packed red blood cell units but not by fluid from clinically-available

washed units. *The Journal of Trauma*, *60*(4), 851–858. http://doi.org/10.1097/01.ta.0000195727.38068.7d

Rather, L. J. (1971). Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus. *Bull N Y Acad Med*, *47*(3), 303–322. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5276838

Rollison, D. E., Howlader, N., Smith, M. T., Strom, S. S., Merritt, W. D., Ries, L. a., … List, A. F. (2008). Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. *Blood*, *112*(1), 45–52. http://doi.org/10.1182/blood-2008-01-134858

Sakagawa, H., Miyazaki, T., Fujihara, M., Sato, S., Yamaguchi, M., Fukai, K., … Ikeda, H. (2007). Generation of inflammatory cytokines and chemokines from peripheral blood mononuclear cells by HLA Class II antibody-containing plasma unit that was associated with severe nonhemolytic transfusion reactions. *Transfusion*, *47*(1), 154–161. http://doi.org/10.1111/j.1537-2995.2007.01078.x

Seghatchian, J., Krailadsiri, P., Dilger, P., Thorpe, R., & Wadhwa, M. (2002). Cytokines as quality indicators of leucoreduced red cell concentrates. *Transfus Apher Sci*, *26*(1), 43–46. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11931377

Senay, S., Toraman, F., Gunaydin, S., Kilercik, M., Karabulut, H., & Alhan, C. (2009). The impact of allogenic red cell transfusion and coated bypass circuit on the inflammatory

response during cardiopulmonary bypass: a randomized study. *Interactive Cardiovascular and Thoracic Surgery*, *8*(1), 93–99.

Shanwell, A., Kristiansson, M., Remberger, M., & Ringdén, O. (1997). Generation of cytokines in red cell concentrates during storage is prevented by prestorage white cell reduction. *Transfusion*, *37*(7), 678–684.

Sholapur, N. (2015). Informing the design of an age of blood crossover randomized controlled trial in patients with myelodysplastic syndromes to study change in quality of life as a response to RBC transfusion. (unpublished masters theis, McMaster Univeristy, 2015). McMaster .

Silliman, C. C., Clay, K. L., Thurman, G. W., Johnson, C. A., & Ambruso, D. R. (1994). Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. *The Journal of Laboratory and Clinical Medicine*, *124*(5), 684–694.

Sparrow, R. L., & Patton, K. A. (2004). Supernatant from stored red blood cell primes inflammatory cells: influence of prestorage white cell reduction. *Transfusion*, *44*(5), 722–730.

Stack, G., & Snyder, E. L. (1994). Cytokine generation in stored platelet concentrates. *Transfusion*, *34*(1), 20–25.

Stark, M. J., Keir, A. K., & Andersen, C. C. (2013). Does non-transferrin bound iron contribute to transfusion related immune-modulation in preterms? *Archives of Disease in Childhood-Fetal and Neonatal Edition*, fetalneonatal–2012–303353.

Steel, D. M., & Whitehead, A. S. (1994). The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today*, *15*(2), 81–88. http://doi.org/10.1016/0167-5699(94)90138-4

Stowell, S. R., Smith, N. H., Zimring, J. C., Fu, X., Palmer, A. F., Fontes, J., … Yazer, M. H. (2013). Addition of ascorbic acid solution to stored murine red blood cells increases posttransfusion recovery and decreases microparticles and alloimmunization. *Transfusion*, *53*(10), 2248–2257. http://doi.org/10.1111/trf.12106

Subramanian, V., Bharat, A., Vachharajani, N., Crippin, J., Shenoy, S., Mohanakumar, T., & Chapman, W. C. (2014). Perioperative blood transfusion affects hepatitis C virus (HCV)-specific immune responses and outcome following liver transplantation in HCV-infected patients. *HPB (Oxford)*, *16*(3), 282–294. http://doi.org/10.1111/hpb.12128

Surinenaite, B., Prasmickiene, G., Milasiene, V., Stratilatovas, E., & Didziapetriene, J. (2009). The influence of surgical treatment and red blood cell transfusion on changes in antioxidative and immune system parameters in colorectal cancer patients. *Medicina (Kaunas)*, *45*(10), 785–791. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19996665

Szende, A., Schaefer, C., Goss, T. F., Heptinstall, K., Knight, R., Lubbert, M., … List, A. F. (2009). Valuation of transfusion-free living in MDS: results of health utility interviews with patients. *Health Qual Life Outcomes*, *7*, 81. http://doi.org/10.1186/1477-7525-7-81

Taniguchi, T., Koido, Y., Aiboshi, J., Yamashita, T., Suzaki, S., & Kurokawa, A. (1999). Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. *Crit Care Med*, *27*(7), 1262–1264.

Tefferi, A., & Vardiman, J. W. (2009). Myelodysplastic syndromes. *N Engl J Med*, *361*(19), 1872–1885. http://doi.org/10.1056/NEJMra0902908

Thabane, L., Ma, J., Chu, R., Cheng, J., Ismaila, A., Rios, L. P., … Goldsmith, C. H. (2010). A tutorial on pilot studies: the what, why and how. *BMC Med Res Methodol*, *10*, 1. http://doi.org/10.1186/1471-2288-10-1

Theodoraki, K., Markatou, M., Rizos, D., & Fassoulaki, A. (2014). The impact of two different transfusion strategies on patient immune response during major abdominal surgery: a preliminary report. *Journal of Immunology Research*, *2014*.

Tinmouth, A., Fergusson, D., Yee, I. C., Hebert, P. C., Investigators, A., & Canadian Critical Care Trials, G. (2006). Clinical consequences of red cell storage in the critically ill. *Transfusion*, *46*(11), 2014–2027. http://doi.org/10.1111/j.1537-2995.2006.01026.x

Tuinman, P. R., Vlaar, A. P., Cornet, A. D., Hofstra, J. J., Levi, M., Meijers, J. C., … Juffermans, N. P. (2011). Blood transfusion during cardiac surgery is associated with

inflammation and coagulation in the lung: a case control study. *Crit Care*, *15*(1), R59. http://doi.org/10.1186/cc10032

Twomley, K., Rao, S., & Becker, R. (2006). Proinflammatory, immunomodulating, and prothrombotic properties of anemia and red blood cell transfusions. *Journal of Thrombosis and Thrombolysis*, *21*(2), 167–174. http://doi.org/10.1007/s11239-006-5206-4

Urner, M., Herrmann, I. K., Buddeberg, F., Schuppli, C., Roth Z'graggen, B., Hasler, M., … Beck Schimmer, B. (2012). Effects of blood products on inflammatory response in endothelial cells in vitro. *PLoS One*, *7*(3), e33403. http://doi.org/10.1371/journal.pone.0033403

Vlaar, A. P., Hofstra, J. J., Determann, R. M., Veelo, D. P., Paulus, F., Levi, M., … Juffermans, N. P. (2012). Transfusion-related acute lung injury in cardiac surgery patients is characterized by pulmonary inflammation and coagulopathy: a prospective nested case-control study. *Crit Care Med*, *40*(10), 2813–2820. http://doi.org/10.1097/CCM.0b013e31825b8e20

Wadhwa, M., Seghatchian, M. J., Dilger, P., Contreras, M., & Thorpe, R. (2000). Cytokine accumulation in stored red cell concentrates: effect of buffy-coat removal and leucoreduction. *Transfus Sci*, *23*(1), 7–16. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10925048

Walter, P. B., Fung, E. B., Killilea, D. W., Jiang, Q., Hudes, M., Madden, J., … Harmatz, P. (2006). Oxidative stress and inflammation in iron-overloaded patients with beta-

thalassaemia or sickle cell disease. *Br J Haematol*, *135*(2), 254–263. http://doi.org/10.1111/j.1365-2141.2006.06277.x

Ydy, L. R. A., Slhessarenko, N., & de Aguilar-Nascimento, J. E. (2007). Effect of perioperative allogeneic red blood cell transfusion on the immune-inflammatory response after colorectal cancer resection. *World Journal of Surgery*, *31*(10), 2044–2051.

Zhou, X., Fragala, M. S., McElhaney, J. E., & Kuchel, G. A. (2010). Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr Opin Clin Nutr Metab Care*, *13*(5), 541–547. http://doi.org/10.1097/MCO.0b013e32833cf3bc

APPENDIX I: SEARCH STRATEGY

Electronic Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R)(1946 to present)

Reference Citations were reviewed.

---

1 exp Blood Group Incompatibility/ or exp ABO Blood-Group System/ or ABO incompatible.mp. or exp Middle Aged/ (3531479)
2 Inflammation Mediators/ or inflammation.mp. or Inflammation/ (348068)
3 1 and 2 (59543)
4 Inflammation/ or inflamation.mp. (108170)
5 1 and 4 (15739)
6 Blood Transfusion/ or Erythrocyte Transfusion/ or Blood Group Incompatibility/ or red blood transfusion.mp. (64247)
7 3 and 6 (127)
8 exp Blood Group Incompatibility/ or exp ABO Blood-Group System/ or ABO incompatible.mp. or exp Middle Aged/ (3531479)
9 Inflammation Mediators/ or inflammation.mp. or Inflammation/ (348068)
10 8 and 9 (59543)
11 Inflammation/ or inflamation.mp. (108170)
12 8 and 11 (15739)
13 Blood Transfusion/ or Erythrocyte Transfusion/ or Blood Group Incompatibility/ or red blood transfusion.mp. (64247)
14 10 and 13 (127)
15 Transfusion.mp. or exp Blood Transfusion/ or exp Blood Component Transfusion/ or exp Erythrocyte Transfusion/ or Platelet Transfusion/ or exp Blood Transfusion, Autologous/ (119658)
16 exp Inflammation Mediators/ or inflammation.mp. or exp Inflammation/ (781812)
17 exp Lipopolysaccharides/ or exp Cytokines/ or exp Macrophages/ or exp Inflammation/ or exp Neutrophils/ or exp Systemic Inflammatory Response Syndrome/ or inflammatory response.mp. or exp Mice/ or exp Sepsis/ (2011564)
18 blood transfusion.mp. or exp Blood Transfusion/ (96876)
19 red blood cell transfusion.mp. or exp Erythrocyte Transfusion/ (7594)

20 exp Blood/ or exp Blood Component Transfusion/ or exp Blood Donors/ or exp Blood Transfusion/ or exp Erythrocytes/ or blood components.mp. or exp Blood Platelets/ (1060312)

21 15 and 18 and 19 and 20 (7089)

22 16 and 17 and 21 (325)

23 exp Cytokines/ or exp Interleukin-6/ or exp Inflammation/ or exp Neutrophils/ or proinflammatory.mp. or exp Tumor Necrosis Factor-alpha/ or Macrophages/ (917660)

24 21 and 23 (860)

25 blumberg n.au. (235)

26 inflammation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (348068)

27 25 and 26 (21)

28 platelets.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (101529)

29 27 and 28 (16)

30 red blood cells.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (35829)

31 27 and 30 (3)

32 26 and 28 and 30 (62)

33 proinflammatory.mp. (49431)

34 inflammatory response.mp. (52542)

35 16 and 26 and 33 and 34 (2958)

36 inflammation complications.mp. (32)

37 30 and 33 (114)

38 19 and 33 (31)

39 Transfusion.mp. or exp Blood Transfusion/ or exp Blood Component Transfusion/ or exp Erythrocyte Transfusion/ or Platelet Transfusion/ or exp Blood Transfusion, Autologous/ (119658)

40 exp Inflammation Mediators/ or inflammation.mp. or exp Inflammation/ (781812)

41 exp Lipopolysaccharides/ or exp Cytokines/ or exp Macrophages/ or exp Inflammation/ or exp Neutrophils/ or exp Systemic Inflammatory Response Syndrome/ or inflammatory response.mp. or exp Mice/ or exp Sepsis/ (2011564)

42 blood transfusion.mp. or exp Blood Transfusion/ (96876)

43 red blood cell transfusion.mp. or exp Erythrocyte Transfusion/ (7594)

44 exp Blood/ or exp Blood Component Transfusion/ or exp Blood Donors/ or exp Blood Transfusion/ or exp Erythrocytes/ or blood components.mp. or exp Blood Platelets/ (1060312)

45 39 and 42 and 43 and 44 (7089)

46 40 and 41 and 45 (325)

47 exp Cytokines/ or exp Interleukin-6/ or exp Inflammation/ or exp Neutrophils/ or proinflammatory.mp. or exp Tumor Necrosis Factor-alpha/ or Macrophages/ (917660)

48 45 and 47 (860)

49 blumberg n.au. (235)

50 inflammation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (348068)

51 49 and 50 (21)

52 platelets.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (101529)

53 51 and 52 (16)

54 red blood cells.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (35829)

55 51 and 54 (3)

56 50 and 52 and 54 (62)

57 proinflammatory.mp. (49431)

58 inflammatory response.mp. (52542)

59 40 and 50 and 57 and 58 (2958)

60 inflammation complications.mp. (32)

61 54 and 57 (114)

62 43 and 57 (31)

63 Transfusion.mp. or exp Blood Transfusion/ or exp Blood Component Transfusion/ or exp Erythrocyte Transfusion/ or Platelet Transfusion/ or exp Blood Transfusion, Autologous/ (119658)

64 exp Inflammation Mediators/ or inflammation.mp. or exp Inflammation/ (781812)

65 exp Lipopolysaccharides/ or exp Cytokines/ or exp Macrophages/ or exp Inflammation/ or exp Neutrophils/ or exp Systemic Inflammatory Response Syndrome/ or inflammatory response.mp. or exp Mice/ or exp Sepsis/ (2011564)

66 blood transfusion.mp. or exp Blood Transfusion/ (96876)

67 red blood cell transfusion.mp. or exp Erythrocyte Transfusion/ (7594)

68 exp Blood/ or exp Blood Component Transfusion/ or exp Blood Donors/ or exp Blood Transfusion/ or exp Erythrocytes/ or blood components.mp. or exp Blood Platelets/ (1060312)

69 63 and 66 and 67 and 68 (7089)

70 64 and 65 and 69 (325)

71 exp Cytokines/ or exp Interleukin-6/ or exp Inflammation/ or exp Neutrophils/ or proinflammatory.mp. or exp Tumor Necrosis Factor-alpha/ or Macrophages/ (917660)

72 69 and 71 (860)

73 blumberg n.au. (235)

74 inflammation.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (348068)

75 73 and 74 (21)

76 platelets.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (101529)

77 75 and 76 (16)

78 red blood cells.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (35829)

79 75 and 78 (3)

80 74 and 76 and 78 (62)

81 proinflammatory.mp. (49431)

82 inflammatory response.mp. (52542)

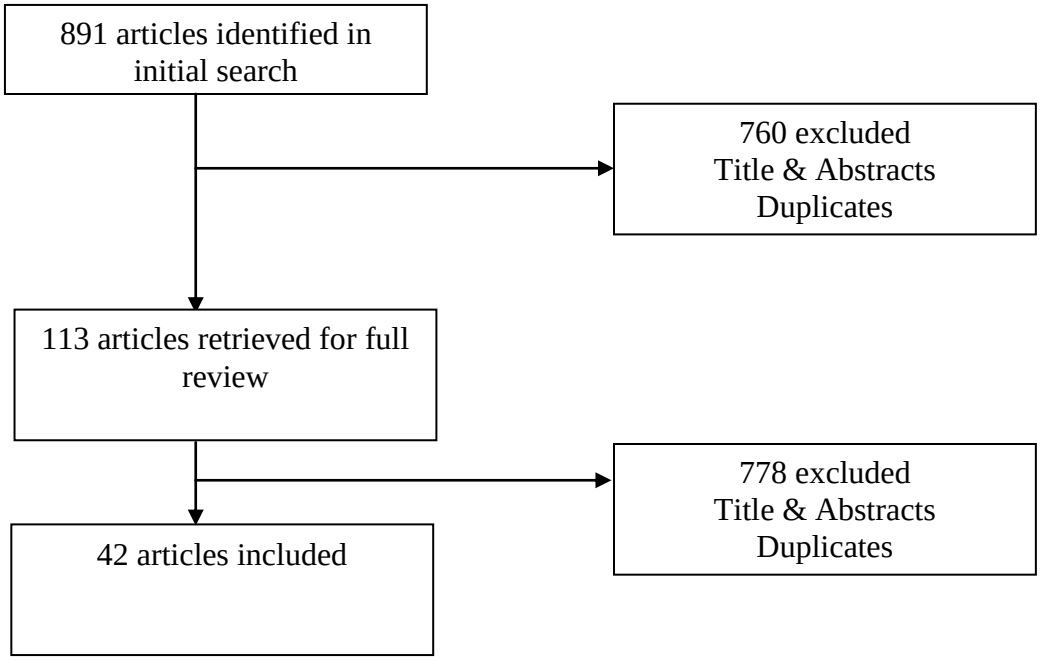
83 64 and 74 and 81 and 82 (2958)

84 inflammation complications.mp. (32)

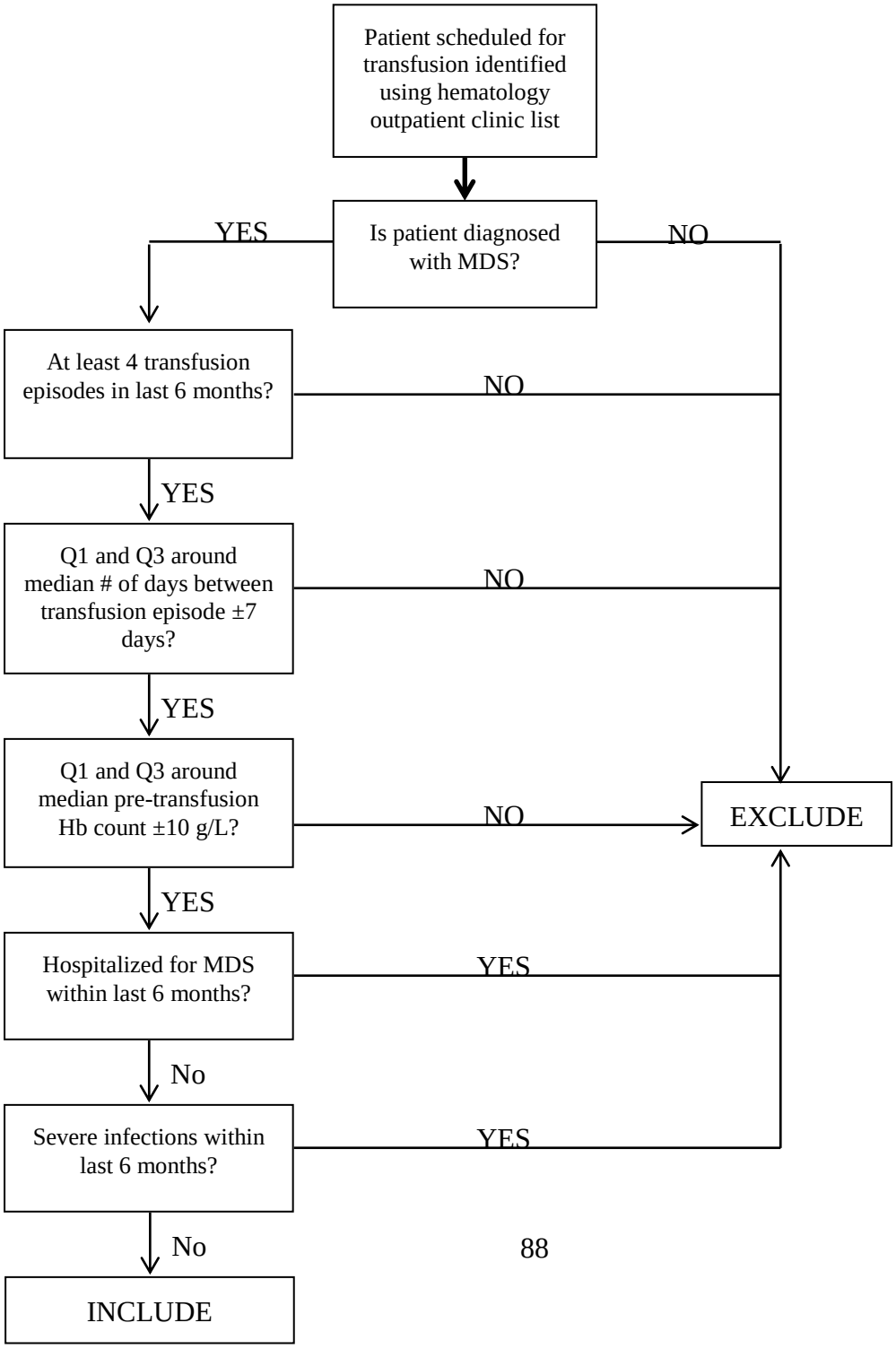
85 78 and 81 (114)

86 67 and 81 (31)

APPENDIX II: LITERATURE REVIEW SELECTION FLOW CHART



APPENDIX III: MDS STABILITY ASSESSMENT ALGORITHM: SCREENING PROCESS FOR RECRUITING PATIENTS WHO ARE STABLE FOR A CROSSOVER RCT (Sholapur, 2015)



APPENDIX IV: MDS DATA ANALYSIS (ICD-10 CODES)

http://apps.who.int/classifications/icd10/browse/2015/en

Myelodysplastic syndromes: any type of diagnosis with ICD=10 codes D46

Incl.: Alkylating agent related myelodysplastic syndrome
Epipodophyllotoxin related myelodysplastic syndrome
Therapy related myelodysplastic syndrome NOS
Excl.: drug induced aplastic anaemia (D61.1)

D46.0 Refractory anaemia without ring sideroblasts, so stated
Note: without sideroblasts, without excess of blasts

D46.1 Refractory anaemia with ring sideroblasts

D46.2 Refractory anaemia with excess of blasts [RAEB]
Note: RAEB I
RAEB II

D46.4 Refractory anaemia, unspecified

D46.5 Refractory anaemia with multi-lineage dysplasia

D46.6 Myelodysplastic syndrome with isolated del(5q) chromosomal abnormality
5 q-minus syndrome

D46.7 Other myelodysplastic syndromes
Excl.: chronic myelomonocytic leukaemia (C93.1)

D46.9 Myelodysplastic syndrome, unspecified
Myelodysplasia NOS
Preleukaemia (syndrome) NOS

APPENDIX V: MDS DATA ANALYSIS (ICD-10 CODES)

http://apps.who.int/classifications/icd10/browse/2015/en

Leukemia: any type of type of diagnosis with ICD-10 codes C90-C95 were excluded

C90.0Multiple myeloma

Kahler disease
Medullary plasmacytoma
Myelomatosis
Plasma cell myeloma
Excl.:solitary plasmacytoma (C90.3)

C90.1Plasma cell leukaemia
Plasmacytic leukaemia

C90.2Extramedullary plasmacytoma

C90.3Solitary plasmacytoma
Localized malignant plasma cell tumour NOS
Plasmacytoma NOS
Solitary myeloma

C91Lymphoid leukaemia

C91.0Acute lymphoblastic leukaemia [ALL]
Note:This code should only be used for T-cell and B-cell precursor leukeamia

C91.1Chronic lymphocytic leukaemia of B-cell type
Lymphoplasmacytic leukaemia
Richter syndrome
Excl.:lymphoplasmacytic lymphoma (C83.0)

C91.3Prolymphocytic leukaemia of B-cell type

C91.4Hairy-cell leukaemia
Leukaemic reticuloendotheliosis

C91.5Adult T-cell lymphoma/leukaemia [HTLV-1-associated]

Acute Chronic	variant

Lymphomatoid
Smouldering

C91.6Prolymphocytic leukaemia of T-cell type

C91.7Other lymphoid leukaemia

T-cell large granular lymphocytic leukaemia (associated with rheumatoid arthritis)

C91.8Mature B-cell leukaemia Burkitt-type

Excl.:Burkitt lymphoma with little or no bone marrow infiltration (C83.7)

C91.9Lymphoid leukaemia, unspecified

C92Myeloid leukaemia

Incl.:leukaemia:

- granulocytic
- myelogenous

C92.0Acute myeloblastic leukaemia [AML]

Acute myeloblastic leukaemia, minimal differentiation
Acute myeloblastic leukaemia (with maturation)
AML1/ETO
AML M0
AML M1
AML M2
AML with t(8;21)
AML (without a FAB classification) NOS
Refractory anaemia with excess blasts in transformation

Excl.:acute exacerbation of chronic myeloid leukaemia (C92.1)

C92.1Chronic myeloid leukaemia [CML], BCR/ABL-positive

Chronic myelogenous leukaemia:

- Philadelphia chromosome (Ph1) positive
- t(9:22)(q34; q11)
- with crisis of blast cells

Excl.:atypical chronic myeloid leukaemia, BCR/ABL-negative (C92.2)
chronic myelomonocytic leukaemia (C93.1)
unclassified myeloproliferative disease (D47.1)

C92.2Atypical chronic myeloid leukaemia, BCR/ABL- negative

C92.3Myeloid sarcoma

Note:a tumour of immature myeloid cells

Chloroma

Granulocytic sarcoma

C92.4Acute promyelocytic leukaemia [PML]

AML M3

AML Me with t(15; 17) and variants

C92.5Acute myelomonocytic leukaemia

AML M4

AML M4 Eo with inv(16) or t(16;16)

C92.6Acute myeloid leukaemia with 11q23-abnormality

Acute myeloid leukaemia with variation of MLL-gene

C92.7Other myeloid leukaemia

Excl.:chronic eosinophilic leukaemia [hypereosinophilic syndrome] (D47.5)

C92.8Acute myeloid leukaemia with multilineage dysplasia

Note:Acute myeloid leukaemia with dysplasia of remaining haematopoesis and/or myelodysplastic disease in its history.

C92.9Myeloid leukaemia, unspecified

C93Monocytic leukaemia

Incl.:monocytoid leukaemia

C93.0Acute monoblastic/monocytic leukaemia

AML M5a

AML M5b

AML M5

C93.1Chronic myelomonocytic leukaemia

Chronic monocytic leukaemia

CMML-1

CMML-2

CMML with eosinophilia

C93.3Juvenile myelomonocytic leukaemia

C93.7Other monocytic leukaemia

C93.9Monocytic leukaemia, unspecified

C94 Other leukaemias of specified cell type

Excl.: leukaemic reticuloendotheliosis (C91.4)
plasma cell leukaemia (C90.1)

C94.0Acute erythroid leukaemia
Acute myeloid leukaemia M6 (a)(b)
Erythroleukaemia

C94.2Acute megakaryoblastic leukaemia
Acute myeloid leukaemia, M7
Acute megakaryocytic leukaemia

C94.3Mast cell leukaemia

C94.4Acute panmyelosis with myelofibrosis
Acute myelofibrosis

C94.6Myelodysplastic and myeloproliferative disease, not elsewhere classified

C94.7Other specified leukaemias
Aggressive NK-cell leukaemia
Acute basophilic leukaemia

C95 Leukaemia of unspecified cell type

C95.0Acute leukaemia of unspecified cell type
Acute bilineal leukaemia
Acute mixed lineage leukaemia
Biphenotypic acute leukaemia
Stem cell leukaemia of unclear lineage
Excl.: acute exacerbation of unspecified chronic leukaemia (C95.1)

C95.1Chronic leukaemia of unspecified cell type

C95.7Other leukaemia of unspecified cell type

C95.9Leukaemia, unspecified

APPENDIX VI: GROUP O RBCS: WHERE IS UNIVERSAL DONOR BLOOD BEING USED?

Rebecca Barty[1]
Menaka Pai[2]
Yang Liu[1]
Donald Arnold[1,3]
Richard J Cook[4]
Nancy M Heddle[1,3]

[1] Department of Medicine, Faculty of Health Sciences, McMaster University, Hamilton ON

[2]McMaster University, Department of Pathology and Molecular Medicine

[3] Centre for Innovation, Canadian Blood Services, Hamilton ON

[4] Department of Statistics and Actuarial Science, University of Waterloo, Waterloo ON

Corresponding Author: Rebecca Barty, MLT, BA, MSc (c)
Clinical Research Coordinator, McMaster Transfusion Research Program
Department of Medicine /Faculty of Health Science, McMaster University
HSC 3H50, 1280 Main Street West, Hamilton, ON L8S 4K1
tel: 905 525 9140 Ext 22945
fax: 905 524 2983
email: bartyr@mcmaster.ca
Reprints will not be available from the author

Sources of Support:
Infrastructure funding for the McMaster Transfusion Research Program is provided in part by Canadian Blood Services and Health Canada.
Dr. Menaka Pai is supported by the E.J. Moran Campbell AFP Internal Career Research Award from McMaster University's Department of Medicine.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Manuscript Word Count (not including abstract or figures): TBD
Abstract Word Count: TBD

Running Title: utilization, blood transfusion, retrospective database

ABSTRACT

BACKGROUND: Individuals with group O blood are designated 'universal donors' as their red blood cells (RBCs) can be transfused to other ABO groups. 15% of donors are Rh negative; their red cells are valuable as they are the only blood compatible with O Rh negative recipients. There have been recurrent shortages of O blood due to low donor prevalence, and use of O RBCs in ABO non-identical recipients; hence, we performed a 12-year retrospective study to determine the utilization of group O Rh positive and Rh negative RBCs by recipient's ABO group in a multisite academic facility. Reasons why group O blood were transfused to non-group O recipients were also assessed.

METHODS: Utilization data from all group O Rh positive and Rh negative RBCs transfused at 3 academic hospitals between April 2002 and March 2014 were included in the study. Autologous and directed donor RBCs were excluded. Recipient data collected included: inpatient, outpatient or emergency department; ABO and Rh type; number of transfusions received; presence of red cell alloantibodies; most responsible diagnosis (ICD-10 code); intervention (CCI code); NICU admission; and indications for transfusion. Descriptive statistics were performed using SAS 9.3.

RESULTS: There were 314,968 RBC transfusions during 75,935 admissions: 151,645 (48.1%) were group O, of which 138,136 (91.1%) RBCs were transfused to group O individuals. Non-identical recipients received 13,509 (8.9%) group O RBCs: in-patients 10,334 (76.5%) and out-patients 3,175 (23.5%). The majority of these group O RBC transfusions were Rh negative 8,378 (62.0%). The percentage of group O RBC transfusions to non-identical recipients by fiscal year varied from 7.8% to 11.1% with a

steady increase from 2011 to 2013. There were 12,360 O Rh negative RBC transfusions given to Rh positive recipients: 7,020 to O Rh positive; 3,348 to A Rh Positive; 1,662 to B Rh positive; and 330 to AB Rh positive.

CONCLUSION: The frequency of O RBC transfusion to non-identical recipients increased annually from 2011 to 2013. Approximately 76.9% of the group O RBC transfusions to non-identical recipients are the result of phenotypic matched group O RBCs for alloimmunization, inventory shortages, and NICU patients. These are areas that could be targeted to change practice, leading to a more sustainable group O RBC supply.

The mandate for blood suppliers is to provide an adequate inventory of safe and effective blood products. Over the past several decades product safety has improved due to rigorous and new standards for blood donor selection, increased sensitivity of laboratory screening tests that have reduced the risk infectious complications, and measures to prevent transfusion related acute lung injury.[1–3] However, ensuring adequate supply has become more challenging because of an increasing number of donor deferrals and increased demand for blood from an aging population.[4] Hence, balancing the RBC (RBC) inventory to meet supply and demand is an ongoing challenge.

Group O RBCs are considered the universal donor as the A and B antigens are not expressed on these cells allowing them to be safely transfused to all individuals. Although group O RBCs can be given to any recipient, the group O individual needing RBC transfusion can only receive RBCs from a group O donor. Hence, the transfusion of group O RBCs to individuals of groups A, B or AB can contribute to an inventory shortage for the group O patient. This situation is even more complex when the Rh type is also considered. Seven percent of donors are O Rh negative.[5] When the O Rh negative patient needs RBCs he/she is restricted typically to the O Rh negative inventory as the transfusion of Rh positive blood to this individual puts them at risk of alloimmunization to D. It is well recognized that there are situations when group O RBCs (Rh positive and Rh negative) are given intentionally to individuals of other ABO groups including: a lack of group specific RBC supply; emergency situations where blood is needed before the recipients' blood group is determined; neonates in centres where the policy is to give only O RBCs; special circumstances such as phenotypically matched RBCs, transplant

patients; requirement for CMV negative RBCs; and to prevent wastage when O RBCs are close to outdating.

Understanding group O RBC utilization patterns and why group O RBCs are given to ABO non-identical (A/B/AB) recipients is the first step in identifying potential policy changes that would contribute to an adequate blood supply. This study was undertaken in three acute care Canadian hospitals to determine the utilization patterns of group O RBCs (Rh positive and Rh negative) by non-identical recipients over a 12-year period and to explore the reasons for the non-group specific practices.

Methods

A retrospective study design was used to explore utilization patterns of group O RBCs over time, by recipient ABO blood group. Data from patients who were transfused RBCs at three acute care hospital sites in Hamilton from April 1, 2002 to March 31, 2014 were included in the study. The Hamilton Integrated Research Ethics Board approved the study.

The Transfusion Registry for Utilization Surveillance and Tracking (TRUST) database was used as the source of data collection. TRUST is a comprehensive database developed by the McMaster Transfusion Research Programs (MTRP) which contains detailed information on all blood products received at the hospital, including product type, ABO and Rh type, final disposition (transfused, wasted, outdated), and demographic and clinical data on all patients admitted to hospital (transfused and non-transfused).

The following information was extracted for each allogeneic donor RBC transfused (autologous and directed donations were excluded): ABO group; Rh type; and, date of collection. For each recipient the transfused information collected included: ABO group; Rh type; most responsible diagnosis (coded using the 10th edition of the International Classification of Disease); interventions (CCI code); date of RBC transfusion; whether or not the patient was alloimmunized; and, the ward/clinical location of the patient at the time of the transfusion.

The reasons for non-identical RBC transfusions were categorized hierarchically, as follows:

1) Urgent massive transfusion (trauma): patients who required ≥ 10 RBC transfusions within 24 hours from the initial transfusion, with any number of consecutive ABO non-identical (O to A, B or AB) transfusion from the initial unit transfused.

2) Neonatal transfusion: patients in the Neonatal Intensive Care Unit (NICU) at the time of transfusion. (Note: current hospital policy is to give only group O RBC transfusions to neonates in the NICU).

3) Alloimmunization: patients who had a positive antibody result in their transfusion history. We assumed that these patients had a requirement for phenotype matched blood.

4) Patients with sickle-cell disease. These patients are given phenotype matched blood at our centre and the phenotyped RBCs are often provided as group O.

5) RBC outdating: if the RBC unit was within one week of the outdate date at the time of transfusion it was classified as outdating (age of unit ≥ 35 days old).

6) Transplant patient: patient receiving a solid organ or hematopoietic stem cell transplant. Transplant protocols often require group O RBCs to circumvent compatibility issues between donor and recipient.

7) Presumed shortage of ABO-identical blood (Other): This was the default category. If none of the above conditions were met, it was presumed that the non-identical RBC transfusion was due to a shortage of ABO-identical blood in the blood bank inventory.

Analysis

To determine the utilization of blood by donor and recipient group a cross tabulation table was constructed by ABO group alone and by ABO and Rh type combined for the entire data set. The number of RBC units of group O Rh positive and O Rh negative given to ABO non-identical individuals were also analysed by year over the 12 years of the study period. The transfusion of group O RBCs to non-group O recipients was analysed by out-patients receiving transfusion, and by 19 diagnostic categories for transfused in-patients based on the ICD-10 high level diagnostic codes for most responsible diagnosis. The reasons for the transfusion of group O RBCs to non-group O recipients were analysed using the hierarchical definitions summarized in the methods. The analyses were performed using SAS 9.3 (SAS Institute Inc, Cary, NC).

Results

There were 314,968 RBC units transfused during 75,935 admissions (in and out-patient) during the 12-year study period. Approximately half of the transfused RBCs were group O (48.1%; 151,645 units). Of the group O RBCs, 8.9% (13,509) were transfused to

recipients of another blood group: 8,004 to Group A, 4,664 to Group B, and 841 to Group AB. (Table 1).

Of the 138,137 group O RBC units transfused 76.3% (115,651) were O Rh positive: of these: 95.0% (109,824) were given to O Rh positive recipients; 0.6% (696) were transfused to O Rh negative recipients; and 4.4% (5,131) were transfused to non-group O patients. There were 789 (0.7%) O Rh positive RBC units transfused to Rh negative recipients. Of the 35,994 group O Rh negative red cells transfused: 57.2% (20,596) were given to O Rh negative recipients; 19.5% (7,020) were transfused to O Rh positive recipients; and 23.3% (8,378) were transfused to patients who were group A, B or AB. A total of 34.3% (12,360) O Rh negative donor RBC units were transfused to Rh positive recipients. Table 2 summarizes the number of donor RBC units by ABO group that were transfused to the different ABO groups and the O Rh negative RBC units that were transfused to Rh positive recipients by ABO group.

In-patient transfusions accounted for 77.3% (117,149/151,165) of the group O RBCs transfused: the remaining 22.7%; 34,496/151,645 of RBCs were transfused in the out-patient setting. The highest out-of-group utilization was seen in group A Rh positive inpatient recipients (10.7%) and group A Rh negative outpatients (6.2%) transfused with O Rh negative RBCs.

The group O Rh positive RBCs transfused to in-patients showed that 95.3% (84,743) were transfused to O Rh positive recipients; 0.8% (696) were transfused to O Rh negative recipients; 3.9% (3,481) were given to the ABO non-group O recipient; and 0.9% (789) to Rh negative recipients. For in-patients, O Rh negative RBCs were transfused as follows:

56.2% (15,864) were transfused to O Rh negative recipients; 19.5% (5,512) to O Rh positive recipients; 24.3% (6,853) to non- group O recipients; and 35.6% (10,057) to Rh positive recipients.

In the outpatient setting 93.4% (25,081) of group O Rh positive RBC transfusions were transfused to O Rh positive recipients, none were transfused to O Rh negative recipients; and, 6.2% (1,650) were transfused to non-group O recipients. Hence, 100% of the outpatient group O Rh positive blood transfused went to Rh positive recipients. For O Rh negative RBCs transfused to outpatients, 60.9% (4,732) were transfused to O Rh negative recipients, 19.4% (1,508) to O Rh positive recipients, 19.6% (1,525) to non-group O recipients, with a total of 29.7% (2,303/7,765) transfused to Rh positive recipients.

The percentage of O RBCs transfused to non-group O recipients was assessed by fiscal year. From 2002 to 2007 the proportion of group O RBCs given to non-group O individuals was consistently around 7.8% with a spike to 10.1% in 2008. During fiscal years 2009 and 2010 utilization returned to a baseline of 7.8%. Over the last three fiscal years (2011 – 2013); however, there has been an increase in this proportion to 11.1% (Figure 1a). When the percentage increase over time is plotted by recipient ABO group there is a trend towards higher group O blood use in group A individuals since 2011.

Figure 2a and 2b illustrates trends over time for O Rh Negative blood give to Rh positive recipients (overall and by blood group). The overall percentages by year ranged from 35.3 % to 54.0% with highest increases in 2012 and 2013. This increase is due primarily to O Rh negative blood being transfused to O Rh positive recipients (Figure 2a).

Reasons why non-group O patients received group O RBCs (Rh positive and Rh negative) included: alloimmunization (2,903; 28.1%); shortage (2,671; 25.8%); NICU (2,373; 23.0%); urgent massive transfusion (trauma) (1,002; 9.7%); transplantation (770; 7.5%); outdating (575; 5.6%); and patients with sickle-cell disease requiring phenotypic matched group O RBCs (40; 0.4%). The most common diagnosis for group A, B, or AB inpatients receiving group O RBCs were neoplasms, urgent massive transfusion (trauma), and diseases of the circulatory system (Figure 3). For the utilization of O Rh negative blood the top three diagnostic categories were conditions during the perinatal period, trauma and neoplasms diagnosis (Figure 3). The most common indications for ABO non-identical transfusion of O Rh positive and O Rh negative blood were an alloantibody in the recipient, presumed shortage of group-specific blood, and neonates in the NICU (Figure 4a-4c).

Discussion

Balancing supply and demand of whole blood derived blood products is a challenge for blood suppliers around the world. More recently, one of the challenges for blood suppliers is providing an adequate supply of group O RBCs, and ongoing shortages of O Rh negative RBCs. In this study half of all RBCs transfused over a 12-year period were group O and 8.9% of O RBCs were transfused to non-group O recipients. Furthermore, 23.3% of O Rh negative RBCs were transfused to recipients who were not group O and 34.3% of O Rh negative RBCs were transfused to Rh positive recipients. Hence, the use of group O blood for other blood groups and the use of O Rh negative blood for Rh positive individuals only increases the likelihood of a shortage of supply. Although our

data were retrospective we were able to create an algorithm to identify a presumed reason why ABO group and Rh specific blood were not used. The greatest use (>1000 units) of O RBCs in non-group O recipients was categorized as: shortage/other; patients with antibodies; patients in NICU; and urgent massive transfusion (trauma). Approximately one third (30.3%; 2073units) of the O Rh negative RBC given to Rh positive recipients were in the category of shortage/other. It is important to understand the reasons why group O RBCs are being used for A, B, and AB individuals instead of group specific RBCs, in order to explore areas where policy changes could help to alleviate inventory shortages. For example, if group O RBCs are preferentially phenotyped to ensure availability of compatible red cells for alloimmunized patients, this will make it more difficult to maintain an adequate group O RBC supply. The solution would be to also phenotype a number of A, B, and AB RBCs, making all ABO group specific blood available for transfusion. Automated large scale phenotyping platforms make this a feasible option. Many hospitals have a policy of transfusing only group O blood to neonates; hence, policy changes to give group specific RBCs to this patient population could also be considered. Finally, massive transfusion protocols that use only O Rh negative red cells for all trauma patients could be reconsidered to provide Rh positive blood for males and older females. Turnaround time for blood sampling and group and screen testing could also be improved so that less O Rh negative blood is issued in the setting of massive hemorrhage. The increasing trend of group O blood utilization in non-group O patients illustrates the need to consider these types of policy changes which

should increase group O inventory and reduce the number of group O RBCs in the category of shortages/other.

There were limitations to our study. Database-driven studies are by nature retrospective. Analyses are limited by how diagnostic data were coded. Clinical and demographic information (including the most responsible diagnoses) housed in the TRUST database are collected by abstractors trained in interpreting and coding health information. The reason why O RBCs were given to non-group O individuals was not coded in TRUST and it is possible that out algorithms to classify these reasons could have resulted in some misclassifications. Additional work would be required to explore the shortage/other category, possibly through a manual chart review. Alternatively, reasons for O RBCs being transfused to other groups would require prospective data collection which is time consuming and more costly. Although some misclassifications may have occurred, the data does provide an understanding of areas where policy changes could improve the availability of group O RBCs.

Our study has several strengths. At the time of data extraction, the TRUST database included a complete dataset of transfusion, laboratory, and clinical information at the patient level for 12 years. This extensive, rich data source allowed us to make pragmatic observations about recipients of out-of-group transfusions.[6,7] The comprehensive time span of the TRUST database allowed for an analysis of utilization trends over time. Finally, the electronic approach to data collection enables a large cohort to be studied with minimal resources, thus minimizing: data collection; errors through manual data collection; and, the potential for missing data. The scope of this data set, which is drawn

from a large, multi-site academic tertiary care hospital corporation, also increases generalizability. Patients from the pediatric, cardiac, orthopedic, hematology, oncology, general, medicine, critical care, and trauma settings were included in the analysis.

The need to manage the blood supply – particularly the supply of scarce blood products – has been highlighted in other research. Several investigators have discussed the reduction of the donor pool due to the aging population. Katalinic et al., observed that these changing demographics also led to an increased burden of disease, and an increased demand for blood.[8] Their study projected that the demand for RBCs would grow at the same rate as the population disease burden.[8] In 2007 Greinacher et al., had forecasted that by 2015 there would be increased demand in blood with significant shortfalls of donor blood.[9] Drackley et al., used mathematical modeling to predict that demand for blood would be outpaced in less than a year after their publication.[10] It is essential to continuously be aware of the impact of increased demand and decreased donations on blood supply. However, inventory crisis could be avoided not just by recruitment of new donors and advances in manufacturing processes, but also through proper management by blood suppliers and end-users.

Finally, inventory concerns of group O RBC may not be the only factor to consider, as recent data also suggest a clinical impact when group O blood is transfused to non-group O individuals. Several investigators have found an increased incidence of sepsis and multiple organ failure in marrow allograft recipients receiving transfusions.[11–13] ABO non-identical blood components have been associated with platelet refractoriness[14–16]; increased titres of HLA-AB antibodies[15]; immune complex formation [17,18]; and reduced

survival in acute leukemia[19]. A recent analysis conducted by the McMaster Transfusion Research Program has further explored an association between out-of-group RBC transfusions and in-hospital mortality. Pai et al., found that group A patients receiving group O RBCs had a 79% higher risk of death (RR=1.79; 95% CI: 1.20, 2.67; p=0.0047) compared to those receiving ABO identical blood. This finding persisted when trauma patients (≥ 6 units transfused within 24 hours), who typically have higher mortality (that often correlates with the amount of blood transfused) for other reasons, were excluded from the analysis.[20] These observations raise the hypothesis that transfusion of group O RBCs may come with a risk of harm that has previously been unrecognized.

To our knowledge this is the first study to address patterns of group O RBC use in non-group O patients using 12 years of data. Understanding the reasons for this practice could lead to policy changes that improve inventory availability and patient safety.

List of tables and figures

Acknowledgements

Infrastructure funding for the McMaster Transfusion Research Program is provided in part by Canadian Blood Services and Health Canada.

Table 1: Summary of the number of donor RBCs by ABO group that are transfused to recipients who were group A, B AB and O

		Number of RBCs Transfused			
		Recipient Blood Group			
		A	**B**	**AB**	**O**
Donor Blood Group	**A**	116,936	0	4,087	1*
	B	0	34,372	553	0
	AB	0	0	7,374	0
	O	**8,004**	**4,664**	**841**	**138,136**
	Total	124,940	39,036	12,855	138,137
% of transfused RBCs that were group O		6.4	11.9	5.5	0.0

*Patient was a transplant patient that had not sero-converted

Table 2: Summary of the number of donor RBCs by ABO group that are transfused to recipients of different ABO groups and O Rh negative RBCs transfused to Rh positive recipient

Recipient ABO	# of group O RBCs transfused	O Rh negative RBC Transfused to Rh positive Recipients	
		#	%
A	8,004	3,348	27.1
B	4,664	1,662	13.4
AB	841	330	2.7
O	138,136	7,020	56.8

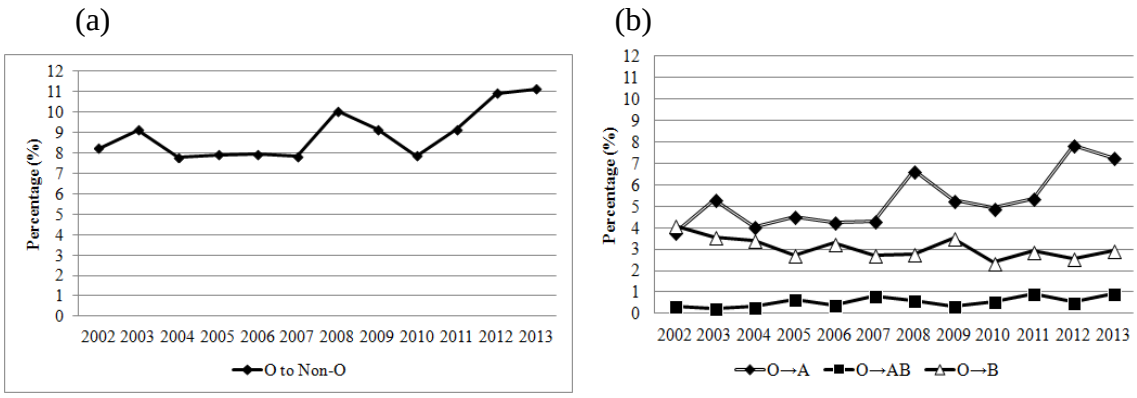


Figure 1a: Percentage of O RBCs transfused to non-group O recipients by fiscal year
Figure 1b: Percentage of O RBCs transfused to group A, AB, and B recipients by fiscal year

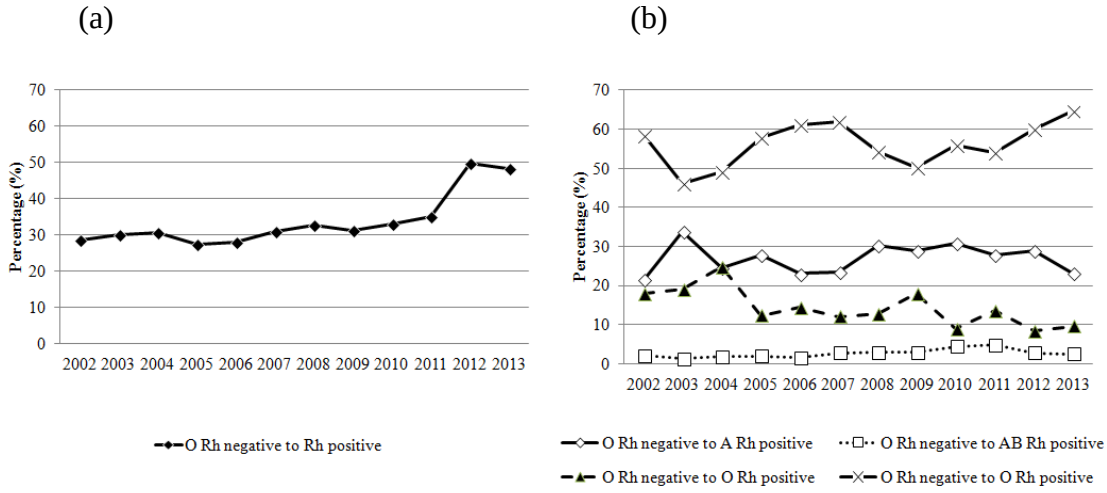


Figure 2a: Percentage of O Rh negative RBCs transfused to Rh positive recipients by fiscal year
Figure 2b: Percentage of O Rh negative RBCs transfused to group A Rh positive, O Rh positive, AB Rh positive, and B Rh positive by fiscal year

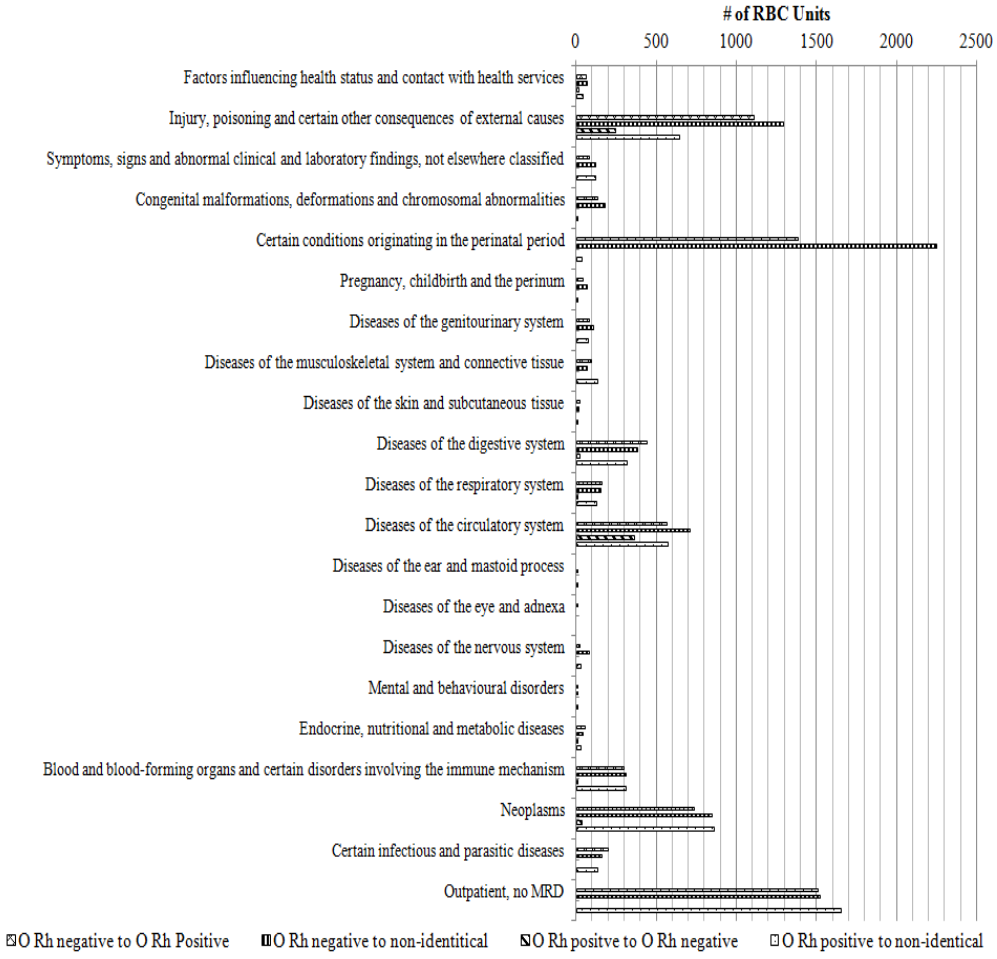


Figure 3:Non-identical Transfusions by Most Responsible Diagnosis (MRD) coded using ICD-10 for in-patients

Supplement attached has the ICD-10 code for each of the MRD.

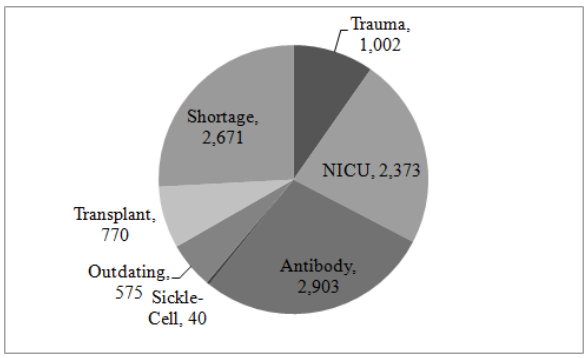


Figure 4a: Pie graph illustrating the presumed reasons why O RBCs (n=10334) were transfused to non-group O recipients

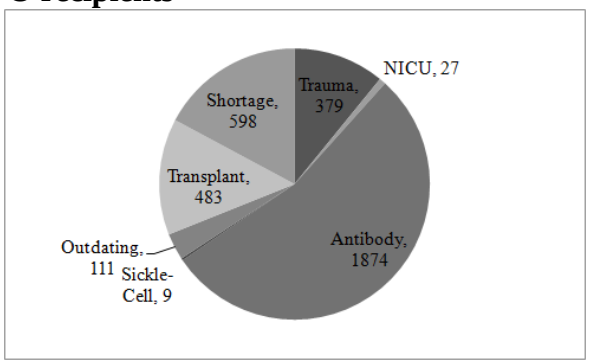


Figure 4b: Pie graph illustrating the presumed reasons why O Rh positive RBCs (n=3,481) were transfused to non-group O recipients

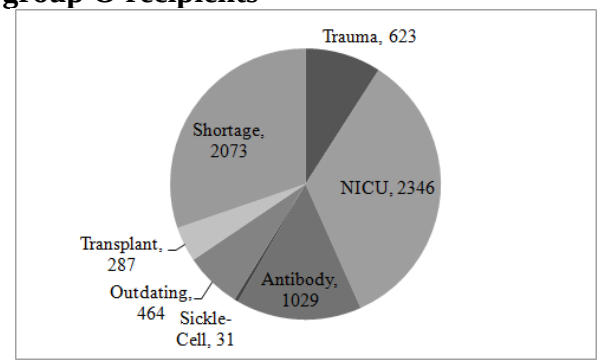


Figure 4c: Pie graph illustrating the presumed reasons why O Rh negative RBCs (n=6,853) were transfused to non-group O recipients

References

1. Bihl, F., Castelli, D., Marincola, F., Dodd, R. Y. & Brander, C. Transfusion-transmitted infections. *J. Transl. Med.* **5,** 25 (2007).

2. Farrugia, a. The mantra of blood safety: Time for a new tune? *Vox Sang.* **86,** 1–7 (2004).

3. Lin, Y., Saw, C. L., Hannach, B. & Goldman, M. Transfusion-related acute lung injury prevention measures and their impact at Canadian Blood Services. *Transfusion* **52,** 567–574 (2012).

4. Ali, A., Auvinen, M. K. & Rautonen, J. The aging population poses a global challenge for blood services. *Transfusion* **50,** 584–588 (2010).

5. Canadian Blood Services. The Facts About Whole Blood. (2015). at <https://www.blood.ca/en/bloodtype>

6. Schulman, K. L. *et al.* A checklist for ascertaining study cohorts in oncology health services research using secondary data: Report of the ISPOR oncology good outcomes research practices working group. *Value Heal.* **16,** 655–669 (2013).

7. Motheral, B. *et al.* A checklist for retrospective database studies - Report of the ISPOR task force on retrospective databases. *Value Heal.* **6,** 90–97 (2003).

8. Katalinic, A., Peters, E., Beske, F. & Pritzkuleit, R. Projection of morbidity 2030 and 2050: Impact for the National health system and blood supply. *Transfus. Med. Hemotherapy* **37,** 155–159 (2010).

9. Greinacher, A., Fendrich, K., Alpen, U. & Hoffmann, W. Impact of demographic changes on the blood supply: Mecklenburg-West Pomerania as a model region for Europe. *Transfusion* **47,** 395–401 (2007).

10. Drackley, A., Newbold, K. B., Paez, A. & Heddle, N. Forecasting Ontario's blood supply and demand. *Transfusion* **52,** 366–374 (2012).

11. Benjamin, R. J. & Antin, J. H. ABO-incompatible bone marrow transplantation: the transfusion of incompatible plasma may exacerbate regimen-related toxicity. *Transfusion* **39,** 1273–1274 (1999).

12. Benjamin, R. J., McGurk, S., Ralston, M. S., Churchill, W. H. & Antin, J. H. ABO incompatibility as an adverse risk factor for survival after allogeneic bone marrow transplantation. *Transfusion* **39,** 179–187 (1999).

13. Heal, J. M. & Blumberg, N. The second century of ABO: and now for something completely different. *Transfusion* **39,** 1155–1159 (1999).

14. Heal, J. M. *et al.* The role of ABO matching in platelet transfusion. *Eur J Haematol* **50,** 110–117 (1993).

15. Carr, R., Hutton, J. L., Jenkins, J. A., Lucas, G. F. & Amphlett, N. W. Transfusion of ABO-mismatched platelets leads to early platelet refractoriness. *Br J Haematol* **75,** 408–413 (1990).

16. Heal, J. M., Blumberg, N. & Masel, D. An evaluation of crossmatching, HLA, and ABO matching for platelet transfusions to refractory patients. *Blood* **70,** 23–30 (1987).

17. Heal, J. M., Masel, D. & Blumberg, N. Platelets (Plt) Coated with Abo Immune-Complexes (Cic) from Refractory Patients Are Associated with Increased Adhesion to Monocytes. *Transfusion* **33,** S16–S16 (1993).

18. Heal, J. M., Masel, D. & Blumberg, N. Interaction of platelet Fc and complement receptors with circulating immune complexes involving the ABO system. *Vox Sang.* **71,** 205–211 (1996).

19. Heal, J. M., Kenmotsu, N., Rowe, J. M. & Blumberg, N. A possible survival advantage in adults with acute leukemia receiving ABO-identical platelet transfusions. *Am J Hematol* **45,** 189–190 (1994).

20. Pai, M. *et al.* Exposure to ABO non-identical blood associated with increased in-hospital mortality in patients with group A blood (Manuscript submitted for publication.). (2015).

APPENDIX VII: CLASSIFICATION AND DESCRIPTIONS OF THE GRADES OF BLEEDING

WHO Bleeding Grade and Characteristics	
Grade 1*	
• Mucocutaneous Hemorrhage (oral blood blisters) • Petechiae (lesions <2 mm in size) • Purpura less than 2.54 cm (1") diameter • Ecchymosis (lesions ≤10 cm in size)	• Oropharyngeal bleeding • Conjunctival bleeding • Epistaxis <1 hour in duration and not requiring intervention • Abnormal vaginal bleeding (non-menstrual) with spotting (<two pads per day)
Grade 2*	
• Ecchymosis (lesions > 10 cm in size) • Hematoma • Epistaxis >1 hour in duration or packing required • Retinal hemorrhage without visual impairment	• Abnormal vaginal bleeding (not normal menses) using > 2 pads/day • Melena, hematemesis, hemoptysis, hematuria, hematochezia • Bleeding from invasive sites, musculoskeletal bleeding
Grade 3**	
• Melena • Hematemesis • Hemoptysis • Hematuria – including intermittent gross bleeding without clots • Abnormal vaginal bleeding	• Hematochezia • Epistaxis • Oropharyngeal • Bleeding from invasive sites, musculoskeletal bleeding, or soft tissue bleeding
Grade 4	
• Debilitating bleeding including retinal bleeding with visual impairment (defined as a field deficit and there must be a consult note documenting visual impairment). • Non-fatal CNS bleeding with neurological signs and symptoms • Fatal bleeding from any source.	

*Does not require red cell transfusion

** requiring red cell transfusion specifically for support of bleeding within 24 hours of onset.

APPENDIX VIII: INFLAME-P SUGGESTED VARIABLES FOR DATA COLLECTION

Patient Demographics:
Patient age (month and year)
MDS diagnosis, time of diagnosis (month and year if known)
Previous history of transfusion
Vitals (pre-, during and post-transfusion)
Medications
Adverse event reactions

Clinic Visit Information:
Edmonton Symptom Assessment System (ESAS)
Physical exam (signs and symptoms of disease status (i.e. heart, lungs, spleen, and liver))

Routine/Clinic Visit Laboratory Information:
Testing dates and Times (specimen collection, receipt, and testing)
Complete blood count (CBC)
Creatinine
Electrolytes
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Bilirubin
Group and screen
Iron studies (as performed every three to four months)

Patient Specific Laboratory Biomarkers:
Subgroup of A
Lewis type
C-Reactive Protein
Cytokines (Il-6, IL-8, TNF-α, IL-1β)
BPI
CD40 Ligand
Circulating Immune Complexes

RBC Unit Specific Laboratory Markers:
Product identifiers (product name, ABO, Rh type, unit number, source code, product collection and/or expiry dates
anti-A and -B titres for group O RBC units only

Hospitalization Admissions:
Admission Date (and time if known)
Discharge Date (and time if known)

Infection Episode:
Infection Date onset (and time if known)
Infection resolution Date (and time if known)
Laboratory culture as required
Requirement antibiotics to treat (type, oral, iv, dose)
Requirement of hospital admission
Requirement of oxygen
Use and pressure of fluids to support blood pressure
Requirement of intubation
Diagnosis septic shock

Patient Questionnaire to assess success of patient blinding:
Do you know the ABO of the RBC you received?
how do you know?;
And, what ABO group do you think you received?

APPENDIX IX: IDENTIFIED BIAS DEFINED, RISK RELATED TO INFLAME-P AND THE PLAN TO MINIMIZE THE IDENTIFIED BIAS

Type of Bias Definition	**Potential Risk in INFLAME-P** *Plan to Minimize the Risk (**PMR**)*
Allocation of Intervention Bias: if the sequence is known it may introduce a **selection bias.** **Selection bias:** when the study population does not represent the target population.	After the first treatment episode is given, the subsequent treatment will be identifiable because blocks of 2 are being used and study personnel and HCPs will not be blinded ***PMR:*** *TMS & Research staff are not blinded. After the administrative check by the nursing team the RBC unit will be covered by an opaque bag in order to blind the patient. The person conducting the laboratory measures will be blinded to the treatment allocation.*
Referral Bias: occurs when characteristics differ between settings.	Two sites participating: one a cancer centre; the second a non-cancer academic hospital. Classification of MDS may vary between centres (IPSS Score) ***PMR:*** *Stratification by centre and risk type.*
Ascertainment Bias*: when the study sample does not reflect the cases in the population. **Misclassification:** sensitivity and/or the specificity of the procedure to detect effect is flawed. **Non-differential:** since all the measures will be done the sample way.	Inflammatory response ***PMR:*** *Objective outcome measure*

Reporting Bias is when participants give answers in the direction they perceive are of interest. **Differential misclassification bias: i**s also a type of **Information bias**. **Information bias:** occurs during data collection (three main categories are: misclassification, ecological fallacy, and regression; however there are others i.e., Hawthrone effect) **Reporting bias differential misclassification:** occurs with misclassification is different in the group being compared.	Inflammation detected as a result of other reasons (not the transfusion itself). ***PMR:*** *Study inclusion is based on the being "stable" as defined by the MDS Stability Assessment Algorithm. The tool covers a 6-month observational period, therefore patients should remain stable for the study duration. Short testing duration (follow-up for each transfusion episode is 96 hours).* Previous exposure to ABO non-identical blood products (RBCs, platelets, and/or plasma) status ***PMR:*** *Effect Unknown; however, the crossover design should negate differences due to past exposure.*
Attrition Bias: difference between treatment groups due to missing data or loss to follow up.	Compliance with the transfusion schedule ***PMR:*** *Transfusion dependent MDS patients are conditioned to frequent clinic visits and transfusions*. Missing serial timed testing & risk of dropout. Participants may dropout before crossover transfusion ***PMR:*** *Mobile service agency/research staff to collect samples at the patient's home.* *Scheduling of the transfusion start time early morning.* *Reviewed testing schedule with study participant.*
Treatment Access Bias*: differential degree of access to a treatment due to cultural, geographical, or economic reasons (in this case a blood supply issue)	Blood inventory shortages could impact the provision of the appropriate ABO group RBC transfusion. ***PMR:*** *Confirm availability of the blood supply once clinic schedule is known.*

*not usually subject to this bias in a clinical trial