INFORMING THE DESIGN OF A CROSSOVER TRIAL IN MDS PATIENTS

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

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TITLE: 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

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PREFACE

The age of blood debate has generated significant interest in transfusion research over the past decade. While there have been numerous studies examining the impact of red blood cell (RBC) storage time on various clinical outcomes, I was interested in understanding if age of blood was associated with improved post-transfusion health-related quality of life (HR-QoL) in patients who are chronically transfused. To study this association, I developed a preliminary research question:

> *"Is it feasible to conduct a crossover randomized controlled trial to determine if the freshest available RBCs (target ≤7 days) are associated with improved post-transfusion health related quality of life compared to transfusions with the oldest available RBC products (target ≥35 days) in adult, transfusion-dependent patients with Myelodysplastic Syndromes (MDS)?"*

As the feasibility issues were identified, it became evident that further pilot work was necessary to refine the research question and inform the design of the study. The scope of this dissertation is focused on gathering data to refine the research question and includes:

1. Development of a theoretical framework to establish biological plausibility that may suggest blood storage time is associated with HR-QoL in chronically transfused patients;
2. Conducting a systematic review to identify and summarize previous studies that have examined the association between blood storage time and HR-QoL;
3. Determining if patients with MDS are appropriate to study in a crossover trial by conducting a chart review; and,
4. Conducting a qualitative study to determine if the MDS population is suitable for a study where HR-QoL is the outcome of interest.

The pilot information presented in this thesis will be used to inform a future age of blood transfusion trial in patients with MDS.

ABSTRACT

Patients with myelodysplastic syndromes (MDS) frequently receive red blood cell (RBC) transfusions to alleviate symptoms of anemia and improve health-related quality of life (HR-QoL). Patients can sometimes continue to feel unwell after transfusion and the age of the transfused RBCs could contribute to this observation. Three pilot studies were conducted to inform the design of a randomized crossover trial to determine if fresh blood to MDS patients could improve HR-QoL post-transfusion.

A systematic review was performed to inform the background and rationale for the trial. The results showed a dearth of literature addressing the research question. Only two clinical trials have been conducted to date where fatigue and HR-QoL were the primary outcomes of interest. Although results of the trials were negative, several limitations and generalizability issues warrant additional research in this area.

Crossover designs necessitate patients have a stable prognosis while being observed; hence, a chart review of adult MDS patients was conducted to assess clinical stability using the following criteria: interval of days between transfusions; pre-transfusion Hb; number of hospital admissions; and severe infections. Results indicated that the majority of patients who had received greater than 3 transfusions within the 6-month observation period had stable disease and were appropriate for a crossover trial. The criteria defining stability will be useful for identifying eligible patients.

Finally, an applied qualitative study in adult MDS patients in Hamilton was conducted to inform the selection of an appropriate outcome measure (i.e. HR-QoL tool). Short semi-structured interviews were conducted with participants to elicit information about anemia related symptoms and changes in well-being in response to transfusion. The results of the study support clinical observations that suggest patients do not immediately recover post-transfusion. Findings indicate that an appropriate HR-QoL tool should be short, be disease specific, and have a short recall period. Currently, the Quality of Life-E tool, validated in patients with MDS, fits most of these criteria.

In conclusion, data from the systematic review and the two pilot studies suggest that it may be feasible to conduct an age of blood crossover trial in MDS patients where the primary outcome of interest is HR-QoL.

DEDICATION & ACKNOWLEDGEMENTS

I dedicate this thesis to my beloved parents, Atiya and Rauf Sholapur, and my two younger brothers Asim and Aamir. Your constant love, support, and guidance throughout the years have been instrumental in my success. I express my most heartfelt thanks for your patience and encouragement through this journey. Thank you for always believing in me, I could not have done it without you!

I would like to acknowledge and extend my sincerest gratitude to my supervisor, Professor Nancy Heddle, for her tremendous efforts and guidance throughout the development of this thesis. She has been an excellent role model, brilliant mentor, and a great resource every step of the way.

I would like to thank my thesis committee members and external reviewer Dr. Mark Crowther, Dr. Richard Cook, and Dr. Ronald Barr for their time, insights, expertise, and contributions to this dissertation. I also wish to thank Dr. Brian Leber and Dr. Christopher Hillis for their insight and contributions.

I am also very grateful to the McMaster Transfusion Research team. I extend a special thanks to Shannon Lane for her guidance in qualitative research methods; Anushka Jaffer for her contributions as the second reviewer for the systematic review; and Zakia Islam and Faiza Khokhar for their help with data abstraction and verification for the chart review component of this thesis.

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LIST OF ABBREVIATIONS

2,3-DPG	2,3-diphosphoglycerate
AJ	Anushka Jaffer
AML	Acute Myeloid Leukemia
ASH	American Society of Hematology
ATP	Adenosine triphosphate
BC	Buffy coat
BFI	Brief Fatigue Inventory
cGMP	Cyclic guanosine monophosphate
CH	Christopher Hillis
COPD	Chronic obstructive pulmonary disease
CRF	Case report form
DVT	Deep vein thrombosis
eNOS	Endothelial nitric oxide synthase
EORTC QLQ-C30	European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire 30
EPO	Erythropoietin
EQ-5D	EuroQol 5 Dimension
FACT-An	Functional Assessment of Cancer Therapy - Anemia
FDA	Food and Drug Administration
FK	Faiza Khokhar
Hb	Hemoglobin
HiREB	Hamilton integrated Research Ethics Board
HR-QoL	Health-related quality of life
IPSS	International Prognostic Scoring System
IQR	Interquartile range
JCC	Juravinksi Hospital and Cancer Center
LASA	Linear Analog Self-Assessment
MC	Mark Crowther

MDS	Myelodysplastic Syndromes
MFI	The Multidimensional Fatigue Inventory
MTRP	McMaster Transfusion Research Program
NACRS	National Ambulatory Care Reporting System
NH	Nancy Heddle
NO	Nitric oxide
NS	Naushin Sholapur
O_2	Oxygen
PROs	Patient reported outcomes
PS	Phosphatidylserine
RBC	Red blood cell
RCC	Red cell concentrates
RCT	Randomized controlled trial
SAGM	Saline, adenine, glucose, and mannitol
SF-36	Short Form-36
SJHH	St. Joseph's Hamilton Healthcare
SL	Shannon Lane
SNO-Hb	S-nitroshemoglobin
SNO	S-nitrosthiol
SOP	Standard operating procedure
TRUST	Transfusion Registry for Utilization, Surveillance, and Tracking
Quality of Life-E	QOL-E
VAS	Visual Analog Scale
ZI	Zakia Islam

DECLARATION OF ACADEMIC ACHIEVEMENT

N. Sholapur developed the components of the thesis with input from N. Heddle (supervisor) and M. Crowther (first chair). N. Sholapur also developed the systematic review research proposal and drafted the advanced search strategy, which was reviewed and refined by L. Banfield (librarian). N. Sholapur and A. Jaffer (research assistant) screened identified titles and abstracts for inclusion. N. Sholapur reviewed full texts of eligible articles, then appraised and summarized each study.

The research question, research proposal, and ethics application for the chart review of patients with MDS was prepared by N. Sholapur and revised by N. Heddle and M. Crowther. N. Sholapur drafted the case report forms, which were reviewed and refined by N. Heddle and M. Crowther. Data was abstracted by F. Khokhar (research assistant) and verified by N. Sholapur. Data was entered by N. Sholapur and verified by Z. Islam. Outcome measures (i.e. criteria to define patient stability) were developed by N. Sholapur and reviewed by C. Hillis (transfusion research fellow).

Additionally, N. Sholapur developed the research question, proposal, and ethics application to conduct a qualitative study in patients with MDS, which were reviewed by N. Heddle and M. Crowther. A semi-structured interview guide was drafted by N. Sholapur and refined by N. Heddle, M. Crowther, and S. Lane (qualitative research coordinator at MTRP). N. Sholapur conducted and transcribed all interviews verbatim. Inter rater reliability was conducted by N. Sholapur and S. Lane. N. Sholapur coded and analyzed all interviews.

CHAPTER 1: Introduction and Theoretical Framework

Patients with myelodysplastic syndromes (MDS) most commonly present with refractory anemia, resulting in chronic fatigue due to impaired red blood cell (RBC) production, low hemoglobin (Hb) levels and poor tissue oxygenation. The role of chronic fatigue on patient health related quality of life (HR-QoL) is significant (1,2). Fatigue is characterized by an extreme sense of malaise, tiredness, exhaustion, or feeling sick that extends beyond usual weariness from physical exhaustion (2). Aside from being unable to participate in day-to-day activities, patients who experience fatigue may have associated decreased mental alertness, physical weakness, and lack of concentration (1). Since fatigue prevents patients from fully participating in their lives, they may also experience feelings of loneliness or isolation, which further reduce overall QoL (2).

The use of recombinant human erythropoietin (EPO) is often indicated for patients who experience anemia (3) to boost RBC production in the bone marrow. However, not all patients respond to this treatment (3). In order to alleviate symptoms of anemia, albeit transiently, patients with MDS are managed with regular and ongoing RBC transfusion therapy (4,5). The goal of transfusion is to improve RBC counts and Hb levels, thereby improving oxygen delivery to tissues and subsequently overall HR-QoL. This assertion is supported by literature indicating that Hb levels are positively correlated with HR-QoL (1,6). Despite the short term benefits of transfusion, there are a number of complications associated with transfusion dependency that adversely affect HR-

QoL (i.e. iron over load, increased hospital visits for compatibility testing and transfusion, and hours of chair time spent in outpatient care) (7). Aside from Hb levels, literature also suggests that transfusion-dependency (6,7), co-morbidities (6), and patient perceptions of illness (6) are also major determinants of HR-QoL.

While transfusion can temporarily relieve fatigue, clinicians in the field report that sometimes patients continue to feel unwell with pre-transfusion symptoms persisting for hours to days later (Crowther, Mark. Conversation with: Nancy Heddle & Naushin Sholapur. 2012 Apr 02). Patients also report the arbitrary onset of physical symptoms associated with transfusion that resolve within a few hours. Knowing that changes occur in the quality of the red cell concentrate (RCC) as it is stored prior to transfusion, we hypothesize that blood storage time may be associated with post transfusion HR-QoL in patients who are transfusion dependent.

1.1 – Is it biologically plausible for blood storage time to be associated with post-transfusion HR-QoL? A theoretical framework

The optimal storage time of RCCs is an area of high interest in transfusion research. Current Food and Drug Administration (FDA) and Health Canada regulations allow RCCs to be stored *in vitro* for up to 42 days before transfusion. Blood banks adopt a “first in, first out” policy, where patients are administered the oldest available unit in order to optimize storage time and minimize product wastage (8,9). In 2008 an inventory management toolkit developed by the

Ontario Regional Blood Coordinating Network was introduced to 160 hospitals in Ontario, Canada (10) encouraging blood banks to transfuse the oldest available unit to minimize product outdating (11).

When whole blood is processed, RBCs are separated and mixed with an additive solution (12) containing saline, adenine, glucose, and mannitol (SAGM solution). The RCC is leukoreduced and stored between 1-6°C (12). Although the storage conditions of the RCC minimize cell injury, accumulation of by-products from glucose metabolism that cannot be eliminated due to *ex vivo* storage, result in a series of time dependent biochemical and mechanical changes collectively referred to as "storage lesions" (8,13–16). These storage lesions entail intracellular, membrane and structural, and medium changes that can potentially compromise tissue oxygenation and cause inflammatory responses when transfused (8,15). Storage lesions may account for the delays in recovery experienced by patients who receive chronic transfusion therapy and the arbitrary onset of physical symptoms.

1.1.1 – Intracellular changes in RBCs

The RBC is optimized in its structure and physiology to achieve its primary function of facilitating the transport of oxygen (O_2) and nutrients to tissues, and carbon dioxide towards the lungs. Human RBCs measure approximately 8μm in diameter, have a biconcave shape (as opposed to spherical, which is typical to animal cells), are anucleate, and most importantly contain high concentrations of

Hb molecules in their cytoplasm. While Hb mediates the transport of O_2 to -tissues, 2,3-diphosphoglycerate (2,3-DPG), a known allosteric modifier of Hb, plays a significant role in O_2 release and diffusion. 2,3-DPG is produced through the Rapoport-Luebering shunt of glycolysis (17) and exists in concentrations slightly greater than that of Hb (18). Near respiring tissue 2,3-DPG binds with high affinity to deoxygenated Hb which induces a conformational change, thereby facilitating the release of remaining O_2 and promoting diffusion (8,17,19,20).

Within two weeks of red cell storage, levels of 2,3-DPG are completely depleted and virtually undetectable (17). The decline in 2,3-DPG occurs due to changes in enzymes specificity of diphosphoglycerate mutase and phosphatase (the enzymes involved with the production of this biomolecule) as a result of decreasing pH levels (18). Therefore, this decline can be detrimental to the effectiveness of the transfusion since it impedes O_2 release to tissues, despite the increased number of RBCs in circulation. However, since normalization of 2,3-DPG can begin a few hours after transfusion and is completely restored within 72 hours (21), it seems likely that there may be delays in experiencing the full benefits of the transfusion based on storage time.

Other intracellular changes associated with increased storage time include the depletion of adenosine triphosphate (ATP) and reduced nitric oxide (NO) metabolism (19). As storage time increases and glucose is consumed, studies (22) demonstrate that levels of ATP decline to 55-60% of their baseline value after 5-6 weeks of storage (17,23). Since ATP drives a number of cellular

processes, depletion can result in a decrease of active transport, antioxidant reactions, and disruptions in membrane phospholipid distribution (15). Since the RBC's primary energy source is depleted, it becomes highly vulnerable to oxidative stress, making it susceptible to hemolysis. Additionally, several studies discuss the role of ATP in hypoxic vasodilation (17,24–26). RBCs are able to detect hypoxic conditions (such as those in patients with refractory anemia) and regulate blood flow via the release of ATP through the membrane bound cystic fibrosis transmembrane protein receptor (17). Released ATP stimulates vasorelaxation in an endothelial-mediated process (25), facilitated by the activation of P_2Y purinergic receptors (26) and endothelial NO synthase (eNOS) (24,27).

NO is synthesized by eNOS not only in response to RBC released ATP, but also by platelet derived factors, shear stress, acetylcholine, and cytokines (28). In addition to this, NO equivalent S-nitrosthiol (SNO) is also released by RBCs to regulate the flow of blood (19,29). S-nitrosthiol binds covalently to the highly conserved β_{93}cys thiol residue of Hb forming an S-nitroshemoglobin (SNO-Hb) complex (19,30). Under hypoxic conditions, SNO is released from the Hb within RBCs proportional to the extent of hypoxia. In the classical pathway of vasodilation, NO diffuses into smooth muscle cells (31) where it interacts with soluble guanylate cyclase, promoting the generation of cyclic guanosine monophosphate (cGMP) (32). The activation of cGMP dependent protein kinase type I induces vasorelaxation by several mechanisms (32). In storage, levels of

SNO fall within 3 hours *ex vivo,* therefore, there may be reduced O_2 delivery to hypoxic tissues even with fresh blood transfusions (19).

1.1.2 – Morphological Changes to the RBC and Cell Membrane Loss

Studies demonstrate that by two weeks of storage, RBCs exhibit a number of changes in membrane composition, which affect their cell structure and ability to deform (8,13–16). Analysis of the cell membrane after 42 days of storage *in vitro* shows significant reduction in the total amount of phospholipids and cholesterol, indicating overall membrane loss (14). In rat models, a five-fold increase in the number of deformed and rigid cells was observed with increased storage time (8,13). In patients undergoing posterior spinal fusion surgery, long periods of storage (>38 days) were associated with significantly reduced deformability compared to fresh blood (<14 days of storage), with no signs of reversing within three days post-transfusion (16).

Aside from the aforementioned intracellular changes that RBCs undergo (decrease in levels of 2,3-DPG, ATP, NO, and pH), levels of potassium (8,31), lactate (8,15), and various pro-inflammatory cytokines increase (8). These changes, in conjunction with further alterations including: phospholipid vesiculation; protein oxidation; and lipid peroxidation, contribute to significant changes in the cell membrane composition (8,15,16,31). Membrane loss and changes to the phospholipid, cholesterol, and protein ratio significantly impact membrane fluidity, lipid sorting, bilayer thickness, and packing efficiency of

membrane compounds (14). Ultimately, these changes result in reduced membrane deformability affecting the unique ability of RBCs to change shape and traverse through microcirculation (8).

RBC shape and cell membrane properties both play a crucial role in the red cells' primary function. The biconcave cell shape facilitates O_2 delivery since it maximizes the surface area to volume ratio, which increases diffusion of O_2 across the plasma membrane into tissues (33). The RBC structure is maintained by the composition and integrity of its membrane and the underlying two dimensional spectrin molecules (33). Remodelling of the spectrin molecule cytoskeleton gives RBCs the unique property of deformability (33). Studies indicate that ATP depletion may contribute to the observed RBC change in shape from biconcave to spiculated echinocyte during storage (20). Since spectrin remodelling is proposed to be fuelled by ATP (34), which is shown to rapidly deplete during storage, the membrane composition and spectrin network have an important role in the RBC function. Changes to either component (such as those observed with increased storage time) result in reduced deformability of the RBC membrane. Reduced deformability may impede the RBCs capacity to pass through the spleen, which could result in accelerated removal of stored RBCs (35).

Finally, increased storage time is associated with greater phosphatidylserine (PS) and decreased CD47 expression on cell surface (36). PS is a component of the phospholipid, which is usually expressed on the

cytosolic side of the plasma membrane. Externalization of PS, due to asymmetric membrane loss (14), signals phagocytosis. The increased expression of PS may be linked to leukocyte contamination since studies indicate that this phenomenon is primarily observed with RCCs that have not been leukoreduced; and those that do not show PS externalization (20). Conversely, CD47 is a ubiquitous cell surface glycoprotein and acts as a marker for "self" since it is able to inhibit macrophage activity by interacting with signal regulatory protein α (37). In storage, there can be a 10-60% decline of CD47 expression in RBC cell membranes, which may lead to the elimination of these "foreign" cells (20).

The consequences of macrophage-mediated erythrophagocytosis are more far reaching than simply causing a decrease in viable RBCs for circulation. The cumulative effect of storage lesions associated with prolonged blood storage time seems to be hemolysis of cells *in vitro* and reduced 24 hour RBC survival *in vivo* post-transfusion (38). FDA regulations mandate that RBC products must have greater than 75% 24-hour post-transfusion recovery. However, Hod & Spitalnik focus on the subset of RBCs that are cleared from circulation after transfusion due to storage lesion induced damage (38). Excess non-transferring bound iron released by the macrophage/monocyte system from rapidly cleared senescent RBCs may lead to inflammatory responses through the activation of inflammasome activity and increased risk of infection (38). Evidence for this hypothesis comes from rat models (39) that show a rapid initial clearing of RBCs which induced pro-inflammatory cytokine responses (38,40). In a study with

healthy human volunteers (41) who were transfused with autologous fresh blood (3-7 days) vs. old blood (>40 days), there were differences in circulating non-transferrin bound iron and ferritin (38). The increase in non-transferrin bound iron was also hypothesized to increase the risk of infection, which was observed in both rat models and human studies (38).

1.1.3 – Metabolic changes associated with RBC storage lesions

Consumption and metabolism of glucose results in the accumulation of by-products that cannot be cleared *ex vivo*. The accumulation of metabolites results in several changes to the storage medium of the RCCs associated with increased storage time. These changes have been briefly mentioned in relation to the intracellular and membrane changes that RBCs undergo (specifically, the consequences of decreased pH levels which result in depletion of 2,3-DPG). In addition to the aforementioned changes, cold storage and decrease in levels of ATP affect the Na^+-K^+ ATPase, which actively transports Na^+ outside of the cell against the concentration gradient (17). The expulsion of Na^+ leads to the removal of water through osmosis, therefore the retention of Na^+ can cause swelling of the cell, which impedes circulation and efficient O_2 delivery (17).

Furthermore, there is an increase in lactate, cytokines, and free Hb in the storage medium of RCCs, which could compromise the efficacy of the transfusion (8). Red blood cells can scavenge NO since Hb binds to NO with high affinity (31). Hemolysis of RBCs that have experienced oxidative damage release

free Hb, which can scavenge NO at a rate 1000 times faster than NO contained by RBCs (31). Therefore, transfusion of older blood products with cell-free Hb can further deplete NO, promoting vasoconstriction in hypoxic patients.

1.2 – Summary of the storage lesion

The numerous time dependent biochemical and mechanical changes that RBCs undergo while stored *in vitro* can potentially reduce transfusion efficacy in three ways: (1) compromise O_2 delivery to tissues due to occlusion of blood vessels and/or insufficient release of O_2 at sites of perfusion; (2) promote pro-inflammatory responses that are mediated by non-transferrin bound iron from senescent RBCs that are rapidly cleared from circulation; and, (3) increased risk of infection. In patients with MDS who require transfusion to manage chronic fatigue, it seems plausible that storage lesions associated with the transfusion of older RBCs could account for poor HR-QoL post-transfusion and the arbitrary onset of physical symptoms as compared to fresh. While laboratory studies and rat models demonstrate the consequences of storage lesions, the results should be interpreted cautiously when extrapolated to humans. Considering that some of these changes begin to reverse upon transfusion with the normalization process beginning within a few hours, depending on the length of storage the hypothesized negative consequences of transfusing older units of RBCs may be short lived, rather than permanent.

CHAPTER 2: A Systematic Review of the Literature to Examine the Impact of Blood Storage Time on Patient Reported Outcomes

A key goal of chronic transfusion therapy in the management of patients who experience refractory anemia is to improve overall HR-QoL; therefore, it is essential to incorporate patient-reported outcomes (PROs) as outcomes in the evaluation of this therapy. PROs are measures that are informed by patients without the interpretation of a clinician or other healthcare provider (42). These outcomes (reported through the use of diaries, interviews, and/or questionnaires) specifically pertain to perceptions of illness, treatment satisfaction or burden, HR-QoL, disease impact, and symptom burden (42).

In the consumer model of healthcare, as described by Baron-Epel & colleagues, the "relationship between the patient and the treating physician is based upon the mutual goal of optimizing the patient's health" (43). Patient expectations influence the degree to which these expectations are fulfilled by physicians and impact satisfaction in the patient-provider relationship (43). PROs are important for enhancing patient-provider communication since they inform patient values and treatment goals (44), which may encompass values outside of a purely biomedical sphere and include psychosocial elements. For example, patient advocacy for greater comfort and functionality has promoted an emphasis on symptom management in patient care (45). Considering the need for patient-centered healthcare (44), PROs are an important aspect of establishing the patient perspective, which can then guide treatment decisions.

Recently, there has been a move towards the incorporation of PROs as end-points in clinical research, specifically the assessment of symptom burden, which can be both disease and/or treatment related (45). In patients who receive chronic transfusion, the use of PROs can give providers greater insight about the role of this therapy in patient perceptions of their illness and treatment.

Current research in the transfusion medicine field is geared towards ascertaining the clinical impact of blood storage time. Systematic reviews and meta-analysis related to this question are primarily interested in physiological outcomes, morbidity, and mortality (46–49). In light of this, we conducted a systematic review with the objective of identifying and summarizing previous research that has been conducted pertaining to the impact of blood storage time on PROs across all populations. We could not anticipate being able to conduct a meta-analysis since PROs are comprised of a number of highly heterogeneous outcomes.

2.1 – Methods and Materials

The process of developing a comprehensive search strategy was informed by the Cochrane Handbook for Systematic Reviews (50) and the checklist developed by the Cochrane Haematological Malignancies Group (51). A high level scan of the literature was conducted to identify text words from the age of blood and PRO literature. Synonyms for each text word were identified to increase sensitivity of the search. Each text word was searched using truncation

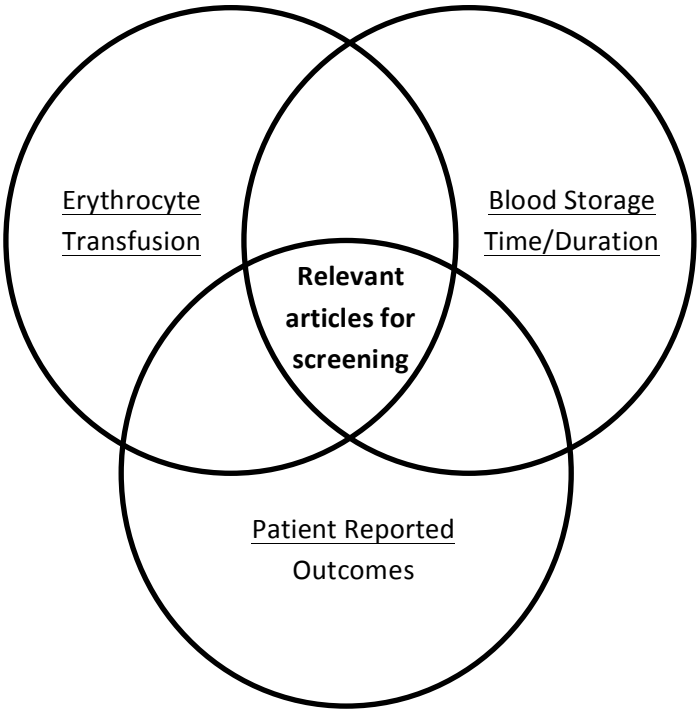


Figure 1: Approach to search strategy

where applicable to determine database specific "controlled vocabulary" (MeSH terms for the Medline database and EMTREE for Embase). Using the MeSH terms identified, a comprehensive search strategy was developed (Appendix A). All terms related to "red blood cell transfusion", "blood storage time", and the "patient reported outcomes" concepts were grouped using the Boolean operator "OR". The three concepts were combined using "AND" (Figure 1). Articles with time-trade off and/or quality adjusted life years outcomes were included in the search in the event that a PRO was assessed as a secondary outcome. In addition to this, literature with a primary focus on fresh frozen plasma, platelet transfusions, and genotyping were searched and excluded from the results by using the Boolean operator "NOT" in order to remove irrelevant articles that the initial search strategy identified. The search was limited to include only articles with "red blood cell transfusion" related terms in the title or abstract. The results were further limited to include only articles that were written in English and conducted in the human population. There were no year limits.

The online clinical trials registry (clinicaltrials.gov) was also searched for any ongoing studies using the search terms “age of blood”, “blood storage time”, “fresh blood”, “old blood”, and “standard issue blood”. All results were searched to identify studies measuring at least one PRO where the intervention was blood storage time. Finally, reference lists of four age of blood systematic reviews (46–49) were also searched to determine if there were any other relevant studies.

Since the purpose of this systematic review was to identify all age of blood research with at least one PRO outcome, studies of all designs (case reports/series, observational, and experimental) were included. Also included were abstracts, posters, conference proceedings, and published original research. Articles were only eligible if the intervention/exposure was blood storage time and at least one of the outcomes in the study pertained to a PRO: HR-QoL, patient satisfaction, fatigue, and well-being. Review articles, commentaries, opinion pieces, and articles that were unavailable online or in print were also excluded.

Literature was searched across two Ovid databases: Medline (1946 – March 26, 2014) and Embase (1974 – March 26, 2014). Final search results from each database were exported to Endnote X6.0.1 (Thomson Reuters, Philadelphia, PA, 2012) reference manager and duplicates were deleted. Two members of the research team (NS & AJ) independently reviewed the titles of each article to identify literature pertaining specifically to age of blood. Based on titles, the articles were coded as “yes”, “no”, or “maybe” relevant. Codes that

were discrepant between reviewers were considered to be articles that were "maybe" relevant. Abstracts of titles coded "yes" and "maybe" were then reviewed in duplicate and coded for inclusion or exclusion. Abstracts that were coded "yes" or "maybe" were retrieved for a full text review by one reviewer (NS) and a final decision for inclusion was made. An update to the literature search was conducted on July 1, 2015.

2.2 – Results

The initial search yielded a total of 2,329 articles (1,320 from Medline and 1,009 from Embase). Four hundred and twenty six duplicate articles were identified and subsequently deleted; 1,903 remained for screening. While 140 articles were related to the age of blood, only two studies reported on at least one PRO and were eligible for the review (Figure 2) (52,53). Of these two studies, one was only available as an abstract from the American Hematology Society (ASH) Annual Meeting (53). Though both studies were randomized, Mynster and colleagues used a parallel arm approach (52); whereas, Seitelbach et al. (53), used an N-of-1 approach. An overview of each study is summarized in Table 1.

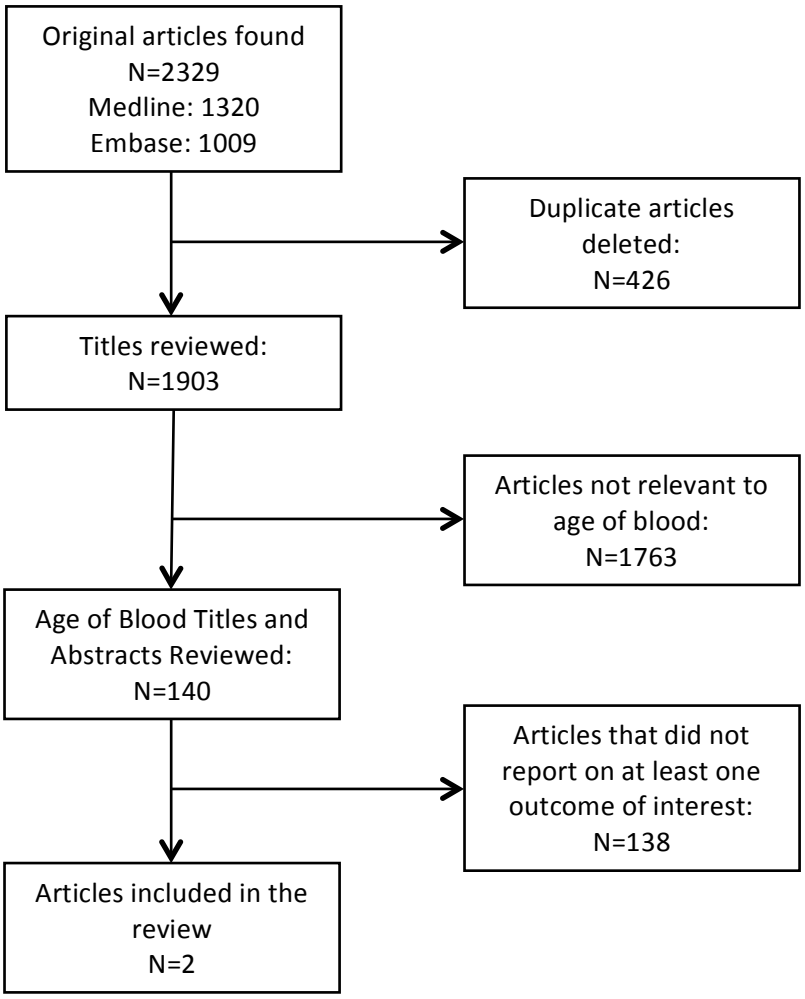


Figure 2: Screening Process and Study Selection

Table 1: Overview of the studies reporting the age of transfused blood and PROs

	Mynster et al, 2007 (52)	**Seitelbach et al, 2011** (53)
Sample Size	N = 22	N = 20
Years of Enrolment	April 2004 – February 2005	Not reported
Design	Parallel arm, randomised	N-of-1 trials
Country	Copenhagen, Denmark	London, Canada
Population	Patients admitted to the surgical gastroenterology department with Hb < 6.0mmol/L, complaints of fatigue, and no active bleeding or signs of blood pressure instability	18 years or older; transfusion dependent (at least 1 unit of RBCs every 4 weeks)
Setting	Surgical gastroenterology department	Not reported
Intervention	2 units of leukoreduced BC* RCCs stored for less than 1 week	Four fresh transfusions (<7 days of storage – product details not specified
Comparator	2 units of leukoreduced BC RCCs stored for more than 3 weeks	Four standard issue transfusion (7-42 days of storage) – product details not specified
PRO Measure	Change in self-estimated fatigue (measured using a visual analog scale)	Quality of Life (measured using a visual analog scale and the FACT-An** questionnaire)
Summary of Main Findings	2.8 point reduction in intervention arm vs. 0.4 in standard; p>0.05. No statistically differences between groups	There were no differences in QoL between fresh blood transfusions vs. standard issue

*BC = Buffy Coat; **FACT-An = Functional Assessment of Cancer Therapy - Anemia

Additionally, we found one more eligible age of blood RCT (the TRANSFUSE study) through the online clinical trials registry (54). Quality of life measured using the EuroQol-5 Dimension (EQ-5D) questionnaire at 90-day post-randomization is listed as a secondary outcome. However, since the study is currently ongoing, there are no results to report. The manual search for studies using references of systematic reviews did not yield any results. An update to the literature search in July 2015 yielded a total of 228 additional articles (144 from Embase and 84 from Medline). None of these articles were relevant.

2.2.1 – Is RBC storage time associated with fatigue?

The study conducted by Mynster and colleagues evaluated the impact of blood storage time on anemia-associated fatigue (52). Twenty-two patients who were admitted to the surgical gastroenterology department, with Hb levels of less than 60 g/L, complaining of fatigue, and having no active bleeding or signs of pressure instability were enrolled into the study and included for analysis. Patients were randomized to receiving 2 units of either fresh blood (stored <1 week) or standard issue blood (stored >3 weeks). Fatigue was self-estimated using a visual analog scale (VAS) prior to transfusion and 2-8 hours post-transfusion; zero indicated no fatigue and 10 was used to indicate maximum fatigue. The results of the study indicated an overall decrease in fatigue scores: scores decreased from a median of 6.6 (range 0.1-9.9) to 4.7 (range 0.6-10.0); p=0.02. Although there was a 2.8 point decrease in fatigue score among the

group of patients receiving fresh blood (median storage time = 4.5 days) compared with a reduction of 0.4 in patients who received standard issue blood (median storage time = 27 days), these results were not statistically significant (confidence intervals were not reported). Finally, the results showed an overall increase in Hb levels after transfusion for all patients (5.2 ± 0.6 to 6.4 ± 0.7 mmol/L); however, once again, there were no significant differences between groups. Although the sample size was small (n=22), the study was powered to detect a difference of 30% on the visual analog scale.

2.2.2 – Is blood storage time associated with patient quality of life?

Seitelbach et al.'s (53) series of N-of-1 trials examined if blood storage time affects HR-QoL and Hb in transfusion-dependent patients aged ≥18. Participants were assigned to receive four fresh (<7 days of storage) and four standard issue (7-42 days of storage) RBC transfusions. Quality of life was measured using a VAS and the Functional Assessment of Cancer Therapy – Anemia (FACT-An) questionnaire. These tools were administered prior to the transfusion and 24 hours post-transfusion. Twenty participants comprising of patients with myelodysplastic syndromes (MDS), β-Thalassemia, myeloproliferative neoplasms, Diamond-Blackfan anemia, and chronic anemia of underdetermined etiology were enrolled. Nine of 20 patients in the study completed at least six transfusions and were included in the "within-subject between patient" analysis. An overall between group analysis of QoL scores was

conducted for all study participants using a paired t-test and mixed model approach. The results did not show any statistically significant difference in QoL or Hb levels in either analysis. Since this study was published only as an abstract, numerical results are not currently available.

2.3 – Discussion

Although the studies that were included in this systematic review (52,53) were prospective and randomized, there are several limitations that should be addressed. It was difficult to assess the quality of both the Mynster et al. (52) and Seitelbach et al. (53) studies since there were gaps in the reporting. Specifically, the method of randomization and allocation concealment was not presented in either article thereby making it difficult to assess the quality of each study. There is also a lack of reporting in the results (particularly in the Setielbach et al. (53) study, which was published as an abstract, where no numerical results were provided), creating difficulty in drawing meaningful conclusions.

In both studies a small sample size was a major limitation. In the Mynster et al study (n=22), although there was a large difference in the overall reduction of fatigue between patients transfused with fresh blood compared to those transfused with old blood, the results were not statistically significant (52). It is difficult to ascertain how robust the results are since the study does not report confidence intervals. However, considering the extremely wide ranges of overall fatigue scores, a larger sample size may be needed for more precise results. The

article acknowledges that the "study population may be too small and too heterogeneous to find a low impact of red cell storage time on post-transfusion cytokine concentrations [a secondary outcomes] and fatigue". Additionally, the population studied in the Mynster et al. (52) study are patients who experience anemia from non-acute gastrointestinal bleedings. Disease and treatment burden in these patients may be vastly different compared to the MDS population, therefore the results of the study have limited generalizability.

Similar to Mynster et al. (52), the patient population in the Seitelbach et al. (53) study was also small (n=20; 9 included in the analysis) and heterogeneous. However, the small sample size and heterogeneity in the population were managed using a series of N-of-1 trials. N-of-1 studies are used to guide treatment decisions for individual patients (55). In a traditional N-of-1 trial, upon enrolment patients undergo pairs of successive treatment periods in random order (i.e. the patient will undergo both treatments one after another; however the order in which they receive these treatments will be determined by randomization). Once the patient has completed a cycle, the process is replicated and they receive another pair of treatments (55). These cycles can continue until effectiveness, equivalence, or harm has been demonstrated (55); it is recommended that patients complete at least three cycles (56,57). Each trial is analyzed separately to compare outcomes within each patient and establish optimal care for the individual. Since the patient acts as their own control, the trial is extremely efficient to establish treatment effects for individual patients.

Multiple N-of-1 trials can be combined in an N-of-1 series (such as the approach used by Seitelbach et al. (53)'s study) to estimate population treatment effects. However, this approach may require increased participants to ensure that the gains in within-patient variability achieved by multiple crossovers are balanced to estimate population variance and ensure the results are generalizable (58). Since crossover designs use multiple patients they are better for estimating population treatment effects. Therefore, while Seitelbach et al. (53)'s study uses a between group analysis comparing fresh blood to old blood where all participants were included, further research with a larger sample size should be conducted to estimate treatment effects for the population.

Finally, a third limitation of both studies is the short time frame in which fatigue and QoL are measured after each transfusion. In the study conducted by Mynster et al. (52), fatigue is measured 2-8 hours later, whereas in the study by Setielbach et al. (53), QoL is measured 24 hours post-transfusion. Since some biochemical changes (i.e. decreases in 2,3-DPG) are reversed *in vivo* between 4-72 hours, depending on the age of the blood transfused (21), there may be delays in experiencing the full benefits of transfusion in patients who were transfused aged blood. Moreover, since there were no repeated measures, changes in QoL over a defined period of time could not be assessed. While the short timeframe in which the outcome measures were administered can capture immediate changes in HR-QoL, it is not sufficient to capture more long terms changes associated with blood storage time.

2.4 – Limitations

This review was useful for identifying articles that assessed the association between age of blood transfused and various PROs. The search strategy was comprehensive. A librarian reviewed the strategy and provided feedback, which was incorporated to ensure the search would yield the greatest possible fraction of relevant articles. Additionally, the search did not specify any particular patient population therefore it was highly sensitive.

However, because of the "high sensitivity" of this search strategy, searching the databases by identifying articles purely on intervention (i.e. only combining the concepts of "RBC Transfusion" with "Blood Storage Time" using the Boolean operator "AND") yielded >20,000 articles. In order to narrow the search, it was essential to streamline the results by specifying the PROs of interest, despite the risk of excluding articles with a PRO endpoint that was not specified. We mitigated this risk by searching the literature to identify all possible text words that could be used to index and identify literature with a PRO endpoint. A final limitation of the review was that a meta-analysis could not be conducted. Only two studies were identified. Both consisted of heterogeneous populations and outcomes and one was presented only in abstract form with no numerical results; therefore a meta-analysis could not be conducted.

2.5 – Conclusion

Although both studies conclude that there was no association between blood storage time and fatigue (52) or HR-QoL (53), in light of the aforementioned limitations and concerns with generalizability, the findings may be susceptible to type II error; thus, there is a need to conduct further research. Future research studying this association will not only provide insight about the role of transfusion in managing patients with MDS and improving quality of care, but also engender a greater understanding of the *in vivo* consequences of the storage lesion, thereby improving our knowledge of blood products. While the study by Seitelbach et al., (53) demonstrates the feasibility of using N-of-1 studies in the management of individual patients the feasibility of conducting a crossover trial has not been explored. Therefore, additional pilot work is warranted and necessary to inform the design of future trials that use PROs as endpoints.

CHAPTER 3: Can MDS patients be studied using a crossover design? A chart review

To address the ultimate research question, we contemplated using a crossover randomized controlled trial (RCT) instead of a parallel arm design. In crossover trials, study participants receive all interventions, with each intervention being separated by a "washout" period (59–63). Participants are randomized to the order in which they receive the interventions (Figure 3).

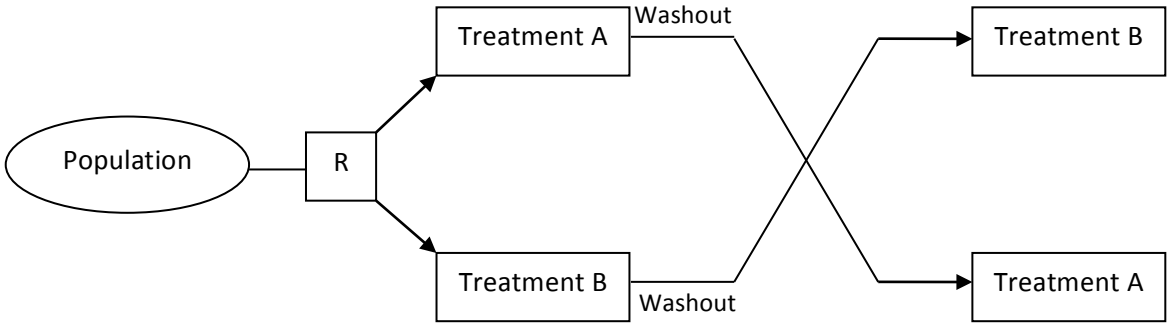


Figure 3: Schematic of a Crossover Randomized Controlled Trial

A crossover design has several advantages over a parallel arm design that potentially make it appropriate for this proposed study. The primary advantage of the crossover design is being able to measure and compare the same subject's response to a particular intervention. Since patients are self-controlled with a concurrent control group, crossover trials allow not only *within* subject, but also *between* group comparisons (59,62,64). Furthermore, since crossover designs have reduced between subject variability (62), they typically require fewer participants to achieve statistical power (59,61). Despite these

advantages, crossover trials in MDS patients are not widely used in comparison to parallel arm RCTs since they are only appropriate under specific circumstances (61). For a crossover design to be appropriate three specific criteria must be met.

Firstly, *the effects of the intervention being studied must have a rapid onset and be transient* (61,65). A major drawback of crossover trials is their susceptibility to carryover effects when an adequate washout period has not be established (59–63). Carryover of treatment effects is highly problematic since it interferes with the evaluation of the treatments being studied (61). The effects of RBC transfusion in this population are typically experienced within a few hours. Patients generally return to clinic for another transfusion when their symptoms return; hence, we would not expect carryover to be an issue in this study.

Secondly, *the intervention being studied cannot be a curative treatment.* If the intervention cures the disease or improves the subject's condition permanently, the baseline characteristics of the subject in each period of the trial will be different, making them incomparable (61). For patients with MDS, chronic transfusion with RBCs is used to transiently raise low Hb levels. Once transfused RBCs are cleared by the immune system, the patient should return to their baseline health status unless their disease state is worsening.

Finally, *crossover trials should be used in populations with a stable prognosis over the timeframe of the study* since it is essential for the study subject's baseline characteristics to be the same at the beginning of each treatment period (61). If the patient's disease worsens or improves naturally over the course of the trial the treatment periods will not be comparable. The International Prognosis Scoring System (IPSS) is currently used to place patients with MDS in various risk strata based on their bone marrow aspirates, number of cytopenias, and karyotype (66). The prognosis of patients with MDS can be variable since the clinical and physiological manifestation of this population is highly heterogeneous. The three year and five year survival for patients with MDS is approximately 42% and 29% respectively (67). Additionally, a subset of patients is at risk for acute myeloid leukemia (AML) progression. Despite the variability in prognosis, patients may still be stable over the time frame of the study provided their baseline characteristics remain the same. Since we expect patients with MDS to be treated with RBC transfusion therapy at least once every month, the expected study timeframe is approximately two months. However, since transfusion is mandated based on clinical requirement, it is possible for patients to be on study for a longer period of time and their condition could change during this period. To determine if MDS patients would meet this criterion of disease stability over a four to six month period, we conducted a retrospective chart review.

3.1 – The Research Question for the Chart Review

Are patients with MDS a stable population (defined as patients who receive RBC transfusion at regular intervals; have a consistent pre-RBC transfusion trigger; have less than three hospital admissions for causes unrelated to MDS or other chronic co-morbidities; and do not contract severe infections), to study in a crossover RCT where the intervention of interest is RBC transfusion over a six month period of time?

3.2 – Secondary Study Objectives

1. To determine if a six-month timeframe is appropriate for conducting a crossover transfusion trial; and,
2. To determine possible eligibility and feasibility considerations by collecting additional baseline data to characterize the patient population.

3.3 – Methods

3.3.1 – Study Design and Overview

We conducted a retrospective chart review of adult patients with MDS treated at the St. Joseph Hamilton Healthcare[1] (SJHH) and Juravinski Hospital and Cancer Center[2] (JCC) hematology outpatient clinics. Information pertaining to RBC and platelet transfusions, haematology lab tests, hospitalizations, and

[1] SJHH 50 Charlton Ave. E., Hamilton, ON, L8N 4A6.
[2] 711 Concession St., Hamilton, ON L8V 1C3

other relevant variables (Appendix B) were collected for a six-month observation period (January 1 to June 30, 2013). An overview of the study is illustrated in Figure 4.

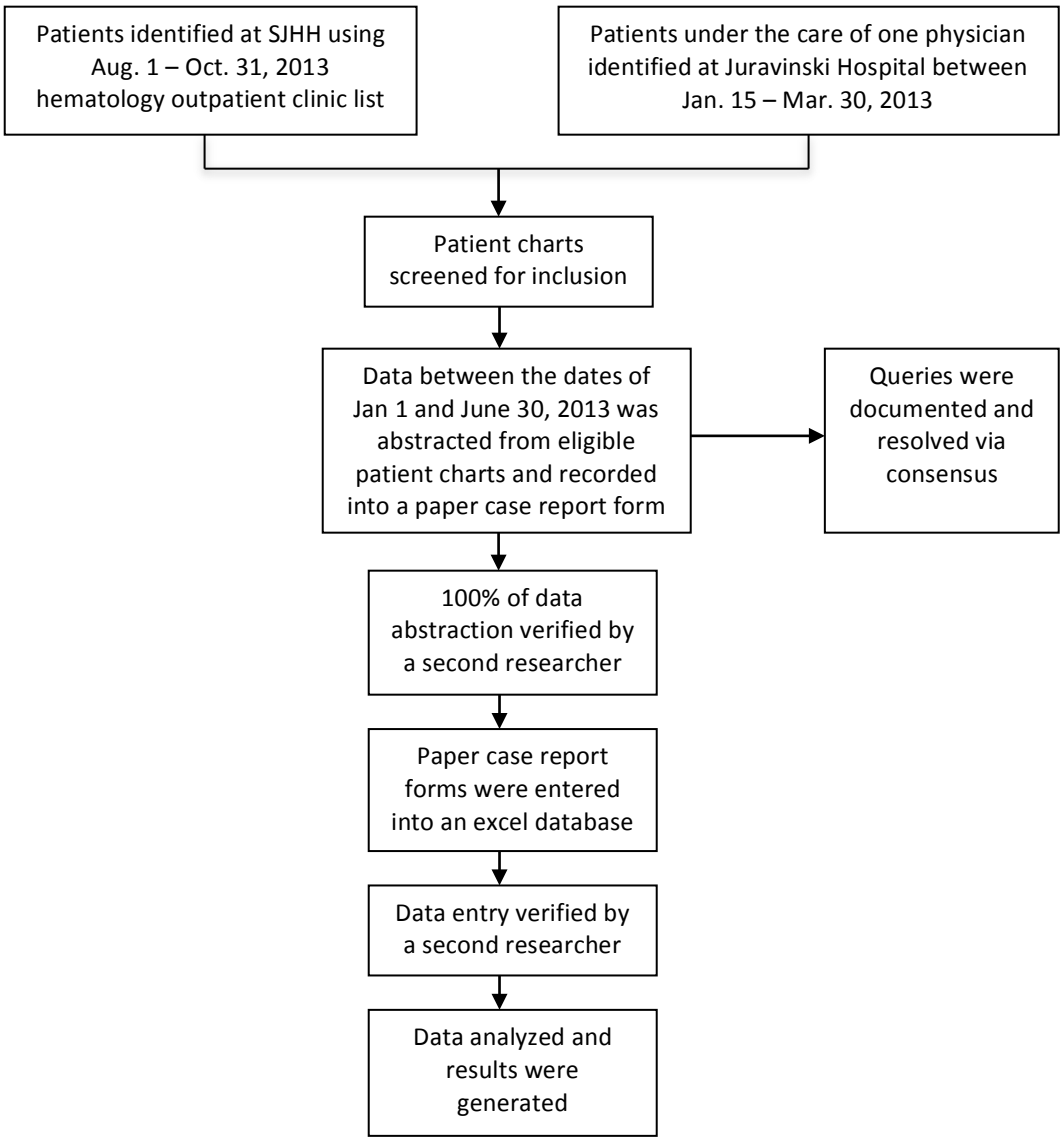


Figure 4: Overview of Patient Identification, Screening, and Data Management

3.3.2 – Patient Identification and Eligibility

Adult patients with MDS at SJHH were identified using the August 1 – October 31, 2013 haematology outpatient clinic list. The same approach was used to identify eligible patients at the JCC between January 15 and March 30, 2014 with one main difference: due to logistical reasons only patients under the care of one haematologist could be identified.

Patients that were identified were screened for eligibility. Patients at Juravinski Hospital were considered to be eligible if they had a confirmed diagnosis of MDS based on analysis of bone marrow aspirate. However, since bone marrow aspirates are not routinely done at SJHH, patients with suspected MDS based on clinical presentation as per physician documentation in clinic notes were also considered eligible. Patients with MDS were only included if: they were ≥18 years old at the start of the observation period, diagnosed with MDS and followed (as per visit history) for at least six months prior to the start of the observation period.

Data from electronic health records and charts of all patients who met these criteria between the dates of January 1 and June 30, 2013 were reviewed and recorded onto a paper case report form (CRF). Since this was an exploratory and descriptive chart review, a sample size of 20-25 charts was deemed appropriate based on feasibility (each site was estimated to treat approximately 20-30 patients each).

3.3.3 – Data Abstraction and Management

Paper CRFs were developed by the study coordinator (NS) and revised by two experts, one in the field of MDS and another in research methodology (MC & NH). Case report forms were pilot tested by the coordinator (NS) with one chart to assess clarity and determine where additional definitions were required. The CRF was revised and a standard operating procedure (SOP) was developed by the coordinator (NS) to document and regulate the data abstraction process. Two members of the research team, the coordinator (NS) and a student research assistant (FK) used the SOP to abstract one chart in duplicate independently of each other. Data abstraction was compared and discrepancies were resolved through consensus. The CRF and SOP were further refined and definitions were clarified. One member of the research team (FK) abstracted all data to the final version of the paper CRFs. Patient health records at SJHH were reviewed through Provider Portal. Health records at the JCC were reviewed through Meditech and Soveira. Where information was unavailable online, the physical patient chart was accessed through Health Records to retrieve missing data. The study coordinator (NS) verified 100% of the data abstracted on paper CRFs.

Baseline demographic information was collected for each patient including age, gender, blood type, IPSS scores, and pre-existing co-morbidities at the beginning of the observation period. New conditions that developed during the course of the observation period were also noted.

In addition to the aforementioned baseline data, information pertaining to hospital admissions and infections was also retrieved. Patients were considered to be hospitalized if they were admitted as an in-patient for a minimum of 12 hours. Hospital admissions were identified through electronic health records (Provider Portal, Meditech, and Soveira) using discharge summaries and visit histories. Clinic notes were also searched for mention of hospital admission in the event that patients were admitted outside of the study sites. The date of admission and discharge, reason for admission, and diagnosis on discharge were recorded.

Similarly, infections were recorded if patients had a positive microbiology culture during the observation period, or if there was documentation of infection in the clinic notes. The start and stop date of the infections were recorded when available, along with whether or not it was severe, and any additional details such as type of infection (viral/bacterial) and treatment information. An infection was considered to be severe if it persisted for ≥14 days with symptoms; required IV antibiotics or hospitalization to treat; required oxygen, fluids to support blood pressure, or intubation; or resulted in septic shock (68).

Blood product utilization information, hematology blood work, and various liver enzyme test results were also collected. Information related to RBC transfusions included: date of transfusion, donor blood type, number of units transfused, transfusion reactions, and product unit number. The date of transfusion, the donor blood type, and the product unit number were used to

retrieve the collection date of the unit using the Transfusion Registry for Utilization, Surveillance, and Tracking (TRUST) database housed at the McMaster Transfusion Research Program (MTRP[3]). The collection date of the unit and date of transfusions were used to determine the age of the blood at time of transfusion. Transfusion reactions were recorded if they were documented in a clinic note, listed in the blood bank history, or recorded in nursing progress notes. Finally, information pertaining to platelet transfusions were also captured and included: date of transfusion; donor blood type; number of units; and, possible transfusion reaction information.

Data from paper CRFs were entered into an excel spreadsheet for analysis by the coordinator (NS). Another student research assistant (ZI) verified data entry. Discrepancies between paper CRFs and the database were logged in an Excel spreadsheet and corrected in the database by the coordinator (NS).

3.3.3 – Ethical Considerations

We received approval from the Hamilton Integrated Research Ethics Board (HiREB) to conduct this study. The study complied with all regulations, guidelines, and policies to protect patient privacy. Patient charts reviewed for the study were given a unique subject ID code, which was used on each CRF. Paper CRFs and the electronic database were stored in a locked cabinet and secure

[3] HSC-3H50 McMaster University; 1280 Main St. W.; Hamilton; ON; L8S 4K1

server respectively, at the coordinating center. Access to the computer where the database is stored is limited by a password that is changed regularly.

A document linking the subject ID and patient chart was temporarily stored using an "on-the-fly" encryption data storage device. This document was permanently deleted upon completion of the study. We did not seek informed consent since this study was retrospective and minimal risk to patients. Additionally, no patient identifiers except for year of birth/age were collected.

3.5 – Analysis

Descriptive statistics were used to analyze the data. Means and standard deviations were reported for continuous variables along with medians and interquartile ranges (IQRs) where appropriate. Proportions were used to describe categorical variables.

3.3 – Outcome Measures

The primary outcome of this study was proportion of patients who have a stable diagnosis over a defined six-month period. In order to meet this study objective, an outcome measure assessing patient stability was required. Traditionally, prognosis of patients with MDS and transformation to AML is often established using the International Prognostic Scoring System (IPSS) score, calculated at diagnosis (69). However, this score may not necessarily be indicative of patient stability. Furthermore, in this study, IPSS score was not used

since a subset of the population (patients treated at SJHH) did not routinely have a bone marrow aspirate done, which is required to calculate an IPSS score. We instead used a combination of other criteria to assess patient stability, including:

1. The dispersion around median number of days between each transfusion was assessed; *patients were considered stable if the first and third quartile were within ±7 days of the median.*

Patients are typically transfused at a Hb trigger of approximately 80 g/L to replace RBCs that cannot be produced in the bone marrow. Once transfused RBCs disappear from the patient's circulation, another transfusion is required to maintain adequate Hb levels and prevent fatigue. Therefore, increasing interval of days between transfusions indicates an improved disease state since patients are able to sustain their RBC levels for longer periods of time. Conversely decreasing interval of days between transfusions is indicative of worsening disease. The ideal patient for the proposed trial should receive transfusions at regular intervals; however, clinic bookings and patient schedules can also affect the interval between transfusions so this had to be taken into account when defining a 'regular interval'. We anticipated being unable to conduct a time-to-event (survival) analysis due to the short observation period and limited data; therefore, we used the dispersion around median number of days between each transfusion episode.

2. The dispersion around median pre-RBC transfusion Hb count; *patients were considered to be stable if the first and third quartile were within ±10 g/L of the median.*

We also considered pre-transfusion Hb counts to avoid perceived stability as a result of individualized transfusion protocol. For example, patients may have clinical orders to receive RBC transfusions every two weeks should their Hb counts fall below a specific threshold (most frequently 80 g/L). These orders may be re-evaluated at each follow-up visit, which can range from weekly to biannually depending on patient, provider, and hematology clinic. Therefore, a patient who receives transfusions at a regular schedule may still be unstable if there is a high level of dispersion around their median pre-transfusion Hb count.

3. Number of hospital admissions; *patients were considered stable only if they had been admitted to hospital less than 3 times within the six month observation period and if those hospital admissions were unrelated to MDS or other chronic co-morbidities.*

Frequent admission to hospital for MDS or co-morbidities may indicate advanced disease state and can affect not only regularity of RBC transfusion, but also overall well-being.

4. Number of infections; *patients were considered stable if they did not contract any severe infections during the observation period*.

Since patients with MDS may experience neutropenia, which can make them susceptible to infection, patients were considered stable if they did not contract any severe infections during the observation period.

These stability assessment criteria were peer reviewed by a hematologist specializing in the management and care of patients with MDS.

3.6 – Results

Twenty-one of 52 (40.4%) patients screened were eligible for the purposes of the study (mean age: 79.52 ± 6.03 years; 6 female). Most patients (26; 50%) who failed screening did not have a diagnosis of MDS; three of 52 (5.8%) were not diagnosed with MDS six months prior to the observation period. Two patients (3.8%) were excluded due to lack of data since their primary treatment center was not within Hamilton (Figure 5).

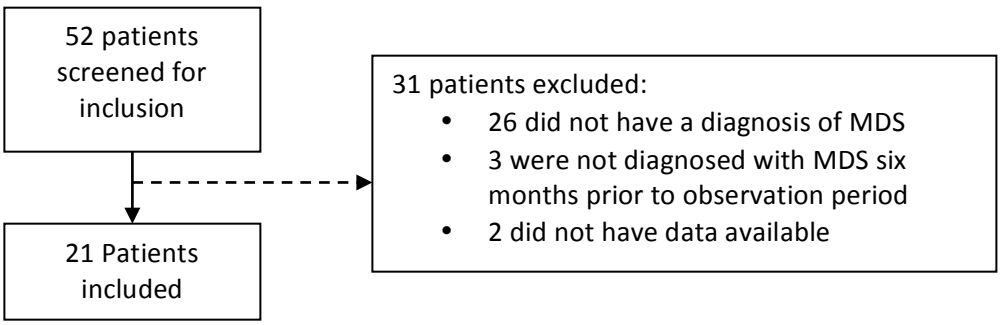


Figure 5: Final number of patients included into the chart review

A significant proportion of patients (17/21; 81%) had at least one comorbid illness present at baseline. Hypertension, diabetes, and cardiac disease were among the most frequently reported. Patients were also reported to have various respiratory disorders at baseline including: asthma, COPD, asbestosis, pulmonary fibrosis, and/or obstructive sleep apnea (Table 2 summarizes additional baseline information).

Table 2: Demographic and Baseline Characteristics of 21 Patients with MDS

	SJHH (n=11)	**Juravinski Hospital** (n=10)	**Total** (N=21)
Age (Mean ± SD)	81.90 ± 5.58	76.9 ± 5.63	79.52 ± 6.03
Female, n (%)	6 (54.6)	0 (0)	6 (28.6)
IPSS Score, n			
Low	2	2	4
Intermediate-1	--	3	3
Intermediate-2	--	3	3
High	1	2	3
Comorbid Illness, n (%)			
At least one	9 (81.8)	8 (80)	17 (81)
Hypertension	3 (27.3)	4 (40)	7 (33.3)
Diabetes	4 (36.4)	3 (30)	7 (33.3)
Arthritis	2 (18.2)	0 (0)	2 (9.5)
Cardiac Disease	4 (36.4)	3 (30)	7 (33.3)
Stroke	1 (9.1)	0 (0)	1 (4.8)
Respiratory Disease	3 (27.3)	2 (20)	5 (23.8)
	Asthma, COPD, Asbestosis, Pulmonary Fibrosis, Obstructive Sleep Apnea		
Other, n (%)	8 (72.7)	7 (70)	15 (71.4)
	Thalessemia trait, glaucoma, chron's disease, gout, dislipidemia, pneumonia, chronic kidney disease, bladder cancer, vasculitic neuropathy, GERD, GI disease, hypercholesterolemia, ovarian cancer, macrocytic anemia,		

The IPSS score was only available or could be calculated for 13/21 (62%) of patients. Although the number of patients in the intermediate-1 and 2 risk strata may be underestimated, there seems to be an overall equal distribution of patients in each category. Overall, patients experienced a median of 5 (IQR, 2, 12) transfusion episodes over a six-month time period. The median interval between each transfusion episode was 14 days (IQR, 7, 21 days). The mean rate of transfusion episode was 1.5 (SD, 0.33) transfusions/month (Figure 6).

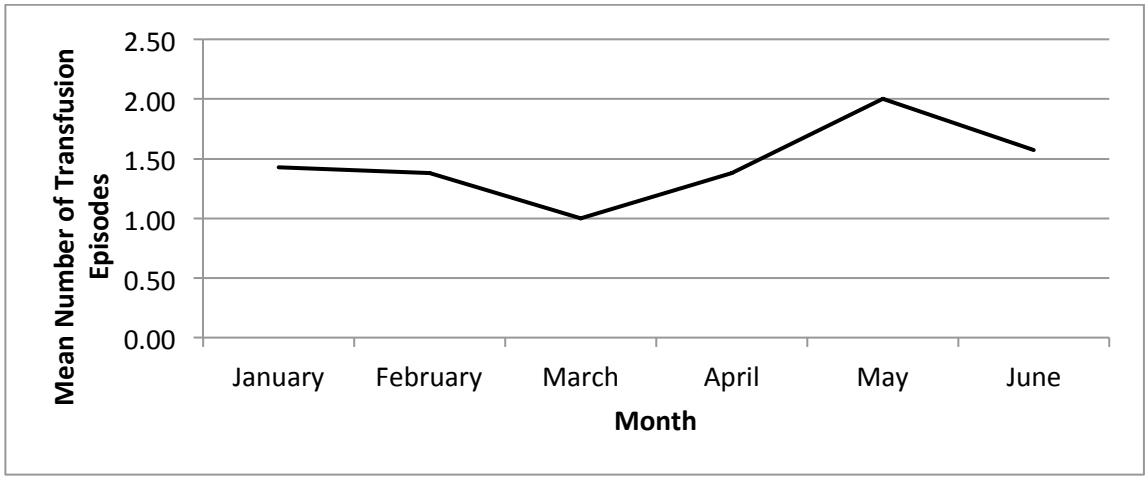


Figure 6: Mean Distribution of RBC Transfusion Episodes between Jan 1 and Jun 30, 2013

3.6.1 – Stability of Patients with MDS

Since transfusion schedules are unique to each patient based on clinical requirement, there is a high level of heterogeneity, which likely accounts for the dispersion of data around the median interval of days between transfusion episodes. To understand how stable patients are on an individual level, summary statistics were generated for each patient who received greater than three transfusions (Table 3). Only patients who had more than three transfusion

episodes were included since a minimum of three time intervals was required to generate medians and IQRs.

Table 3: Summary statistics for patients who received greater than 3 transfusion episodes

Pt. ID	Median # of Units Transfused (IQR*)	Median Interval of days between Tx (IQR)	Median pre-transfusion Hb counts (IQR)	# of Hospital Admissions	# of Severe Infections
P01	2 (0)	21 (20.5, 21)	75 (72, 77)	0	0
P03	2 (0)	35 (33.75, 36.25)	84 (84, 87)	0	0
P05	2 (0)	14 (13, 14)	84.5 (82.25, 89)	0	0
P06	2 (0)	7 (7, 8)	80 (78, 85.75)	0	0
P07	2 (0)	42 (35, 42)	101 (96, 101)	0	0
P08	2 (0)	28 (21.5, 28)	87.5 (84.5, 88.5)	0	0
P09	1 (0)	28 (28, 35)	81 (80, 85)	0	0
P10	2 (0)	35 (30.5, 37.75)	95 (94, 96)	0	0
P12	2 (0)	15 (14, 20)	77 (72.25, 80.5)	0	0
P13	2 (0)	14 (12.5, 16.5)	84 (79.75, 87)	0	0
P14	2 (1)	11 (4.25, 20)	89.5 (84, 92)	0	0
P16	2 (0)	12.5 (5.5, 14)	91.5 (79.75, 97.5)	0	0
P17	1 (1)	7 (6.25, 7)	92.5 (86, 96)	0	0
P18	2 (1)	14 (13.75, 14.25)	87 (85, 91)	1	0

*IQR = Interquartile range

Fourteen of 21 had more than 3 transfusion episodes (eight patients at SJHH, six patients at Juravinski Hospital). Most patients received a median of 2 units each transfusion episode. Each of the 14 patients was assessed using the four pre-defined criteria.

1. The dispersion around median number of days between each transfusion

As expected, the dispersion around median interval of days between transfusion episodes for each of these 14 patients was quite narrow. Only 3 patients had an IQR of more than seven. Two of these patients may still be considered appropriate for the purposes of the trial since the first and third quartile are within seven days of the median. One patient (P14), however, had an IQR>14 and could not be considered stable.

2. The dispersion around median number of days between each transfusion

The median pre-transfusion Hb count was 86 g/L (IQR, 79, 90). The variation around the median seems to decrease significantly when assessing each individual patient. For instance, all but one patient (P16) had an IQR≤10 around their median pre-transfusion Hb count. The one patient whose lower limit of the IQR exceeded 10 was transfused at a median pre-transfusion Hb count of 91.5 (IQR; 79.75, 97.5). Despite this patient having the acceptable amount variation in the interval of days between transfusion episodes (median of 12.5

days; IQR, 5.5, 14), there seems to high level of variability in the transfusion trigger. Therefore, this patient may not be ideal for the purposes of the study.

3. Number of hospital admissions

Only one patient (P18; from the cohort treated at the JCC) was admitted to the hospital during the six-month observation period for one night due to symptoms consistent with pulmonary edema. The patient had previous triple vessel coronary artery disease with bypass in 2001; it is unclear whether hospital admission was related to cardiac disease. Therefore, the patient was not considered unstable for the purposes of this review since the episode seemed to be acute and relatedness to co-morbidity is unconfirmed.

4. Number of severe infections

Three patients (all of whom were part of the SJHH cohort) contracted infections as confirmed by microbiology culture. Two patients had asymptomatic bacteriuria and one had a chest infection; none were considered serious.

In summary, of the 21 patients included into this study, 14 (66.7%) had more than three transfusion episodes in 6 months, making them appropriate candidates for the purposes of the larger trial. Of these 14 patients, two did not seem appropriate for the trial on account of the high variability in interval of days between transfusion (P14) and pre-transfusion Hb levels (P16). Therefore, 12/14

(84.7%) could be considered "stable". The above criteria can be applied to the study population during screening to identify and recruit participants who may be considered stable for the proposed trial.

3.6.1 – Age of Blood Transfused

A total of 185 transfusions and 333 RCCs were transfused to 21 patients over a six-month observation period. Although all transfusions were compatible with the recipient, 55/333 (16.5%) units were not group specific. The age of RBCs transfused ranged between 8 to 41 days. The median age of blood transfused was 24 (IQR, 20, 31) days. Figure 7a illustrates the distribution of age of blood transfused. Figure 7b illustrates the distribution by site.

Figure 7a: Distribution of Age of Blood Transfused

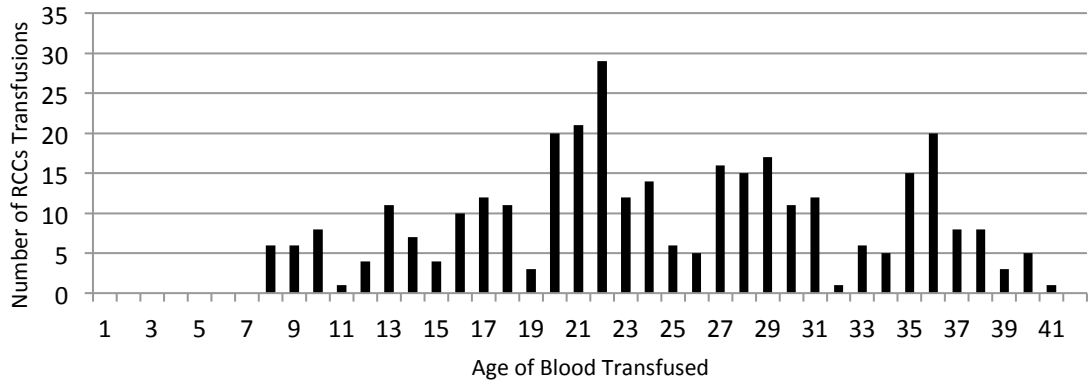
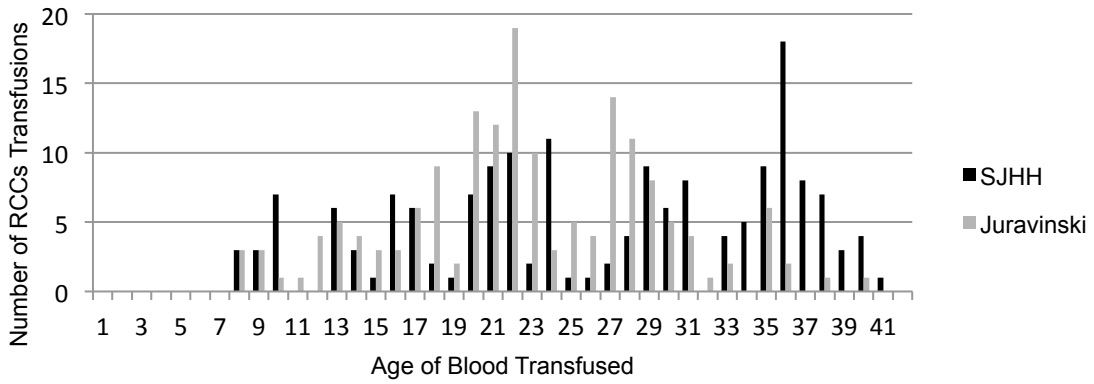


Figure 7b: Distribution of Age of Blood Transfused by Site



The age of blood most frequently dispensed was 36 days at SJHH (18/168; 10.7%) and 22 days at the Juravinski Hospital (19/165; 11.5%). The median age of blood transfused at the SJHH was 28 days old, (IQR, 20, 35) compared to 22 days at the Juravinski Hospital (IQR, 18, 27). Based on these figures, it will be essential to determine the feasibility of transfusing very fresh (1-7 day old) at both sites and very old (35-42 day old) blood particularly at the JCC.

3.7 – Discussion

There are two main advantages of using a crossover study design: 1) reduced between-patient variation; and, 2) higher efficiency, thereby requiring fewer participants to achieve statistical power (59,61,62). These two advantages are especially relevant when studying patients with MDS.

MDS is a rare hematological disorder characterized by a hypercellular bone marrow and ineffective hematopoiesis (4), more specifically, deficiencies in

the differentiation process of hematopoietic stem cells (70). While the pathophysiology of MDS is multi-factorial and has yet to be fully elucidated, *de novo* cytogenetic and molecular alterations in tumour suppression genes of hematopoietic stem cells have been suggested to play a role (4,5). Depending on the location and combination of genetic lesions, the clinical presentation and prognosis for patients with MDS can be highly diverse (69). Consequently, patients with MDS may experience a series of peripheral blood cytopenias (4,5,70). A crossover design can minimize the between-patient variation that is characteristic of such heterogeneous populations since each participant acts as their own control.

With reduced between-patient variation, a smaller sample size can be used while maintaining the power of the trial. This feature of the design is especially relevant since patients with MDS are rare; the incidence of MDS is approximately 3-4 in 100,000 individuals each year aged ≥ 65 years (66,71,72). Furthermore, chronically transfused patients with MDS who have a stable prognosis are rarer still, considering 25-40% of cases progress to AML (70,73). Finally, of the 52 patients who were screened from the outpatient hematology clinics, only 14 (26.9%) fit our eligibility criteria and had received greater than three transfusions in 6 months. Based on these figures, it is anticipated that patient accrual may be slow and the lower sample size may improve feasibility of detecting significant differences in outcome, should such differences actually exist.

Although there are advantages to using crossover methods when studying patients with MDS, ultimately the appropriateness of this design hinges on the stability of the patient's disease over the expected duration of the trial. Since patients only received a median of 5 (IQR, 2, 12) transfusions over a six-month observation period, there was not enough data to conduct a time-to-event analysis or regression. Therefore, measures of central tendency and dispersion were used to describe the population. Overall, despite the diversity in clinical presentation and prognosis, there was not a high degree of variability around the median interval of days between transfusions (median was 14; IQR, 7, 21). The mean distribution of transfusions was also fairly consistent (maximum fluctuation was 2 transfusions). The same seems to be true of pre-transfusion Hb value (IQR; 79, 90). Furthermore, the dispersion around medians seems to decrease drastically when these summary statistics are calculated for each individual patient. Only 2/14 patients analyzed were not considered to have a stable prognosis. This suggests that as a population, patients with MDS are sufficiently stable to study using a crossover design.

The results of this study also suggest that a six-month time frame is reasonable to conduct a trial considering 12/21 (57.1%) had at least 4 transfusions within that time and were considered to be stable. These patients could be enrolled and randomized in the proposed trial at least twice, which would make patient accrual far more feasible.

Aside from patient accrual, another potential feasibility issue that may need further piloting is the availability of fresh blood in the blood bank inventory. The "first in, first out" policy suggests blood banks dispense the oldest available product in order to minimize wastage due to expiry. The results of this chart review show that of the 333 units dispensed, there were no products fresher than 8 days. This may pose a potential feasibility issue since the age parameters for fresh blood in the proposed trial are between 1-7 days old. If RBC products less than 8 days are consistently unavailable, alternative arrangements will need to be made. For instance, blood transfusion services that participated in the ABLE trial were able to maintain an inventory of fresh (<8 days old) RBC products (74). In the event that fresh blood was unavailable, the freshest available component was to be transfused. This strategy seemed to be successful for the ABLE trial since the mean age of fresh blood transfused was 6.1 (SD, 4.9) days. The blood transfusion services at SJHH in Hamilton participated in the ABLE trial; hence there is local experience in using this strategy to provide fresh blood.

This chart review was conducted over two sites – a benign hematology care center (SJHH) and a malignant hematology-oncology care center (JCC). It appears patients treated at SJHH may be more appropriate for the trial since 8/11 patients reviewed received greater than three transfusions and seemed to be stable, compared with 4/10 at the Juravinski Hospital. However, since patients at the JCC were only recruited from one hematologist specializing in MDS care, there may be a referral bias; it is possible patients in the care of other

hematologists may be appropriate and were missed during screening. Another limitation of the study is patients at SJHH did not have a bone marrow aspirate done to confirm diagnosis of MDS. Since bone marrow biopsy can be a fairly intrusive and uncomfortable procedure, patients at SJHH are not routinely receive this procedure unless they are eligible for active MDS treatments other than supportive care. Therefore, patients treated at SJHH who did not have a confirmed diagnosis of MDS were still included in this study if they were strongly suspected of having MDS based on clinical documentation.

3.8 – Conclusion

While the data suggest that is possible to conduct a crossover trial where the exposure being studied is RBC transfusion in this population, identifying these patients may be challenging considering the limited number of individuals who were eligible, had received greater than 3 transfusions, and were stable over a six-month observation period (12/52 patients screened). The results of this study indicate that using the hematology outpatient clinic list (usually prepared one week prior to the clinic) to identify patients is a reasonable approach. Once patients who are scheduled for transfusion are identified, they can be pre-screened for a diagnosis of MDS using patient electronic health records. An advantage of pre-screening patients is reduced burden on outpatient clinic staff to determine patient diagnosis. Patients who are identified to have MDS can be

further assessed for stability using the retrospective data in TRUST and if eligible, approached during clinic for recruitment.

The criteria used to define patient stability in this chart review can be applied as eligibility criteria to ensure that only patients who are "stable" are recruited. Adult patients with MDS will be included:

- If they have had at least 4 transfusion episodes over the past 6 months;
- The first and third quartile around the median number of days between each transfusion episode is within ±7 days; and,
- The first and third quartile around the median 24-48 hour pre-transfusion Hb count is within ±10 g/L.

Patients would be excluded from the study if:

- They were admitted to hospital for issue related to MDS or other chronic co-morbidity within the past six months; or,
- If they had contracted or have any severe infections within the past six months.

Figure 8 demonstrates the proposed Stability Assessment Algorithm that can be used for screening and identifying patients with stable transfusion requirements. Further validation studies are needed to ensure this tool can predict patient stability prospectively.

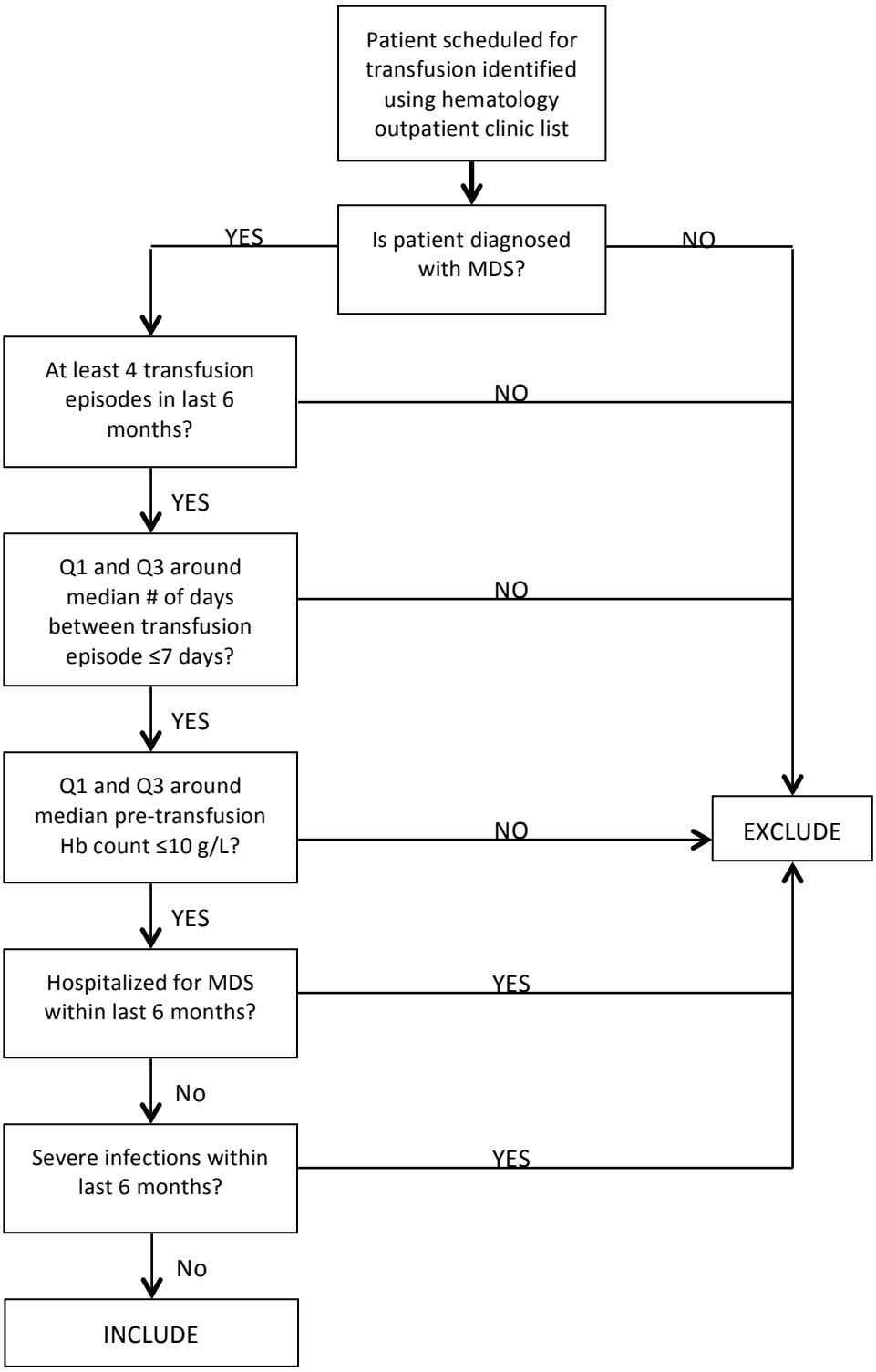


Figure 8: Stability Assessment Algorithm for screening and recruiting patients who are stable for a crossover RCT

CHAPTER 4: Using an applied qualitative approach to characterize post-transfusion well-being in patients with MDS

Literature studying HR-QoL outcomes in patients with MDS is primarily focused on understanding the impact of various interventions or supportive care (75). Patients with MDS experience poorer HR-QoL compared to age and sex matched individuals of the general population (1). While there are several factors that contribute to poor HR-QoL among patients (75), fatigue due to refractory anemia is a major determinant since it significantly interferes with activities of daily living (1,7,75). Compounding this issue, literature identified in a systematic review conducted by Platzbecker et al. (7), suggests patients with MDS who are transfusion dependent experience worse HR-QoL compared with those who are transfusion independent (1,76,77). The review outlines several consequences of transfusion dependency which can include: risk of infection (78); RBC or platelet transfusion related complications (79); febrile or allergic reactions (80); skin rashes (81); and, transfusion related iron overload, which may increase the risk of morbidity and mortality (82). Despite these results, there seems to be a correlation between increase in Hb and HR-QoL scores; these observations suggest that RBC transfusion may improve HR-QoL transiently (7).

Clinicians anecdotally report that patients may continue to feel unwell despite RBC transfusion, sometimes experiencing the onset of additional physical symptoms despite transfusion. These short-term changes in patient well-being after a transfusion have not been systematically characterized.

Therefore, we undertook this study to understand how transfusion can impact a patient's well-being and to characterize transfusion related symptoms that patients may experience. The results of this study will be used to inform the selection of an appropriate outcome measure with appropriate face validity for the proposed trial.

4.1 - The Research Objectives

To explore the symptoms and changes in well-being that patients with MDS experience as they are transfused with RCCs.

4.2 – Methods

4.2.1 – Study Design

An applied qualitative approach using a semi-structured interview guide was employed to meet the study objectives. Applied qualitative research studies "develop, monitor, or evaluate a policy or practice, using qualitative techniques as an alternative to, or to complement, other approaches" (83). The focus of applied qualitative research is "collecting and generating data to further our understanding of real-world problems" (84). Unlike structured questionnaires and interviews that consist of fixed questions with pre-coded response choices, semi-structured interviews are comprised of open-ended questions that allow participants to share rich and unique perspectives related to the research objective (85,86). These responses can be analyzed using primarily an inductive

approach where observations and patterns from textual data are used to make broad generalizations and derive theories (87). Conversely, a deductive approach to the analysis (typical of quantitative methods) uses the data to test previous theories/hypotheses (87). A combination of inductive and deductive approaches were used to analyze the data from this study to systematically characterize the symptoms and changes in well-being participants experience when transfused with RCCs. High level codes, derived from the study objectives, were applied to the data deductively; specific sub-codes were also generated from the data inductively.

4.2.2 – Sampling and Recruitment Strategy

Consecutive adult patients with MDS, scheduled to receive RCCs were recruited from the hematology outpatient clinics of SJHH and Juravinski Hospital between August 1, 2013 and March 31, 2014. Patients were excluded if they were unable to speak English or provide informed consent. A charge nurse pre-screened potential patients. If eligible, permission to approach the patient for the purposes of a research study was obtained by the nurse. The study coordinator (NS) provided details of the study, answered any questions or concerns, and provided a copy of the consent package. Patients were encouraged to read the consent form and inform the study coordinator of their decision in person during their appointment or via phone.

Adequacy of sample size in a qualitative study can be dependent on several factors; while a heterogeneous population may require many participants to achieve data saturation, large sample sizes may impede the completion of a detailed qualitative analysis (88). It was estimated that approximately 10-15 participants would be required to elicit a full range of perspectives and achieve data saturation. Data saturation is achieved when there is repetition or 'informational redundancy' of themes in the data (89). Considering patients with MDS are rare, it was estimated that approximately 20-30 patients who met the eligibility criteria would be available to approach for recruitment in clinic. However, recruitment was slow since only a subset of patients who arrived in clinic had a diagnosis of MDS and were eligible to participate in the study. Patient recruitment was closed when all patients within the data collection phase had been approached. Written informed consent was obtained from all participants prior to interviews and a copy of the consent form was provided for the patient's records. This study was approved by HiREB.

4.2.3 – Data Collection and Participant Interviews

Semi-structured interviews (lasting approximately 20-30 minutes) were conducted with participants either while they were waiting to receive their RBC transfusion or during their transfusion. The study coordinator (NS) developed the first draft of the interview guide. This initial draft was reviewed and refined by a researcher experienced with qualitative interviewing (SL) and an expert in

research methodology (NH). A member of the research team (MC) with expertise in the management and care of patients with MDS reviewed the revised interview guide.

The researcher experienced in qualitative interviewing (SL) supervised the first participant interview and provided feedback to the study coordinator (NS), who led all the interviews. The interview guide (Appendix D) was further refined to ensure questions were capturing information pertinent to the research objectives in a manner that was not leading. All interviews were digitally recorded and transcribed verbatim. Transcripts were anonymized and checked for accuracy. Final transcripts were imported into NVivo 10 qualitative data software (QSR International Pty; Victoria, Australia) for analysis.

4.2.4 – Qualitative Content Analysis

The techniques of a descriptive qualitative content analysis were employed to analyze and summarize the data using both an inductive and deductive approach. Qualitative descriptive analysis is an approach to the systematic coding, categorizing, and summarizing of textual data to answer research questions that are typically relevant to practitioners or policy makers (90). This approach draws from the tenants of naturalistic inquiry where there is no pre-selection or manipulation of the variables studied; rather codes and categories are derived specifically from the data (90). Two researchers (NS &

SL) were involved with the analysis of data. The analysis process is outlined in Figure 9.

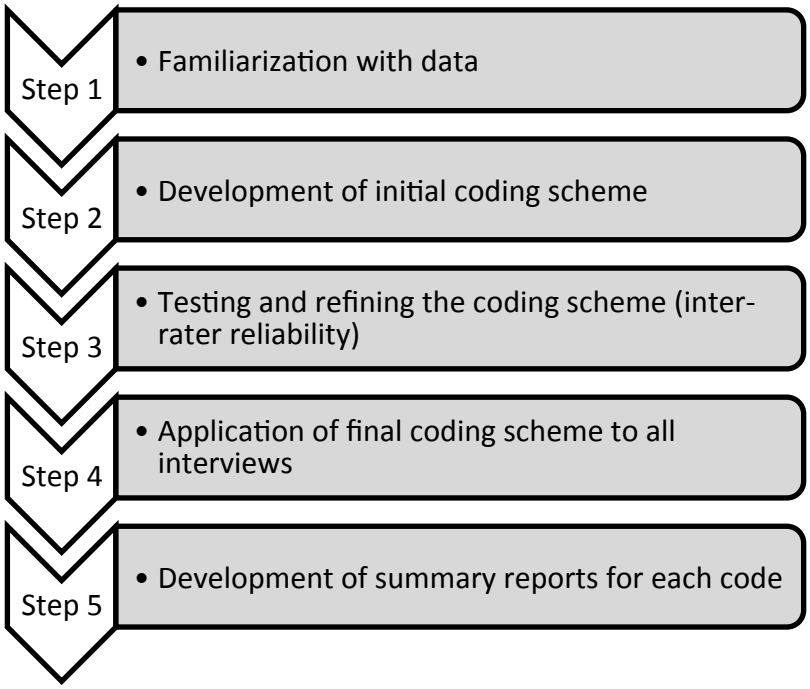


Figure 9: Overview of a Qualitative Content Analysis

As a first step to the analysis process, two researchers (NS & SL) independently reviewed all transcripts to familiarize themselves with the data. First impressions and comments were noted on the interview transcripts. Both researchers convened and used the notes and comments to develop codes that sections of data could be categorized under; this was the initial coding scheme. Both researchers independently applied the initial coding scheme to the same three interviews chosen at random using NVivo. A coding comparison query was conducted to determine percent agreement between coders. Codes with less than 90% agreement were discussed and disagreement was resolved using

consensus. Percent agreement between researchers was high; 18/19 codes had higher than 80% percent agreement, 16 of which had greater than 95% agreement. Codes less than 95% agreement were reviewed and discrepancies were resolved through consensus. Only one code (mental state of mind) had very poor percent agreement (67.9%); discrepancies in this code were not only resolved through consensus but also used to revise the definition of the code to improve clarity. Finally, one researcher (NS) applied the final version of the coding scheme to all interviews; slight changes to the coding scheme relating to organization of categories and labelling were made based on insights that became available through the coding process. Data in each category and subcategory of the coding scheme was descriptively summarized under three broad subheadings: the participant experience of fatigue, impact of RBC transfusion, and side effects of transfusion. Illustrative quotes from participant interviews were selected to add depth and richness to descriptive summaries and convey ideas in the participant's voice.

4.3 – Results

Sixteen participants were approached to participate in this study. Two patients refused due to personal circumstances, one was too ill to provide informed consent and participate in an interview, and one had a significant language barrier for whom an interpreter could not be arranged. One participant was progressing from MDS to AML over the three months prior to the interview.

We included this participant since prior to AML he was a transfusion-dependent patient with MDS whose experiences were considered to be similar enough to the rest of the study sample. Additionally, one participant, despite a significant language barrier, was able to participate since a family member was available to interpret the interview. Since the interview guide did not consist of any psychosocial questions of a sensitive nature, it was considered appropriate for a family member to be present. Therefore, a total of 12 participants were included into the study.

Median age of participants was 77 years (IQR, 72.25, 80); only two were female. Most patients were diagnosed when they presented with extreme fatigue as a result of anemia and were started on RBC transfusion around the same time. Three participants became transfusion-dependent some time after their diagnosis. Four participants reported using other supportive treatments to manage anemia related symptoms: iron infusions, cyclosporine, and platelet transfusions. Demographic characteristics and attributes of participants are summarized in Table 4.

TABLE 4: Demographic Characteristics and Attributes of Patients Interviewed for the MDS Well-Being Study

Interview	Age	Gender	Frequency of RBC Transfusion	Physical Effects of Transfusion	Co-Morbidities
PIH-01	70	Female	Every week	Confusion; headache, Temperature ; Itchy	Diabetes
PIH-02	73	Male	Every 2 weeks	None	Kidney problems, history of lung cancer
PIH-04	78	Male	Every 2 weeks	None	None mentioned
PIH-05	80	Male	Every 2 weeks	None	None mentioned
PIH-06	77	Male	Every 2 weeks	Lightheaded-ness	None mentioned
PIH-07	83	Male	As needed now – history with transfusion	Itchiness; nose bleed if high volume of blood is transfused	MDS in January 2012 – Diagnosed with AML in April, 2013
PIH-08	70	Male	Every week	Allergic reaction to platelets	None mentioned
PIH-09	72	Male	Every 2 weeks	None	None mentioned
PIH-10	82	Male	Every 2 weeks	None	Enlarged heart, muscle injury to both arms - poor motor function
PIH-11	71	Male	Every week	None	Back surgery, ulcers
PIH-12	77	Male	Every week	None	None
PIH-13	87	Female	Every month	Headache	DVT*

*DVT = deep vein thrombosis

4.3.1 – Participant experience of fatigue

All participants experienced fatigue, which seemed to greatly impact their activities of daily living. For example, one participant described being unable to do anything because "[he] had no energy… it was a hard job for [him] to walk upstairs to go to the bathroom" [PIH-02]. Another participant described feeling "completely useless, I couldn't hold a Kleenex in my hand" [PIH-04].

Severity of fatigue between participants prior to transfusion seemed to vary. Participants were asked to rate their levels of fatigue prior to transfusion on a scale of 1 to 10, with 1 representing 'no energy'. While, most participants rated their energy levels as ≤3, two rated it to be a 5 or 6 on a 10-point scale. Interestingly, two participants reported severity of fatigue was not consistent prior to each transfusion. One participant explains that "sometimes I feel good, sometimes I feel bad" [PIH-09]; he explained that it is usually prior to requiring a RBC transfusion that he generally feels worse. The second participant who experienced fluctuations in severity of fatigue prior to transfusion described that "…there was a time when I was getting 2 [transfusions] it would get down to 90 and I would… really feel it… And then there's other times lately, that I might not notice it being that tired. Whether my body's just getting used to doing this or something I don't know. But I, I haven't felt that bad, today having this. I haven't been feeling really that bad" [PIH-10]. One other participant also suggested adapting to consistently low levels of Hb: "…the body adapts and so you can actually live with a lower blood count and not experience the fatigue the same"

[PIH-08]. Finally, one participant received 2 units of RBC units for two consecutive weeks prior to leaving for vacation and reported experiencing significantly improved overall fatigue. In addition to fatigue, some participants identified other symptoms potentially related to anemia, which included loss of appetite, listlessness, shortness of breath, dizziness, sleeplessness, and general feelings of malaise.

4.3.2 - Impact of RBC Transfusion

Participants reported receiving RBC transfusion weekly, biweekly, monthly, or as needed (in the case of one participant). Time to recovery from fatigue and other symptoms of anemia varied between participants. Some participants reported improvements in energy levels immediately during, or few hours post transfusion episode; others stated it could take between 12 to 24 hours before they experience any significant recovery from fatigue: "…I now feel a little bit better. You know. By tomorrow I start to feel much better" [PIH-01]. Sometimes, time to recovery from fatigue varied between RBC transfusion episodes. One participant stated: "I find not much difference right away, okay. It does take a few hours or couple days to completely notice the difference… Some people they say the feel good right away. Now as far as I can tell that's baloney, because I had so many and I never feel good right after transfusion" [PIH-06]. Another participant's daughter noticed, "…a few times that [participants' name] perked up faster" [PIH-13] after receiving RBC transfusion.

RBC transfusion seemed to improve symptoms of poor appetite and lethargy, as well as fatigue. Improvement in fatigue seemed to vary between participants; while some indicate the severity of fatigue is considerably improved post-transfusion, others report less significant changes. It is important to note that RBC transfusion did not alleviate symptoms of anemia completely. As one participant states, "the two units certainly don't take me up to an energy level that would be normal, I don't think" [PIH-05]. A minority of participants reported sometimes not experiencing any improvements or changes after receiving RBC transfusions: "There are some where you feel like… I need another" [PIH-10]. One participant noted that impact of RBC transfusion is dependent on his pre-transfusion Hb count, "[i]f it's a minor top up… I won't feel any different today. But when it's in the 60s or 70s, I can feel the top up" [PIH-08].

In addition to improved fatigue and other anemia related symptoms, one participant revealed the psychosocial impact of RBC transfusion. "I have a better frame of mind, I think. When I go home, my wife and friends say 'oh you can tell he's…been topped up' as they call it. Because he's joking and carrying on. My sense of humor is more pronounced... But, I think I'm a feel a little better in me nature. When I'm reacting with other people. Like, before when I was down, somebody would phone and I couldn't even be pestered to answer the phone because I didn't want to talk to anybody. Now, when I receive my blood, I'm fine" [PIH-02]. Conversely, another participant speaks to the psychological impact of requiring chronic transfusion support, describing it as "just too much going on. It's

just another thing that I have to deal with. It tires me out thinking about it. So then I go home and my home is my refuge right? And I go home and unwind. My breathing is really shallow. So I just lie down and hang in there. I don't have much of an appetite like I used to, so all kinds of stuff" [PIH-11]. This participant felt that the need for chronic RBC transfusion support could be emotionally exhausting: "It's not just an energy level situation with blood transfusions. It's more than that. It's emotional stuff. It's like, uh, it's you feel like you've been hit with a hammer all day every day. You feel stunned" [PIH-11].

While the length of time that transfusion was effective for varied, most participants began to feel the effects of transfusion diminish a few days prior to their next scheduled transfusion episode. For some participants the effect of RBC transfusions only lasts several days, for others the effect lasts between one to two weeks with symptoms returning sometime in the second week. Only one participant described symptom relief post transfusion lasting for up to three weeks.

4.3.3 – Side Effects of Transfusion

A few participants (n=4) reported experiencing side effects of RBC transfusion, which included: confusion, characterized as heaviness in the head [PIH-01]; headache; temperature; itchiness/rash; nose bleed; and/or lightheadedness. These bothersome side effects accompanied some RBC transfusions and did not last very long (up to several hours); only

lightheadedness was reported to last up to two days. According to this participant, the itchiness and nosebleeds were attributed to the number of units and blood pressure. Lightheadedness was attributed to length of time between transfusions: “This time it was pretty bad. Don’t forget, there were 14 days in between, and I think they were a little bit too long. Like when I had blood transfusion every week, I didn’t notice those symptoms” [PIH-06]. While these aforementioned symptoms may or may not be related to the age of RBC unit transfused, they seemed to impact patient well-being.

4.4 – Discussion

Platzbecker et al.’s (7), systematic review discussing the clinical, quality of life, and economic consequences of RBC transfusion dependency, suggests that transfusion may transiently improve patient QoL based on studies that show a positive correlation of QoL with increased Hb levels (1,91–94). However, the review also identified studies that did not show QoL improvements despite increased Hb levels (81,95). This pilot study was undertaken to explore and characterize the changes in well-being patients experience in response to RBC transfusion and identify any transfusion associated side effects that patients with MDS may experience.

To this effect, the results of this study revealed some interesting findings about the impact of RBC transfusion. While RBC transfusions do improve fatigue by correcting anemia they do not completely resolve all symptoms. Patients may

still continue to experience fatigue and compromised HR-QoL despite transfusion. Experiences of the effects of RBC transfusions were diverse between participants. One half of the study population reported experiencing the same changes in well-being after each transfusion episode. The other half indicated within patient variability between transfusion episodes relating to one of the following three: the severity of fatigue prior to transfusion, time to recovery from fatigue after transfusion, or the effect of transfusion. For example, patients may feel better after some transfusion episodes and no different after others. A minority of participants reported several side effects of transfusion. While these side effects did not seem to be particularly bothersome, they appear inconsistently and accompanied only some transfusion episodes and not others. Thus, the results of this study validate the observations of clinicians in the field who express that patients may sometimes continue to feel unwell despite transfusion and experience several bothersome side effects.

Although a qualitative approach was taken in order to gain an in depth and rich patient perspective and reduce ambiguity in responses, there were several challenges and limitations to characterizing changes in well-being after RBC transfusion:

1. MDS affects an aging population who may have several chronic co-morbidities that may influence patient HR-QoL, making it difficult to notice transient improvements in well-being.

Literature suggests that symptom burden and co-morbidities impact the consistency of patient responses in their assessment of functioning (96). In the previous chapter 17/21 patients with MDS had chronic co-morbidities. In this study, 5 participants reported having several co-morbidities, which seemed to increase their disease burden. For example one participant explains that despite having poor appetite, "I had to force myself to eat because I am diabetic. I have to three meals plus the snacks," [PIH-01]. Another participant revealed that, "[t]here's just so much there that's changed in my system. Pain through here, pain through there. It'll come over me in waves, I can't breathe. Sit there and I go into hyperventilation. That happens every once in a while. I feel like I'm breathing but there's nothing" [PIH-11]. Pain has been associated with disagreement between self-reported patient functioning and physician assessed (96). Since the semi-structured interviews did not systematically probe for information about comorbidities or their impact on well-being, this information was not consistently captured and may be under reported.

2. Patients with MDS live a primarily sedentary lifestyle on account of fatigue, which may impede their ability to assess the magnitude by which symptoms improve post-transfusion.

A few participants had difficulty assessing the impact of an RBC transfusion on their symptoms of anemia because they lead highly sedentary lifestyles, which impede their ability to notice transient changes in energy. One participant stated

"it's hard for someone who has problems walking and using their arms to know if they're tired or not… If I was walking like I had been normally, I would notice it" [PIH-10].

3. When caregivers were present during the interview, it could be difficult to elicit only the patient perspective.

Literature asserts that caregiver burden in terminally ill patients with cancer is variable (97). Due to their experiences caring for someone with MDS, caregivers have unique insight into the well-being of patients. However, this can pose a challenge since caregivers may speak for the patient. In this study, two participants opted to complete the interview in the presence of their caregivers who sometimes helped them answer interview questions. Since none of the interview questions were of a sensitive nature and participants were able to verify responses, we were still able to use the information that was provided. We encountered a unique ethical issue in one interview when a caregiver divulged information that the interviewer felt the participant [PIH-04] was highly uncomfortable sharing. To protect patient privacy and confidentiality, that section of the interview was not included in the transcript.

4. The timing of interviews can affect the quality of data since patients seem to experience fluctuations in their levels of fatigue.

Interviews were conducted while participants were receiving their RBC transfusion, which was described as the point at which patients are the most fatigued. A number of participants were fatigued during the interview and may not have provided as in-depth responses to interview questions as they could have if the interview had been conduct sometime post-transfusion. However, since the results of this study show that the time of recovery from fatigue post-transfusion and the effect of transfusion vary, waiting for the transfusion to be completed may not have made any significant differences.

A final limitation of this study was lack of data saturation. Several categories such as patient experiences of fatigue, time to recovery after transfusion episode, and the effect of transfusions were well saturated. However, other categories, namely the side effects of transfusion and the impact of transfusion on mental state of mind (an important component of HR-QoL) were not saturated. Additional interviews may yield more in depth information about the changes in well-being participants experience in response to transfusion.

4.5 – Conclusion

The MDS population was initially identified as appropriate for studying the impact of old vs. fresh blood on post-transfusion HR-QoL using a crossover design. Patients with MDS seemed to be an ideal population considering they are frequent recipients of transfusion and those at low risk for AML are relatively stable over the time period likely to be required to complete the study. Furthermore, the prevalence of MDS is significantly higher than other populations (such as patients with ß-thalassemia or other hemoglobinopathies) that require chronic RBC transfusion therapy. The challenges of characterizing changes in well-being post-transfusion in this population suggest that the disease burden of MDS significant impedes participant's ability to assess short-term improvements in HR-QoL. Therefore, when selecting a HR-QoL tool as an appropriate outcome measure for the purposes of the crossover clinical trial, there are several considerations that should be made:

- The tool should have an emphasis on fatigue as it impacts function, since this is the primary symptom of anemia patients experience;
- The tool should have a short recall period to assess short-term transient changes in HR-QoL;
- The tool should be short in order to reduce responder burden, especially if there will be multiple assessments; and,
- The tool should be disease specific in order to measure changes that relevant to the MDS population.

CHAPTER 5: Final Considerations for Designing a Crossover Trial in Patients with MDS

The scope of this dissertation was to gather pilot data that could inform the design of a crossover RCT to determine if RBC storage time is associated with post-transfusion HR-QoL in patients with MDS. This dissertation incorporates a theoretical framework, a systematic review of the literature, a chart review of patients with MDS, and a qualitative study. The results from these pilot projects shed some insight on key questions and considerations that must be addressed in order to design the proposed crossover trial.

5.1 – Is it ethical to conduct a crossover trial where participants will be transfused with old RBCs?

In order to ethically justify randomizing participants to a particular treatment group, there must be clinical equipoise in the literature. Clinical equipoise "is the assumption that there is not one 'better' intervention present (for either the control or experimental group) during the design of a randomized controlled trial" (98). As previously discussed, RBCs undergo several changes during storage that can compromise the quality of the unit transfused. These storage lesions include: a decline in 2,3-DPG (17), ATP (19,22,23), and nitric oxide (19). Additionally, RBCs exhibit a number of changes in membrane composition resulting in decreased deformability, which impedes the cell's ability to change shape and travel through microcirculation (14,16). Finally, excess non-

transferrin bound iron from macrophages that clear senescent RBCs from older units post-transfusion may interact with reactive oxygen species to promote a pro-inflammatory response (38). The effects of these storage lesions may include compromised tissue oxygenation due to poor RBC circulation and O_2 off-loading, and/or pro-inflammatory responses. Ultimately, older RBCs with storage lesions are hypothesized to have reduced impact on patient QoL (74). The results from our systematic review of the literature identified only two age of blood trials where HR-QoL and fatigue were the primary outcome (52,53); neither demonstrated an association between age of blood and fatigue or HR-QoL. Both of these studies had several limitations that affect the generalizability and validity of the results. Most notably, a small sample size and the short time frame in which HR-QoL was assessed post-transfusion was a limitation for both studies (n=20 and 22). Specifically, the Seitalbach et al. (53), study administered the FACT-An prior to and 24-hours post-transfusion to measure change in HR-QoL (53). However, since the FACT-An has a recall period of 7 days (99), the tool may not be sensitive enough to capture such short term changes in HR-QoL post-transfusion. Conversely, the Mynster et al. (52), study was methodologically sound; however the results lack generalizability since impact of blood storage time on fatigue was studied in patients with anemia from non-acute gastrointestinal bleeding (52), a population that is much different than transfusion-dependent patients with MDS. Thus, there is clinical equipoise in the literature to ethically justify randomizing participants on a particular intervention.

Aside from equipoise, data from the chart review revealed that patients with MDS in Hamilton receive RBCs that are aged a median of 24 (IQR, 20, 31) days old; 18% of units issued were 35 days or older. Transfusion with older RBC units is standard practice in this population; however, if we give fresher red cells to patients this is still within the standard of care. Finally, since we propose to use a crossover design, participants will have a chance to receive both fresh and old RBCs. The proposed clinical trial will seek approval from the local research ethics board and participants will be required to provide informed consent prior to randomization.

5.2 – Are patients with MDS appropriate to study in a crossover RCT where HR-QOL is a primary outcome?

We considered studying patients with MDS to address the ultimate research question because they are frequently transfused with RBCs to manage anemia associated with bone failure syndrome. Since patients with MDS are a heterogeneous population, a crossover design was considered (rather than parallel) to reduce within patient variability and increase the efficiency of the trial; fewer participants would be required to detect an effect, should such an effect truly exist. To be suitable for a crossover RCT where the primary outcome of interest is a PRO, it is essential that this population have a stable prognosis over the projected timeframe of the trial (3-6 months) to ensure comparability across

treatment periods (62) and is able to assess transient changes in well-being post-transfusion.

Results from our chart review indicate that majority of patients with MDS who had at least 3 transfusion episodes within the 6 month observation period were considered to have a stable prognosis based on pre-specified criteria which related to: the frequency of transfusion; pre-transfusion Hb count; hospital admissions; and number of severe infections. Since the proposed trial will aim to recruit transfusion-dependent patients who are expected to require RBCs at least once every 3-6 weeks, participants may be in the study for a maximum of 3 months. Based on our results we do not anticipate that prognosis will worsen within this time frame for most patients using the selection criteria that were developed. Therefore, crossover trial would be appropriate to use to study this population.

The results of our chart review identified one feasibility concern: of the 51 patients screened, 21 were eligible for the chart review, of which only 14 had at least 3 transfusions over the observation period. Thus, only 14/51 (27%) patients scheduled in clinic were eligible and stable. These figures indicate that patient accrual may be slow and that the clinical trial will likely require a comprehensive recruitment strategy to maximize patient enrollment. Advertisements for the clinical trial and involvement from the clinic staff will be imperative.

Finally, while our qualitative study was not intended to determine the appropriateness of studying patients with MDS in a trial where HR-QoL is the

primary outcome, the results identified several challenges that may impede the ability of patients to assess transient changes in HR-QoL as a response to transfusion. These challenges include: chronic co-morbidities compounded with increased age; sedentary lifestyles; caregiver influence; and, timing of interviews. Ultimately, for patients who had severe disease states, burden of MDS symptoms seemed to impede their ability to self-complete HR-QoL measures or even assess transient changes as a response to transfusion. Therefore, patients who are low to intermediate-1 risk MDS may be ideal for the purposes of the clinical trial.

5.3 – What outcome measure(s) should be used to assess change in patient well-being as a response to RBC transfusion?

While there are a number of tools that have previously been used to study QoL in patients with MDS, only a few may be appropriate to study changes in HR-QoL as a response to transfusion. The ideal HR-QoL tool should be a short HR-QoL tool that is disease specific to measure MDS related symptoms and fatigue over a recall period of no more than 7 days. Pinchon et al. (100), conducted a systematic review of the literature to identify HR-QoL tools that have been previously used in the population; these are summarized in Table 5.

Table 5: Validated tools used to study quality of life in patients with MDS

Tool	Validated in Population	Type	Domains/Scales (Items)	Recall Period
European Organization for Research and Treatment of Cancer Core Quality of Life Questionnaire (EORTC QLQ-C30) (101)	Lung cancer	Generic tool for Neoplasms	5 functional scales: physical, role, cognitive, emotional, and social. 3 symptom scales: fatigue, pain, and nausea and vomiting (30 items)	7 days
Short Form 36 (SF-36) (102)	General Population	Generic tool for all diseases	8 scales: vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health (36 items)	Last 4 weeks, acute version is 7 days
The Multidimensional Fatigue Inventory (MFI) (103)	Heterogeneous cancer patients treated with radiotherapy	Symptom assessment tool for cancer related fatigue	5 dimensions: general fatigue; physical fatigue; reduced motivation; reduced activity; and mental fatigue (20 items)	"Lately"
EuroQol 5 Dimension (EQ-5D) (104–106)	Diverse population with various chronic conditions	Generic tool to assess health outcomes across all diseases	5 scales: mobility, self-care, usual activities, pain/discomfort, anxiety/depression. Also includes a visual analog scale	Today
Brief Fatigue Inventory (BFI) (107)	Outpatients with cancer	Generic symptom assessment tool for fatigue	No subscales (9 items)	24 hours

Tool	Validated in Population	Type	Domains/Scales (Items)	Recall Period
Functional Assessment of Cancer Therapy (FACT) (108)	Heterogeneous group of cancer patients	Generic for neoplasms	4 scales: physical, social/family, emotional, and function well-being (27 items)	7 days
Quality of Life-E (QOL-E) (109)	Patients with MDS	Disease specific	5 domains: physical, functional, social, fatigue, and MDS related QoL	7 days
Linear Analog Self-Assessment (LASA) (110)	Patients with breast cancer	Generic for neoplasms	Varies depending on the tool, but similar to the VAS, only consists of a few items that rated on a 10 point scale	7 days
Mental Health Inventory (MHI) (111)	General population	Generic	6 subscales: Anxiety, depression, loss of behavioral/ emotional control, general positive affect, emotional ties, and life satisfaction (38 items)	Past month

Only one study identified by the review (100) used both the SF-36 and the MFI (1) to capture HR-QoL in patients with MDS; another used the MHI (112). The SF-36 and MHI are both generic tools used to measure well-being across multiple populations with a recall period of one month; thus we felt they were not appropriate to use for the purposes of the crossover trial since they lacked sensitivity. Although the MFI is more specific than the SF-36 and the MHI with a much shorter recall period of 7 days, it is specific to the impact on fatigue and patients with MDS may experience additional disease related symptoms.

Pinchon et al's (100) review also identified two studies that used the LASA scale (81,94), which is a short generic tool for cancer patients with a 7 day recall period. The three subscales are highly relevant to the outcomes we are looking to capture: energy levels, daily activity, and overall QoL. However, since QoL in each subscale is rated using a 100-mm analog scale for responses (94,113), important information within each subscale would not be captured; thus a more specific tool would be more appropriate.

The EORTC QLQ C30 (76,92,112,114–116), EQ-5D (1,117), and variations of the FACT tools (81,93–95,117–120) seemed to be the most frequently used instruments to measure HR-QoL in this population (100). After reviewing each instrument, the QLQ-C30 and FACT-An seemed to be most relevant since they focused on the burden of anemia related symptoms. However, there were several items on each tool that lacked relevance to the research question, which may dilute the treatment effect (i.e. "did you feel depressed?", "Have you had difficulty remembering things?", and "did you feel irritable?" [QLQ-C30]). Additionally, the social/family and emotional well-being domains of the FACT tools did not seem highly relevant to the research question.

Therefore, we considered using the QOL-E and the BFI to measure changes in HR-QoL as a response to transfusion. While the QOL-E is not a short tool, it is the only MDS specific instrument that has been validated in transfusion dependent patients (109). Furthermore, the tool would be applied twice per transfusion episode, once at baseline prior to receiving transfusion and one 7

days after transfusion. The BFI is a symptom assessment tool used to assess the severity of fatigue in patients with cancer. Items on the tool assess current fatigue as well as fatigue during the past 24 hours. The short recall period allows patients to rate rapid changes in fatigue and energy levels that tools such as the QOL-E may miss. This is of particular importance since we hypothesize the effect of transfusion is observed immediately after the transfusion and for up to a day later. The utility and feasibility of using these tools in the MDS population require further work to estimate parameters that can inform power and sample size calculations.

5.4 – Conclusion

Quality of life is a multifaceted and complex construct. Health-related QoL is the impact of disease and treatment burden on QoL (121). The use of HR- QoL measures in symptom management trials are appropriate when "there is a desire to gauge the importance patients assign to symptom relief, an interest in gaining information about offsetting treatment impact (side effects), and an interest in testing a compelling conceptual model of the relationship between symptoms and quality of life" (122). Cella et al. (122), asserts that the improvement of HR-QoL is a relevant benefit in symptom management trials, especially if there is an absence of significant therapeutic effect. Furthermore, the introduction of symptom management can introduce bothersome side effects that may also impact patient HR-QoL (122).

Patients with MDS experience poor quality of life on account of various disease-related symptoms, of which fatigue is significant factor (75). Allogenic stem cell transplant remains the only curative option for patients with MDS (5,66). However, since stem cell transplants carry a high risk of mortality and relapse, only a specific group of patients (usually under 65-70 years old with good performance status) are considered to be eligible candidates (123). While a number of alternative treatments to stimulate the bone marrow are available, treatment failure is inevitable (109); thus management of patient symptoms and improvement of QoL is a priority for patients with MDS.

RBC transfusion is one of few supportive care interventions in this population. While transfusion dependency has been previously studied and found to be associated with poor QoL (7) changes in HR-QoL as a direct response to transfusion have not been directly studied. The results of this trial can demonstrate the importance of RBC transfusion in managing patient symptoms. The findings of this trial will inform strategies around transfusion as supportive care to improve patient QoL.

REFERENCES

1. Jansen A, Essink-Bot M, Beckers E, Hop W, Schipperus M, Van Rhenen D. Quality of life measurement in patients with transfusion-dependent myelodysplastic syndromes. Br J Haematol. 2003;121(2):270–4.

2. Flechtner H, Bottomley A. Fatigue and quality of life: lessons from the real world. Oncologist. 2003;8(S1):5–9.

3. Tsiara S, Chaidos A, Gouva M, Christou L, Panteli K, Kapsali E, et al. Successful treatment of refractory anemia with a combination regimen containing recombinant human erythropoietin, low-dose methylprednisolone and nandrolone. J Exp Clin cancer Res. 2004;23(1):47–52.

4. Hofmann W, Koeffler H. Myelodysplastic syndrome. Annu Rev Med. 2005;56:1–16.

5. Killick SB. Myelodysplastic disorders. Medicine. 2009;37(4):186–9.

6. Oliva EN, Finelli C, Santini V, Poloni A, Liso V, Cilloni D, et al. Quality of life and physicians' perception in myelodysplastic syndromes. Am J Blood Res. 2012;2(2):136–47.

7. Platzbecker U, Hofbauer LC, Ehninger G, Hölig K. The clinical, quality of life, and economic consequences of chronic anemia and transfusion support in patients with myelodysplastic syndromes. Leuk Res. 2012;36(5):525–36.

8. Cohen B, Matot I. Aged erythrocytes: a fine wine or sour grapes? Br J Anaesth. 2013; 111(Suppl 1):i62–70.

9. Hébert PC, Chin-Yee I, Fergusson D, Blajchman M, Martineau R, Clinch J, et al. A pilot trial evaluating the clinical effects of prolonged storage of red cells. Anesth Analg. 2005;100(5):1433–8.

10. Barty RL, Gagliardi K, Owens W, Lauzon D, Scheuermann S, Liu Y, et al. A benchmarking program to reduce red blood cell outdating: implementation, evaluation, and a conceptual framework. Transfusion. 2015;55(7):1621-1627.

11. ORBCON. Inventory Management Tool Kit [Internet]. 2014 [cited 2015 Jul 16]. Available from: http://transfusionontario.org/en/cmdownloads/categories/inventory-management-toolkits/

12. Callum JL, Lin Y, Pinkerton PH, Karkouti K, Pendergrast JM, Robitaille N, et al. Bloody Easy 3: Blood Transfusions, Blood Alternatives and Transfusion Reactions. Third. Toronto, Ontario: ORBCON; 2011.

13. Matot I, Katz M, Pappo O, Zelig O, Corchia N, Yedgar S, et al. Resuscitation with aged blood exacerbates liver injury in a hemorrhagic rat model. Crit Care Med. 2013;41(3):842–9.

14. Almizraq R, Tchir JDR, Holovati JL, Acker JP. Storage of red blood cells affects membrane composition, microvesiculation, and in vitro quality. Transfusion. 2013;53(10):2258–67.

15. Vandromme MJ, McGwin G, Weinberg JA. Blood transfusion in the critically ill: does storage age matter? Scand J Trauma Resusc Emerg Med. 2009;17:35

16. Frank SM, Abazyan B, Ono M, Hogue CW, Cohen DB, Berkowitz DE, et al. Decreased erythrocyte deformability after transfusion and the effects of erythrocyte storage duration. Anesth Analg. 2013;116(5):975–81.

17. Almac E, Ince C. The impact of storage on red cell function in blood transfusion. Best Pract Res Clin Anaesthesiol. 2007;21(2):195–208.

18. Hess JR. Measures of stored red blood cell quality. Vox Sang. 2014; 107(1):1-9.

19. Kor DJ, Buskirk CM Van, Gajic O. Red blood cell storage lesion. Bosn J Basic Med Sci. 2009;9(Supplement 1):S21–7.

20. Van de Watering L. Red cell storage and prognosis. Vox Sang. 2011; 100(1):36–45.

21. Heaton A, Keegan T, Holme S. In vivo regeneration of red cell 2,3-diphosphoglycerate following transfusion of DPG-depleted AS-1, AS-3 and CPDA-1 red cells. Br J Haematol. 1989;71(1):131–6.

22. Raat NJ, Verhoeven AJ, Mik EG, Gouwerok CW, Verhaar R, Goedhart PT, et al. The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. Crit Care Med. 2005;33(1):39–45; discussion 238–9.

23. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci U S A. 2007; 104(43):17063–8.

24. Cao Z, Bell JB, Mohanty JG, Nagababu E, Rifkind JM. Nitrite enhances RBC hypoxic ATP synthesis and the release of ATP into the vasculature: a new mechanism for nitrite-induced vasodilation. Am J Physiol Hear Circ Physiol. 2009;297:1494–503.

25. Dietrich HH, Ellsworth ML, Sprague RS, Dacey RG. Red blood cell regulation of microvascular tone through adenosine triphosphate. Am J Physiol Heart Circ Physiol. 2000; 278(4):H1294–8.

26. Harrington LS, Evans RJ, Wray J, Norling L, Swales KE, Vial C, et al. Purinergic 2X 1 Receptors Mediate Endothelial Dependent Vasodilation to ATP. Mol Pharmacol. 2007;72(5):1132–6.

27. Raat NJH, Ince C. Oxygenating the microcirculation: the perspective from blood transfusion and blood storage. Vox Sang. 2007;93(1):12–8

28. Katsuki S, Arnold W, Mittal C, Murad F. Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. J Cyclic Nucleotide Res. 1977;3(1):23–35.

29. Reynolds JD, Ahearn GS, Angelo M, Zhang J, Cobb F, Stamler JS. S-nitrosohemoglobin deficiency: a mechanism for loss of physiological activity in banked blood. Proc Natl Acad Sci USA. 2007;104(43):17058–62.

30. Crawford JH, Isbell TS, Huang Z, Shiva S, Chacko BK, Schechter AN, et al. Hypoxia, red blood cells, and nitrite regulate NO-dependent hypoxic vasodilation. Blood. 2006; 107(2):566–74.

31. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion: role of red blood cell breakdown. Transfusion. 2011;51(4):844–51.

32. Martínez-Ruiz A, Cadenas S, Lamas S. Nitric oxide signaling: classical, less classical, and nonclassical mechanisms. Free Radic Biol Med. 2011;51(1):17–29.

33. Diez-Silva M, Dao M, Han J, Lim CT, Suresh S. Shape and Biomechanical Characteristics of Human Red Blood Cells in Health and Disease. MRS Bull. 2010;35(5):382–8.

34. Gov NS, Safran SA. Red blood cell membrane fluctuations and shape controlled by ATP-induced cytoskeletal defects. Biophys J. 2005;88(3):1859–74

35. Cluitmans JC, Hardeman MR, Dinkla S, Brock R, Bosman G. Red blood cell deformability during storage: towards functional proteomics and metabolomics in the Blood Bank. Blood Transfus. 2012;10(Suppl 2):12–8.

36. Bessos H, Seghatchian J. Red cell storage lesion: the potential impact of storage-induced CD47 decline on immunomodulation and the survival of leucofiltered red cells. Transfus Apher Sci. 2005;32(2):227–32.

37. Oldenborg P, Zheleznyak A, Fang YF, Lagenaur C, Gresham H, Lindberg F. Role of CD47 as a Marker of Self on Red Blood Cells. Science. 2000;288(5473):2051–4.

38. Hod EA, Spitalnik SL. Stored red blood cell transfusions: Iron, inflammation, immunity, and infection. Transfus Clin Biol. 2012;19(3):84–89

39. Gilson CR, Kraus TS, Hod EA, Hendrickson JE, Spitalnik SL, Hillyer CD, et al. A novel mouse model of red blood cell storage and posttransfusion in vivo survival. Transfusion. 2009;49(8):1546–53.

40. Hod EA, Zhang N, Sokol SA, Wojczyk BS, Francis RO, Ansaldi D, et al. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. Blood. 2010;115(21):4284–92.

41. Hod EA, Brittenham GM, Billote GB, Francis RO, Ginzburg YZ, Hendrickson JE, et al. Transfusion of human volunteers with older, stored red blood cells produces extravascular hemolysis and circulating non-transferrin-bound iron. Blood. 2011;118(25):6675–82

42. Patrick D, Guyatt G, Acquadro C. Patient-reported outcomes. In: Higgins J, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011] The Cochrane Collaboration; 2011. Available from: www.cochrane-handbook.org.

43. Baron-Epel O, Dushenat M, Friedman N. Evaluation of the consumer model: relationship between patients' expectations, perceptions and satisfaction with care. Int J Qual Health Care. 2001;13(4):317–23.

44. Deshpande PR, Rajan S, Sudeepthi BL, Abdul Nazir CP. Patient-reported outcomes: A new era in clinical research. Perspect Clin Res. 2011;2(4):137–4

45. Cleeland CS. Symptom burden: multiple symptoms and their impact as patient-reported outcomes. J Natl Cancer Inst Monogr. 2007;77030(37):16–21.

46. Lelubre C, Piagnerelli M, Vincent J-L. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? Transfusion. 2009; 49(7):1384–94.47.

47. Lelubre C, Vincent J-L. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. Crit Care. 2013;17(2):R66

48. Vamvakas EC. Meta-analysis of clinical studies of the purported deleterious effects of "old" (versus "fresh") red blood cells: are we at equipoise? Transfusion. 2010;50(3):600–10.

49. Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: a meta-analysis. Transfusion. 2012;52(6):1184–95.

50. Higgins J, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [Updated March 2011]. The Cochrane Collaboration; 2011. Available from: www.cochrane-handbook.org

51. Cochrane Hematological Malignancies Group (CHMG), Naumann. How to develop a search strategy for a Cochrane Review [Internet]. 2007. Available from: http://chmg.cochrane.org/sites/chmg.cochrane.org/files/uploads/How to develop a search strategy-support-manual.pdf

52. Mynster T, Dziegiel MH, Kofoed K. No Early Effect of Storage Time of Transfused Red Blood Cells on Fatigue and Plasma Cytokines in Patients with Anaemia from Non-Acute Gastrointestinal Bleeding. Transfus Med Hemotherap. 2007;34(6):440–5.

53. Seitelbach M, Chin-Yee I, Kinney J, Chia J, Ormond K, Mahon J, et al. Age of Blood Does Not Affect Quality of Life and Hemoglobin: N-of-1 Trials in Transfusion-Dependent Patients. Blood. 2011; 118(21):ABSTRACT 3369.

54. Australian and New Zealand Intensive Care Research Centre. Standard Issue Transfusion Versus Fresher Red Blood Cell Use in Intensive Care- A Randomised Controlled Trial (TRANSFUSE). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). 2000- [cited 2014 Aug 20]. Available from: http://clinicaltrials.gov/show/NCT01638416 NLM Ifentifier: NCT01638416.

55. Guyatt G, Jaeschke R, McGinn T. Advanced Topics in the Validity of Therapy Trials: N-of-1 Randomized Controlled Trials. In: Guyatt G, Drummond R, Meade MO, Cook DJ, editors. Users' Guide to the Medical Literature: A manual for evidence-based practice. 2nd Edition. New York: McGraw-Hill Companies; 2008. p.179–208.

56. Guyatt G, Sackett D, Adachi J, Roberts R, Chong J, Rosenbloom D, et al. A clinician's guide for conducting randomized trials in individual patients. Can Med Assocation J. 1988; 139(6):497–503.

57. Sedgwick P. What is an "n-of-1" trial? BMJ. 2014;348:g2674–g2674.

58. Zucker DR, Ruthazer R, Schmid CH. Individual (N-of-1) trials can be combined to give population comparative treatment effect estimates: methodologic considerations. J Clin Epidemiol. 2010;63(12):1312–23.

59. Sibbald B, Roberts C. Understanding controlled trials: Crossover trials. BMJ. 1998;316(7146):1719–20.

60. Cleophas TJ, de Vogel EM. Crossover studies are a better format for comparing equivalent treatments than parallel-group studies. Pharm World Sci. 1998;20(3):113–7.

61. Jadad AR. Randomised controlled trials: A user's guide. London: BMJ Books; 1998.

60. Mills EJ, Chan AW, Wu P, Vail A, Guyatt GH, Altman DG. Design, analysis, and presentation of crossover trials. Trials. 2009;10:27

61. Stolberg HO, Norman G, Trop I. Fundamentals of Clinical Research: Randomized Controlled Trials. AJR. 2004;183(December):1539–44.

62. Grady D, Cummings SR, Hulley SB. Alternative Trial Designs and Implementation Issues. In: Hulley SB, Cummings SR, Browner WS, Grady DG, Newman TB, editors. Designing Clinical Research. Second. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 163–82.

65. Nason M, Follmann D. Design and analysis of crossover trials for absorbing binary endpoints. Biometrics. 2010;66(3):958–65.

66. Garcia-Manero G. CME Information: Myelodysplastic syndromes: 2014 update on diagnosis, risk stratification and management. Am J Hematol. 2014; 89(1):97–108.

67. Ma X. Epidemiology of myelodysplastic syndromes. Am J Med. 2012; 125(7 Suppl):S2–5

68. Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events, Version 3.0, DCTD, NCI, NIH, DHHS March 31, 2003 (http://ctep.cancer.gov), Publish Date: August 9, 2006

69. Bejar R. Prognostic models in myelodysplastic syndromes. Hematol Am Soc Hematol Educ Progr 2013; 2013:504–10.

70. Yoshida Y. Characteristics and Pathology of Myelodysplastic Syndromes. The Oncologist. 1996; 1:284–7.

71. Cogle CR, Craig BM, Rollison DE, List AF. Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: high number of uncaptured cases by cancer registries. Blood. 2011; 117(26):7121–5

72. Neukirchen J, Schoonen WM, Strupp C, Gattermann N, Aul C, Haas R, et al. Incidence and prevalence of myelodysplastic syndromes: data from the Düsseldorf MDS-registry. Leuk Res. 2011;35(12):1591–6.

73. Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997; 89(6):2079–88.

74. Lacroix J, Hebert PC, Fergusson DA, Tinmouth A, Cook DJ, Marshall JC, et al. Age of Transfused Blood in Critically Ill Adults. N Engl J Med. 2015; 372(15):1410–8.

75. Thomas ML, Crisp N, Campbell K. The importance of quality of life for patients living with myelodysplastic syndromes. Clin J Oncol Nurs. 2012; 16 Suppl:47–57.

76. Caocci G, Baccoli R, Ledda A, Littera R, La Nasa G. A mathematical model for the evaluation of amplitude of hemoglobin fluctuations in elderly anemic patients affected by myelodysplastic syndromes: correlation with quality of life and fatigue. Leuk Res. 2007; 31(2):249–52

77. Goss TF, Szende A, Schaefer C, Totten PJ, Knight R, Jädersten M, et al. Cost effectiveness of lenalidomide in the treatment of transfusion-dependent myelodysplastic syndromes in the United States. Cancer Control. 2006; 13 Suppl:17–25.

78. Goldberg SL, Chen E, Corral M, Guo A, Mody-Patel N, Pecora AL, et al. Incidence and clinical complications of myelodysplastic syndromes among United States Medicare beneficiaries. J Clin Oncol. 2010; 28(17):2847–52.

79. Gupta P, LeRoy SC, Luikart SD, Bateman A, Morrison VA. Long-term blood product transfusion support for patients with myelodysplastic syndromes (MDS): cost analysis and complications. Leuk Res. 1999; 23(10):953–9.

80. Stiegler G, Sperr W, Lorber C, Fabrizii V, Höcker P, Panzer S. Red cell antibodies in frequently transfused patients with myelodysplastic syndrome. Ann Hematol. 2001; 80(6):330–3.

81. Steensma DP, Heptinstall K V, Johnson VM, Novotny PJ, Sloan JA, Camoriano JK, et al. Common troublesome symptoms and their impact on quality of life in patients with myelodysplastic syndromes (MDS): results of a large internet-based survey. Leuk Res. 2008;32(5):691–8

82. Malcovati L, Porta MG Della, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic

syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005; 23(30):7594–603.

83. Walker R. Applied Qualitative Research. In: Lewis-Beck MS, Bryman A, Liao TF, editors. The SAGE Encyclopedia of Social Science Research Methods. 2004; 93–5.

84. Guest GS, Namey EE, Mitchell ML. Chapter 1: Qualitative Research Defining and Designing. In: Collecting Qualitative Data: A field Manual for Applied Research. Thousand Oaks, CA: SAGE Publications, Inc; 2013.

85. Creswell J. Designing a Qualitative Study. In: Laughton D, Axelsen DE, editors. Qualitative Inquiry and Research Desgin. Thousand Oaks, London, and New Delhi: SAGE Publications, Inc; 1998. p.13–26.

86. Bowling A. Qualitative and combined research methods, and their analysis. In: Research Methods in health: Investigating Health and Health Services. 1st Edition. Philadelphia: Open University Press; 1997. p.311–3.

87. Vaismoradi M, Turunen H, Bondas T. Content analysis and thematic analysis: Implications for conducting a qualitative descriptive study. Nurs Health Sci. 2013; 15(3):398–405.

88. Sandelowski M. Sample size in qualitative research. Res Nurs Health. 1995; 18(2):179–83.

89. Sandelowski M. Theoretical Saturation. In: Given, L. The Sage Encyclopedia of Qualitative Research Methods. Thousand Oaks: SAGE Publications, Inc; 2008. p. 876–7.

90. Sandelowski M. Whatever happened to qualitative description? Res Nurs Health. 2000; 23(4):334–40

91. Oliva EN, Dimitrov BD, Benedetto F, D'Angelo A, Nobile F. Hemoglobin level threshold for cardiac remodeling and quality of life in myelodysplastic syndrome. Leuk Res. 2005; 29(10):1217–9.

92. Hellström-Lindberg E, Gulbrandsen N, Lindberg G, Ahlgren T, Dahl IMS, Dybedal I, et al. A validated decision model for treating the anaemia of myelodysplastic syndromes with erythropoietin + granulocyte colony-stimulating factor: significant effects on quality of life. Br J Haematol. 2003; 120(6):1037–46.

93. Spiriti MAA, Latagliata R, Niscola P, Cortelezzi A, Francesconi M, Ferrari D, et al. Impact of a new dosing regimen of epoetin alfa on quality of life and anemia in patients with low-risk myelodysplastic syndrome. Ann Hematol. 2005; 84(3):167–76.

94. Stasi R, Abruzzese E, Lanzetta G, Terzoli E, Amadori S. Darbepoetin alfa for the treatment of anemic patients with low- and intermediate-1-risk myelodysplastic syndromes. Ann Oncol. 2005; 16(12):1921–7.

95. Casadevall N, Durieux P, Dubois S, Hemery F, Lepage E, Quarré M-C, et al. Health, economic, and quality-of-life effects of erythropoietin and granulocyte colony-stimulating factor for the treatment of myelodysplastic syndromes: a randomized, controlled trial. Blood. 2004;104(2):321–7

96. Cheville AL, Basford JR, Dos Santos K, Kroenke K. Symptom burden and comorbidities impact the consistency of responses on patient-reported functional outcomes. Arch Phys Med Rehabil. 2014; 95(1):79–86.

97. Goldstein NE, Concato J, Fried TR, Kasl S V, Johnson-Hurzeler R, Bradley EH. Factors associated with caregiver burden among caregivers of terminally ill patients with cancer. J Palliat Care. 2004; 20(1):38–43.

98. Cook C, Sheets C. Clinical equipoise and personal equipoise: two necessary ingredients for reducing bias in manual therapy trials. J Man Manip Ther. 2011; 19(1):55–7.

99. Yellen SB, Cella DF, Webster K, Blendowski C, Kaplan E. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. J Pain Symptom Manage. 1997; 13(2):63–74.

100. Pinchon DJ, Stanworth SJ, Dorée C, Brunskill S, Norfolk DR. Quality of life and use of red cell transfusion in patients with myelodysplastic syndromes. A systematic review. Am J Hematol. 2009; 84(10):671–7

101. Aaronson NK, Ahmedzai S, Bergman B, Bullinger M, Cull a, Duez NJ, et al. The European Organization for Research and Treatment of Cancer QLQ-C30: a quality-of-life instrument for use in international clinical trials in oncology. J Natl Cancer Inst. 1993; 85(5):365–76.

102. McHorney CA, Ware JE, Raczek AE. The MOS 36-item short-form health survey SF-36 II pyschometric and clinical tests of validity in measuring physical and mental health constructs. Med Care. 1993; 31(3):247–63.

103. Smets E, Garssen B, Bonke B, De Haies J. The multidimensional fatigue inventory (MFI) psychometric qualities of an instrment to assess fatigue. J Psychosom Res. 1995; 39(5):315–25.

104. Hurst NP, Kind P, Ruta D, Hunter M, Stubbings A. Measuring health-related quality of life in rheumatoid arthritis: validity, responsiveness and reliability of EuroQol (EQ-5D). Br J Rheumatol. 1997; 36(5):551–9

105. Badia X, Schiaffino A, Alonso J, Herdman M. Using the EuroQol 5-D in the Catalan general population: feasibility and construct validity. Qual Life Res. 1998; 7(4):311–22.

106. Group E. EuroQol-a new facility for the measurement of health-related quality of life. Health Policy (New York) [Internet]. 1990 Dec [cited 2014 Dec 27];16(3):199–208. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10109801

107. Mendoza TR, Wang XS, Cleeland CS, Morrissey M, Johnson BA, Wendt JK, et al. The rapid assessment of fatigue severity in cancer patients: use of the Brief Fatigue Inventory. Cancer. 1999; 85(5):1186–96.

108. Cella DF, Tulsky DS, Gray G, Sarafian B, Linn E, Bonomi A, et al. The Functional Assessment of Cancer Therapy scale: development and validation of the general measure. J Clin Oncol. 1993; 11(3):570–9

109. Oliva EN, Nobile F, Dimitrov BD. Development and validation of QOL-E© instrument for the assessment of health-related quality of life in myelodysplastic syndromes. Cent Eur J Med. 2013; 8(6):835–44.

110. Priestman TJ, Baum M. Evaluation of quality of life in patients receiving treatment for advanced breast cancer. Lancet (London, England). 1976; 1(7965):899–900.

111. Veit CT, Ware JE. The structure of psychological distress and well-being in general populations. J Consult Clin Psychol. 1983; 51(5):730–42.

112. Kornblith AB, Herndon JE, Silverman LR, Demakos EP, Odchimar-Reissig R, Holland JF, et al. Impact of azacytidine on the quality of life of patients

with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. J Clin Oncol. 2002; 20(10):2441–52

113. Coates A, Dillenbeck CF, McNeil DR, Kaye SB, Sims K, Fox RM, et al. On the receiving end--II. Linear analogue self-assessment (LASA) in evaluation of aspects of the quality of life of cancer patients receiving therapy. Eur J Cancer Clin Oncol. 1983;19(11):1633–7.

114. Atoyebi W, Bywater L, Rawlings L, Brunskill S, Littlewood TJ. Treatment of myelodysplasia with oral cyclosporin. Clin Lab Haematol. 2002; 24(4):211–4.

115. Giagounidis AAN, Haase S, Germing U, Schlegelberger B, Wilkens L, Büsche G, et al. Treatment of myelodysplastic syndrome with isolated del(5q) including bands q31-q33 with a combination of all-trans-retinoic acid and tocopherol-alpha: a phase II study. Ann Hematol. 2005; 84(6):389–94.

116. Kantarjian H, Giles F, List A, Lyons R, Sekeres M a, Pierce S, et al. The incidence and impact of thrombocytopenia in myelodysplastic syndromes. Cancer. 2007;109(9):1705–14.

117. Gabrilove J, Paquette R, Lyons RM, Mushtaq C, Sekeres MA, Tomita D, et al. Phase 2, single-arm trial to evaluate the effectiveness of darbepoetin alfa for correcting anaemia in patients with myelodysplastic syndromes. Br J Haematol. 2008;142(3):379–93.

118. Meyers CA, Albitar M, Estey E. Cognitive impairment, fatigue, and cytokine levels in patients with acute myelogenous leukemia or myelodysplastic syndrome. Cancer. 2005; 104(4):788–93.

119. Balleari E, Rossi E, Clavio M, Congiu A, Gobbi M, Grosso M, et al. Erythropoietin plus granulocyte colony-stimulating factor is better than erythropoietin alone to treat anemia in low-risk myelodysplastic syndromes: results from a randomized single-centre study. Ann Hematol. 2006; 85(3):174–80.

120. Clavio M, Nobili F, Balleari E, Girtler N, Ballerini F, Vitali P, et al. Quality of life and brain function following high-dose recombinant human erythropoietin in low-risk myelodysplastic syndromes: a preliminary report. Eur J Haematol. 2004; 72(2):113–20

121. Thomas ML. Quality of life and psychosocial adjustment in patients with myelodysplastic syndromes. Leuk Res. 1998; 22 (Suppl 1):S41–7.

122. Cella D, Wagner L, Cashy J, Hensing TA, Yount S, Lilenbaum RC. Should health-related quality of life be measured in cancer symptom management clinical trials? Lessons learned using the functional assessment of cancer therapy. J Natl Cancer Inst Monogr. 2007; (37):53–60.

123. Meers S. The Myelodysplastic Syndromes: The Era Of Understanding. Eur J Haematol. 2015; 94(5):397-90

APPENDIX A: Complete Search Strategy of the Age of Blood Literature with Patient Reported Outcomes

A1) Medline Search Strategy

1 Erythrocytes/ or Erythrocyt*.mp. (201861)
2 Red blood cell.mp. (22101)
3 RBC.mp. (16959)
4 Erythrocyte Indices/ or Red Cell.mp. (27498)
5 erythrocyte transfusion.mp. or exp Erythrocyte Transfusion/ (6321)
6 Erythrocyte Transfusion/ae [Adverse Effects] (1074)
7 Blood Component Transfusion/ (2800)
8 Blood Transfusion/ or packed red cell product.mp. (52448)
9 red cell concentrat*.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] (583)
10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 (272495)
11 Aging/ or age of blood.mp. (188848)
12 Blood Storage Time.mp. (30)
13 Blood Preservation/ (10342)
14 Blood Preservation/ae [Adverse Effects] (247)
15 Time Factors/ (992995)
16 storage time.mp. (3065)
17 storage duration.mp. (310)
18 erythrocyte aging.mp. or Erythrocyte Aging/ (3753)
19 storage lesion.mp. (256)
20 old blood.mp. (268)
21 fresh blood.mp. (1418)
22 aged blood.mp. (65)
23 stored blood.mp. (937)
24 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 (1186849)
25 10 and 24 (26465)
26 Quality of life.mp. or "Quality of Life"/ (192234)
27 exp Patient Satisfaction/ or Patient reported outcomes.mp. or exp Treatment Outcome/ (685186)
28 patient important outcomes.mp. (131)
29 Self Report/ or Questionnaires/ or Self report*.mp. (358429)
30 Well being.mp. (39657)
31 exp Health Status/ or wellness.mp. (107153)
32 time trade off.mp. (771)
33 Quality adjusted life years.mp. or Quality-Adjusted Life Years/ (8453)
34 treatment satisfaction.mp. (1532)
35 treatment burden.mp. (224)

36 exp Fatigue/ or Mental Fatigue/ or fatigue.mp. (67140)
37 exhaustion.mp. (13092)
38 tiredness.mp. (2615)
39 Activities of Daily Living.mp. or "Activities of Daily Living"/ (56387)
40 hemoglobin.mp. or exp Hemoglobins/ (142363)
41 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 (1445290)
42 exp *Plasma/ (7340)
43 *Platelet Transfusion/ (2417)
44 exp *Genotype/ or exp *Genotyping Techniques/ (53555)
45 42 or 43 or 44 (63210)
46 10 and 24 and 41 (4771)
47 46 not 45 (4725)
48 "red cell".ti. or "red cell".ab. (24268)
49 "red cells".ti. or "red cells".ab. (15460)
50 "red blood cell".ti. or "red blood cell".ab. (22031)
51 "red blood cells".ti. or "red blood cells".ab. (33470)
52 "transfused blood".ti. or "transfused blood".ab. (678)
53 "transfused cells".ti. or "transfused cells".ab. (82)
54 48 or 49 or 50 or 51 or 52 or 53 (82365)
55 47 and 54 (1828) limit 55 to (english language and humans) (1320)

A2) Embase Search Strategy <1974 to March 26, 2014>

1 exp erythrocyte/ or erythrocyt*.mp. (267332)
2 red blood cell.mp. (28206)
3 RBC.mp. (24428)
4 Red cell.mp. (30784)
5 erythrocyte transfusion.mp. or exp erythrocyte transfusion/ (14028)
6 exp blood transfusion/ or exp transfusion/ or exp blood autotransfusion/ or exp blood component therapy/ (224736)
7 packed red cell product.mp. (0)
8 exp erythrocyte concentrate/ (5042)
9 red cell concentrat*.mp. (901)
10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 (479218)
11 Age of blood.mp. or aging/ (190893)
12 exp blood storage/ or exp erythrocyte preservation/ (10948)
13 blood storage time.mp. (39)
14 time/ (365207)
15 storage time.mp. (3880)
16 storage duration.mp. (447)
17 erythrocyte aging.mp. or erythrocyte lifespan/ (3226)
18 cell damage/ or storage lesion.mp. (27515)
19 old blood.mp. (382)
20 fresh blood.mp. (2019)

21 aged blood.mp. (87)
22 stored blood.mp. (1251)
23 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 (597772)
24 10 and 23 (21162)
25 quality of life.mp. or "quality of life"/ (299742)
26 patient important outcomes.mp. (158)
27 questionnaire/ or treatment outcome/ or patient reported outcome.mp. or outcomes research/ or self report/ (1093247)
28 well being.mp. or exp wellbeing/ (67625)
29 wellness.mp. (6183)
30 exp health status/ (135509)
31 time trade off.mp. or quality adjusted life year/ (12492)
32 exp patient satisfaction/ or treatment satisfaction.mp. (85336)
33 treatment burden.mp. (403)
34 exp Fatigue Severity Scale/ or exp Fatigue Impact Scale/ or fatigue.mp. or exp fatigue/ (153380)
35 exhaustion/ or exhaustion.mp. (16577)
36 tiredness.mp. (4000)
37 activities of daily living.mp. or daily life activity/ (60185)
38 exp hemoglobin/ or hemoglobin.mp. (231567)
39 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 (1849228)
40 10 and 23 and 39 (4218)
41 exp *fresh frozen plasma/ (1172)
42 *thrombocyte transfusion/ (2851)
43 exp *genotyping technique/ or exp *genotype/ (25661)
44 41 or 42 or 43 (29658)
45 40 not 44 (4179)
46 "red cell".ti. or "red cell".ab. (29691)
47 "red cells".ti. or "red cells".ab. (18926)
48 "red blood cell".ti. or "red blood cell".ab. (27271)
49 "red blood cells".ti. or "red blood cells".ab. (40361)
50 "transfused blood".ti. or "transfused blood".ab. (963)
51 "transfused cells".ti. or "transfused cells".ab. (107)
52 46 or 47 or 48 or 49 or 50 or 51 (100387)
53 45 and 52 (1587)
54 limit 53 to (human and english language) (1009)

APPENDIX B: List of variables abstracted for the MDS chart review

Demographic Information:

- Year of birth
- Gender
- Language (if available)

Baseline Information:

- Patient's blood group
- Co-Morbidities
- IPSS Scores

Hospital Admissions:

- Date of admission
- Reason for diagnosis
- Date of discharged
- Discharge on diagnosis

Infections:

- Start date of infection
- Stop date
- Severity
- Additional notes/details

Packed Red Cell Transfusions:

- Date of transfusion
- Donor blood group
- Number of units
- Age of blood at transfusion
- Interval between transfusion
- Transfusion Reactions

Platelet Transfusions:

- Date of transfusion
- Donor blood type
- Number of units
- Transfusion Reactions

Laboratory Tests

- Hemoglobin
- Hematocrit
- Platelets
- Ferritin
- Creatinine
- Bilirubin
- AST
- ALT

APPENDIX C: Final interview guide used to characterize and explore post-transfusion well-being in patients with MDS

Patient Demographics

I would like to start by asking you some questions about your history with MDS. This will help us to understand you a little bit better by putting your responses into context for us.

1. Can you please state your age?
2. How long have you been living with MDS?
3. How long have you been receiving blood transfusions?
4. How often are you transfused?

Questions pertaining to anemia related fatigue:

These next few questions I will be asking you, will be about your experience with anemia. We are interested in understanding your typical energy levels since it will help us place your experience of transfusion in perspective.

5. One of the symptoms of anemia is fatigue. Do you experience this symptom?
6. What are your energy levels like just before transfusion?
    i. PROBE: Are you tired? If you could rate it on a scale of 1-10, 10 being the most energy you have ever had; what would you rate it as?
7. Do you notice a change in your energy levels during or immediately after your transfusion?
    - FOLLOW UP QUESTION: How long does that change in energy level take? How long does this level of energy last? A day? Week?
    - FOLLOW UP QUESTION: How long does it take for your energy levels to start to fall?

Questions pertaining to well-being:

Now that we've talked a little about anemia related symptoms, I'd like to ask a few questions about your experience specifically around transfusion and any physical symptoms that you may have experienced.

8. Can you describe how you physically feel immediately after receiving a transfusion?
    i. PROBE: Do you feel nauseas, chills, fever, clammy, light-headedness?
9. How often do you experience these symptoms?
    i. PROBE: Do you experience these every transfusion, or just some transfusion?
    - FOLLOW UP QUESTION: How long do these symptoms tend to last?
    - FOLLOW UP QUESTION: Do you get these same symptoms after every transfusion or just some?

APPENDIX D: Final coding scheme applied to all interview transcripts

Co-Morbidities: *Other illnesses participants mention as it relates to their well-being*

Diagnosis with MDS:
- Clinical presentation: *How patients are diagnosed*
- Early management: *Information relating to when patient was started on transfusion support and initial frequency of diagnosis*

State of mind

MDS related symptoms
- Anemia related fatigue

Other therapies or treatments: *Transfusion with other blood products, other MDS therapies, concomitant medications that may influence experience of transfusion, fatigue, or QoL and well-being.*

Patient demographic characteristics and attributes: *Age, gender, etc…*

RBC Transfusion
- Frequency of transfusion: *How often patients receive RBC transfusions*
- Impact of transfusion on symptoms of anemia
  - Duration of treatment effect: *How long the transfusion seems to last*
  - Impact of fatigue
  - Time to recovery: *How long it takes for a participant to "feel better" after they have been transfused*
- Side effects of transfusion
  - Duration of side effects

Sedentary Lifestyle: *Any mention of how having a sedentary lifestyle conflicts with the participant's ability to gage changes in well-being or the effects of transfusion*