

EFFECTS OF CHEMOTHERAPY ON RED BLOOD CELL ALLOIMMUNIZATION
IN CHILDREN

TRANSFUSION-RELATED ALLOIMMUNIZATION IN CHILDREN:
EPIDEMIOLOGY AND EFFECTS OF CHEMOTHERAPY (TRACE-EC)

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ABSTRACT

Background: Red cell transfusions can lead to alloimmunization; however, pediatric alloimmunization frequency has not been well studied, and it may vary among diagnostic subgroups. Subgroups such as pediatric hematology/oncology patients require numerous transfusions during chemotherapy, but the immunosuppressive effect of chemotherapy on alloimmunization is unknown. One study demonstrated reduction in IgM and IgG in pediatric leukemia patients (Martín Ibáñez 2003); hence, one could hypothesize that chemotherapy suppresses alloimmunization.

Objectives: This study aimed to: 1) describe alloimmunization frequency and antibody specificities in transfused pediatric patients; and 2) determine if chemotherapy affects the frequency of alloimmunization.

Methods: A retrospective cohort study of pediatric patients (transfused between April 2002 and November 2011 at Hamilton Health Sciences) was performed. Data were extracted from 3 sources: a blood utilization database; the Laboratory Information System; and chart reviews. The chemotherapy treatment group included pediatric hematology/oncology patients stratified by HSCT status and diagnosis; control cohort included other pediatric patients not diagnosed with cancer. Control patients with hemoglobinopathies were analyzed separately due to increased alloimmunization. Alloimmunization was defined as clinically significant IgG alloantibody formation.

Results: There were 1273 patients in the study: 949 in control group; 324 in study group. Alloimmunization was 1.6% overall: 0.6% (95% CI: 0, 1.47) in study group; 1.3% (95% CI: 0.58, 2.06) in control group. The association between chemotherapy and alloimmunization was not significant (p value = 0.38 Fisher's exact test; OR 0.46, 95% CI: 0.10, 2.09). Due to low outcome rate, logistic regression to explore the association was not feasible.

Conclusions: This is the first study exploring the frequency of alloimmunization in pediatric patients by diagnosis and the association between chemotherapy and alloimmunization. The frequency of alloimmunization was low and no association between chemotherapy and alloimmunization was observed. Low event rate would have contributed to low power.

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LIST OF ABBREVIATIONS

AA	Aplastic Anemia
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloblastic Leukemia
CBS	Canadian Blood Services
CI	Confidence Interval
CMML	chronic myelomonocytic leukemia
CMPD	chronic myeloproliferative disease
CNS	Central Nervous System
CRF	Case Report Form
CSA	Clinically Significant Alloantibody
CSU	Computer Services Unit
DAD	Discharge Abstract Database
DHTR	Delayed Hemolytic Transfusion Reaction
DMS	Data Management System
ESFT	Ewing Sarcoma Family of Tumors
GCT	Germ Cell Tumor
HCC	Hepatocellular Carcinoma
HHS	Hamilton Health Sciences
HIN	Hospital Identification Number
HL	Hodgkin Lymphoma
HSCT	Hematopoietic Stem Cell Transplant
Ig	Immunoglobulin
LIS	Laboratory Information System
MDS	Myelodysplastic Syndrome
MM	Multiple Myeloma
MTRP	McMaster Transfusion Research Program
NBL	Neuroblastoma
NHL	Non-Hodgkin Lymphoma
OR	Odds Ratio
OS	Osteogenic Sarcoma
PAS	Positive Antibody Screen
PHI	Personal Health Information
RBC	Red Blood Cell
RC	Red Cell
RCC	Renal Cell Carcinoma
RMS	Rhabdomyosarcoma
RT	Radiation Therapy
SCD	Sickle Cell Disease
SD	Standard Deviation
SDP	Single Donor Platelets
ST	Solid Tumor
Thal	Thalassemia

TRUST
WT

Transfusion Registry for Utilization Statistics and Tracking
Wilms Tumor

DECLARATION OF ACADEMIC ACHIEVEMENT

The development of the research proposal and ethics application was led by Z. Solh (principal investigator). The Case Report Form was developed by Z. Solh with input from N. Heddle (supervisor), U. Athale (co-investigator), R. Barty (database coordinator), E. Jamula (research assistant), and K. Hamilton (research assistant). The chart review was conducted by Z. Solh, E. Jamula and K. Hamilton. Data cleaning was performed by Z. Solh with input from R. Barty and Y. Liu (biostatistician). Statistical plan and analyses were prepared by Z. Solh with assistance from Y. Liu and G. Wang (biostatisticians).

CHAPTER I: Review of the Literature

1. Blood Groups, Antigens, and Alloantibodies

Red blood cell (RBC) surface antigens have been classified into 29 blood group systems, which include: ABO, Rhesus, Lewis, Kell, Duffy, Kidd, MNS, P and Lutheran. This results in approximately 285 distinct blood group antigens (the majority of which are proteins or glycoproteins) that can coexist in an individual, giving rise to a multitude of phenotypic variations (Murphy 2009, Israels 2002).

The absence of certain antigens from the RBC surface can result in the development of alloantibodies after exposure to those antigens from the environment (e.g. plants, bacteria), blood transfusions, or fetal-maternal bleeds during pregnancy. Some antibodies such as ABO are stimulated by environmental exposure and start to appear when an infant is 3 to 6 months of age. Other blood group antibodies are only stimulated by transfusion and pregnancy, and not every transfused patient will form these antibodies.

It is estimated that 1 to 6 % of transfused patients will become alloimmunized (Heddle 1992, Heddle 1995, Hewitt 1988). For these patients, future transfusions can be challenging as they require antigen negative blood. It is currently not known why some individuals become alloimmunized following transfusions, whereas others do not.

2. Transfusion-Related Alloimmunization

RBC antibodies such as E, Jk^a, c, Fy^a, and K are commonly implicated in transfusion-related alloimmunization (Callum 2011). For the purposes of this research study, we define transfusion-related alloimmunization as the development of a clinically relevant alloantibody by an individual after receiving a blood transfusion. Although the red cell concentrate is the blood product which carries the greatest risk of inducing alloimmunization, transfusion of platelet concentrates may also contribute to this phenomenon due to small levels of red cell contamination in each platelet product.

The majority of the literature pertaining to transfusion-related alloimmunization involves patients with hemoglobinopathy (Azarkeivan 2011). Studies report a rate of alloimmunization in the sickle cell population as high as 29% in children and 47% in adults (Aygün 2002, Harm 2014). Similarly, thalassemia patients were found to have an alloimmunization rate of 28% in Egypt and 37% in Taiwan (Saied 2011, Wang 2006). There have also been studies examining the effects of red cell exchange transfusions (high red cell volume) in sickle cell disease showing that the rate of alloimmunization is 1.5% per unit of red cell exposure (Venkateswaran 2011). The other well researched patient population in terms of alloimmunization is the newborn. In neonates, both term and preterm, alloimmunization is quite rare (Strauss 1999, Floss 1986). Antibody specificity has been studied in thalassemia patients (Makroo 2013, Saied 2011), sickle cell anemia patients (O'Suoji 2013), and patients undergoing elective surgery (Reyhaneh 2013) with antibodies against Rh (E and c) and Kell antigens reported most frequently.

Although these reports have improved our understanding of red cell alloimmunization in chronically transfused patient populations, to our knowledge, there are no data to reflect the frequency of alloantibodies in other hospitalized children. There is one study of alloimmunization in pediatric oncology, but it is focused on patients receiving D-antigen-incompatible platelet transfusions and the question of prophylaxis to prevent D-alloimmunization (Molnar 2002).

Hospital laboratories go to great lengths and cost to ensure the compatibility of the blood product with its recipient by conducting compatibility tests which include an antibody screen and a cross match. This testing has been tailored over the years by our understanding of the incidence and immunogenicities (i.e. immune-stimulating potential) of different red cell antigens and the clinical relevance of the antibodies.

2.1 Alloimmunization

The impact of alloimmunization can be significant: increases the complexity of finding compatible blood for patients; results in added cost to the health care system and inconvenience to the patient; and causes transfusion reactions including potential life threatening events. Alloimmunization can result in acute or delayed hemolysis (Pineda 1999). Acute hemolytic reactions are usually the result of blood group incompatibility between the donor and the recipient of the blood product. In some patients these reactions are not associated with signs or symptoms; whereas, in other patients morbidity can range from mild to severe, and in some cases death has been reported. Symptoms include fever,

chills, hemoglobinuria, and less commonly renal failure, dyspnea and disseminated intravascular coagulation. Delayed hemolytic transfusion reactions (DHTR) are more benign and occur at a rate of 1 in 6,715 units of RBC transfused. DHTRs result from alloantibody formation 3 days to 2 weeks after the transfusion (Callum 2011).

3. Effects of Chemotherapy on Alloantibody Development

Childhood cancer is the leading cause of disease-related mortality in children. Because childhood cancer patients receive bone marrow suppressive chemotherapy, they are often anemic and thrombocytopenic requiring multiple blood product transfusions over the course of their therapy putting them at risk of alloimmunization. What is not known is whether chemotherapy affects the likelihood of alloimmunization (independent of cancer or amount of RC utilization).

Schonewille et al. studied 564 adult patients with malignant hematologic diseases and showed that the rate of alloimmunization was 9% overall. Patients who received more intensive chemotherapy produced alloantibodies at a much lower rate despite requiring more platelet transfusions (Schonewille 1999). The study concluded that transfusion-related antibody formation in the adult oncology patient population was similar to that in other diseases requiring multiple transfusions. Therefore, extensive antigen matching prior to transfusion was found to be unnecessary and only added to increased costs. Some studies have examined other adult oncology populations showing an alloimmunization rate of 15% (Sanz 2013) and 20% (Schonewille 2009). There are no similar studies in

children who receive multiple transfusions for hematologic malignancies or other oncologic diagnoses.

A study of 50 pediatric patients with acute lymphoblastic leukemia (ALL) has shown that immunosuppressive chemotherapy is associated with a quantitative decrease in immunoglobulins with IgG and IgM being the most affected, while IgA is less affected (Martín Ibáñez 2003). As most transfusion-related alloantibodies are of the IgG subtype, it can be hypothesized that transfusion-related alloantibodies are less prevalent in patients who have received immunosuppressive chemotherapy compared to other hospitalized patients who have not. However, this question remains unanswered, especially in the pediatric hematology/oncology literature. Appendix I summarizes the literature on antibody formation in oncology patients. It demonstrates that there are no well-designed studies evaluating the effects of chemotherapy on the formation of red cell transfusion-related alloantibodies in children. Such new knowledge about this growing patient population will be essential in managing their transfusion needs.

4. Study Purpose

This review of the literature indicates that there is a paucity of data on the epidemiology of red cell (RC) alloimmunization in pediatric patients, especially those with malignant hematology/oncology diagnoses. This study will address some of the information gaps in this population including: 1) to describe RC alloimmunization frequency and alloantibody specificities in transfused pediatric patients and their diagnostic subgroups; and 2) to

determine if immunosuppressive chemotherapy affects the frequency of RC antibody formation. This study is novel and contributes to an area of medicine where information is lacking. New knowledge generated from this study is likely to provide evidence to guide clinicians and laboratory personnel about which pediatric subgroups are at higher risk of alloimmunization, and this may shed light on whether the frequency of screening could be reduced in less alloimmunized subgroups to improve the cost/safety balance.

5. Research Questions

Question #1: In pediatric patients who received non-autologous red cell (RC) transfusions at Hamilton Health Sciences (HHS) between April 2002 and November 2011, what is the frequency of RC alloimmunization (defined as an IgG alloantibody known to cause RC destruction), and what are the specificities of the antibodies formed?

Question #2: In pediatric patients who have received at least 1 non-autologous RC transfusion at HHS between April 2002 and November 2011 (Population), is receiving chemotherapy (Intervention) associated with a reduction in the percentage of patients who become alloimmunized (Outcome), compared to other hospitalized pediatric patients (Comparator), when the outcome is measured during the patient's clinical course (Timing) using a retrospective cohort study (Study design)?

6. Hypothesis (for Question #2)

Chemotherapy is associated with a lower percentage of alloimmunized patients.

CHAPTER II: Methods

1. Study Overview

This is a retrospective cohort observational study using data collected from a patient transfusion registry between April 2002 and November 2011.

2. Description of Study Population

The study patient population is composed of pediatric patients transfused at Hamilton Health Sciences (HHS) between April 2002 and November 2011. The patient sample was obtained from three locations where children are transfused at HHS: McMaster University Medical Centre, Hamilton General Hospital, and the Juravinski (formerly Henderson) Hospital.

2.1 Sample Size

The database utilized for this study contains data from 2002; hence, the number of pediatric patients treated and transfused at HHS since 2002 dictates the sample size that is available (a convenience sample). The sample is considered representative, as there is no opportunity to volunteer or to refuse participation in the study. A formal sample size calculation was not possible due to lack of data regarding the relationships between confounders, exposure, and outcome.

2.2 Inclusion Criteria

Appendix II is a flow diagram illustrating the inclusion and exclusion criteria for the patient population. Patients were eligible for the study if they met all of the following inclusion criteria:

- Received at least one non-autologous red cell transfusion
- Transfusion occurred between April 2002 and November 2011
- Patient age was older than 4 months and younger than 17 years and 364 days at the time of the first transfusion.

To explore the association between chemotherapy and alloimmunization, it was necessary to identify patients who would receive chemotherapy as part of their disease treatment (study group) and a control group who would not receive chemotherapy. Eligible patients were selected from the database and included in the **study group** if they were diagnosed with a malignancy based on eligible ICD-10 criteria found in Appendix III and received any chemotherapeutic agent. This is possible because the database includes the variables ‘Most Responsible Diagnosis’ and ‘Primary Diagnosis’ which are coded using this ICD-10 coding scheme (these two variables are very similar but ‘Most Responsible Diagnosis’ is regarded as the one that results in the greatest length of stay). The oncologic diagnoses were selected based on published data regarding the most common pediatric cancers (Pizzo and Poplack 2011) which require chemotherapy: acute lymphoblastic leukemia, acute myeloblastic leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, Ewing sarcoma family of tumors, osteogenic sarcoma, Wilms tumor, neuroblastoma, germ cell

tumor, rhabdomyosarcoma, hepatoblastoma, and renal cell carcinoma. This group includes those who have undergone chemotherapy alone or chemotherapy in conjunction with hematopoietic stem cell transplantation (HSCT). As chemotherapeutic agents are a heterogeneous group of bone marrow and/or immune suppressive drugs with various mechanisms of action, we were interested in their collective effect and not the effect of any single type of agent.

The **control group** was defined as: patients who meet the eligibility criteria but who are not diagnosed with a malignancy (based on ICD-10 codes) and thus have not undergone the intervention of interest (chemotherapy). Based on literature confirming that thalassemia and sickle cell disease patients have a very high rate of red cell alloimmunization, the control group was classified into four categories that are frequently transfused: 1) Thalassemia; 2) Sickle Cell Disease; 3) Aplastic Anemia; and 4) Other. This categorization was used for subgroup analysis.

Neonates were not included due to evidence in the literature confirming a lack of alloimmunization in children less than 4 months of age (Strauss 1999, Strauss 1993, Floss 1986, Ludvigsen 1987, Pass 1976, Rawls 1984).

2.3 Exclusion Criteria

Patients who met the inclusion criteria were excluded if they were diagnosed with any of the following as the ‘Primary Diagnosis’ or the ‘Most Responsible Diagnosis’:

- a rheumatologic condition
- a primary immune deficiency
- an autoimmune anemia
- a CNS tumor

Rheumatologic conditions were excluded as they receive immunosuppressive agents, which could bias the outcome measurement. Patients with humoral immune deficiencies were excluded due to their intrinsic ineffectiveness in forming antibodies to red cell antigens. Autoimmune anemias were also excluded due to the presence of auto-antibody which makes it challenging for the lab to detect alloantibodies. Although central nervous system (CNS) tumors are among the most common tumors in children (20% of pediatric cancers), the majority of patients do not require transfusions and the chemotherapy used is generally not bone marrow suppressive, so these patients were excluded. Section 7 of this chapter discusses these and other covariates.

3. The Database

The McMaster Transfusion Research Program (MTRP) retains the Transfusion Registry for Utilization Statistics and Tracking (TRUST), which is a large database of all patients admitted to HHS and includes comprehensive transfusion data on any patient who has received a blood product transfusion at HHS from 2002 to the present time. The demographic and medical information in TRUST comes from the Discharge Abstract Database (DAD) which is collected from the patient's medical record following

discharge. The blood product information in TRUST comes from the Laboratory Information System (LIS) and is captured in real-time. It is considered the institutional gold standard source for blood product inventory and transfusion information. TRUST has been utilized by the Ministry of Health, Canadian Blood Services (CBS), and HHS for various purposes including blood demand forecasting, trace-back investigations after transfusion reactions, best practice comparison, benchmarking, and health outcomes research.

4. Data Collection Procedure

Data for the study were collected from three sources. The first was the TRUST database which contains patient demographics, blood product details, and transfusion information. The second data source was the Laboratory Information System (LIS) which contains additional information about antibody screening that was not captured in TRUST. The third source was the patient medical record which contains chemotherapy drug names and dates of administration.

4.1 TRUST Data

The data variables abstracted from TRUST are listed in Appendix IV, and these included but were not limited to: age, sex, primary diagnosis, types of blood products transfused, and number/dates of transfusions. Information about Albumin and Factor Concentrate infusions were not collected due to the absence of alloantibody formation from these products. Intravenous immunoglobulin (IVIg) infusion data were examined only for

patients found to have a clinically significant alloantibody. Information about red cell, platelet, plasma and cryoprecipitate infusions was collected due to the possible presence of alloantibodies in these products.

4.2 LIS Data

LIS data were available from 2002 to 2012. The patient's initial antibody profile was obtained from the first antibody screen available in LIS. To track disappearance or reappearance of antibodies, all subsequent available antibody profiles within the study period were also obtained from LIS until the patient turned 18 years of age. These data were not available in TRUST. Variables abstracted from LIS included:

- Antibody Screen Date
- Antibody Screen Result (positive/negative)
 - o If positive, antibody specificity result

4.3 Review of Patient Medical Records

The data regarding chemotherapy drug names and dates of administration were not available in TRUST or LIS, so a review of medical records was performed. Data about cancer relapse or second malignancy (diagnosis, dates of chemotherapy), chemotherapy-free periods, and date and number of HSCTs were obtained in order to confirm temporal associations between cancer treatments and red cell alloantibody development. Because patients are referred to another hospital for HSCT (provincial transplant centre), a comprehensive list of all our hospital's pediatric patients that ever underwent a HSCT

was utilized to prospectively flag patients (i.e. before chart review) who have had a transplant, as an extra step to ensure HSCT data were not missed.

If key data were missing from TRUST, patient chart reviews were conducted to retrieve those specific data items. For example, TRUST obtains diagnostic codes from inpatient visits only – not outpatient visits. There were 36 eligible patients whose diagnoses were not available in TRUST because they had received red cell transfusions in an outpatient setting only. These patients required a chart review to ascertain if they belonged in the control, study or exclusion groups (see Chapter III for further details).

Because three different research personnel (including the principle investigator) performed the chart reviews using a Case Report Form (CRF), it was necessary to validate the reliability of the CRF and the reproducibility of the CRF data. For this purpose, five charts were chosen at random before the start of data collection for a triplicate chart review, and it was determined that the three sets of CRF responses to the five-chart pilot review correlated well. During the actual chart review, difficulties encountered with finding data were reviewed with the study PI who reviewed the chart in more depth in order to search for the relevant response. This second review by the PI may have contributed to bias, but it was necessary to minimize missing data points. Bias from the PI may be the result of preconceived opinions about the standards of care of patients with a specific oncologic diagnosis.

At the end of the full study sample chart review, thirty charts (ten per CRF user) were randomly chosen (computer generated random sample) and redone by a different person in order to re-validate each other's data collection results. Discrepancies were resolved by discussing each questionable data point with the study PI who substantiated the correct response. In cases where there were still missing or unknown data points, this was indicated within the CRF (see Chapter III for further details).

5. Design of the Case Report Form

A CRF for manual data collection was developed using the TeleForm software program (Cardiff TeleForm, Vista, CA). Once the data were filled into the form manually, the forms were scanned and the software program detected vacant data points and illegible handwriting, and it prompted the researcher to correct or clarify these data. After successful scanning, the data were exported into a database that utilizes the ACCESS software. The initial CRF version was pilot tested to uncover any issues, and amendments were made until a final CRF version was approved for data collection. For example, the initial CRF version did not include a way to document status post HSCT (deceased or alive) or patients that were lost to follow-up. A data collection training session was held between the PI, the two research assistants, and the MTRP coordinator. The session addressed CRF use and data collection procedures, and it ultimately led to the development of an instruction manual which standardized the approach to CRF data entry. Please refer to Appendix V for the final CRF version.

6. Study Outcomes and Measurement

6.1 Primary Outcome

The primary outcome for this study was alloimmunization defined as the development of an IgG RC alloantibody after transfusion. To measure the primary outcome of ‘alloantibody formation’, antibody screen results were obtained from LIS and were reported as a binary outcome: ‘Yes’ for a positive screen and ‘No’ for a negative screen. This outcome was also measured as a categorical variable by determining the specificity of the alloantibody formed. This was necessary in order to draw conclusions about the clinical significance of the alloantibody. For example, if a screen was positive but the alloantibody was a passive anti-D and not an active red cell alloantibody formation by the patient, it was determined that in fact no alloimmunization had occurred. Autoantibodies and naturally occurring IgM antibodies were considered not to be in keeping with clinically significant red cell alloimmunization. A transfusion medicine expert classified the antibodies to identify those that met the criteria for active RC alloimmunization.

6.2 Secondary Outcomes

The secondary objective of this study is to measure the effect of immunosuppressive chemotherapy on the frequency of red cell antibody formation.

7. Minimizing Bias

Bias in research refers to a systematic deviation from the truth. Observational studies such as this one have built-in bias due to the absence of randomization, but it is important to

minimize bias or at least recognize sources of bias in order to be able to interpret the results in light of their limitations. However, bias can still occur from confounders that are not identified or covariates that are not equally distributed (see below).

7.1 Confounders

A confounder is a variable that is correlated with the exposure of interest and the outcome of interest without lying on the causal pathway between this exposure and this outcome.

Several confounders were identified *a priori*. Neonates and patients with immunodeficiency were excluded because these patients lack the ability to form antibodies and falsely decrease the actual frequency of alloimmunization (selection bias).

Patients who receive immunosuppressive drugs other than chemotherapy, such as those with rheumatologic conditions, were excluded from the control group because

immunosuppressive drugs are a confounder: their mechanism of action is similar to the exposure of interest (chemotherapy) and they affect the outcome (alloimmunization).

Immune anemias were also excluded due to their potential confounding effect i.e. they are associated with the outcome due to their antibody screen positivity, and they are associated with the exposure due to their treatment with immunosuppressive drugs.

Selection bias was minimized in the study group by excluding CNS cancer patients because these patients in general do not receive immunosuppressive chemotherapy (explained further in chapter III). HSCT and some medications that mask or inhibit

antibodies (asparaginase, rituximab) are used to treat oncology patients and may influence alloimmunization, so data on the use of these drugs were collected.

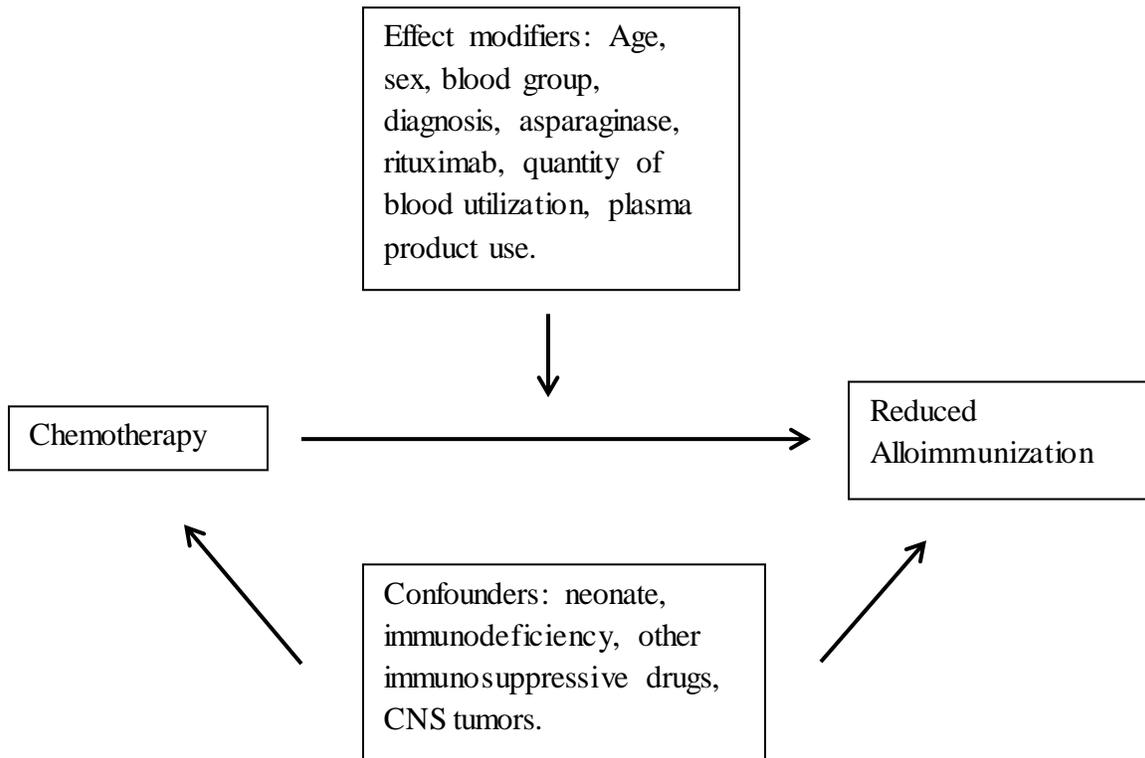


Figure 1: Relationship between exposure, outcome, effect modifiers, and confounders.

7.2 Other Covariates

Covariates are variables of interest which may affect the outcome. Covariates that may influence the validity of the data were considered in the data collection and analysis plan, and various techniques were implemented to minimize bias. To assess if the exposed and unexposed groups were similar or differed in ways other than the exposure (chemotherapy), baseline data regarding age, sex, blood group, and diagnosis were collected.

Blood utilization was considered an effect modifier. This variable was measured and reported as a continuous variable using the median number of red cell transfusions per patient. Plasma based products which may contain alloantibodies and cause an antibody screen to be positive (but not because of red cell antigen presentation to the patient) were considered an effect modifier. Data on plasma containing blood products (platelets, frozen plasma, cryoprecipitate) were collected. Data regarding IVIg use in alloimmunized patients were collected to examine if a transient antibody appears in a temporal association following IVIg infusion then disappears.

8. Data Management and Quality Control

The PI coordinated all aspects of the study engaging the assistance of MTRP staff where necessary. MTRP specializes in transfusion-related research and has an extensive track record in coordinating single and multi-centre randomized trials and observational studies. The infrastructure for study coordination and data management is well established within this group.

The data are stored on a secure server in the Computer Services Unit (CSU) at McMaster University. The server is maintained regularly and monitored daily for any problems. All data are backed-up daily onto tapes (a password protected secret computer drive) which are stored in an off-site location. Only the study biostatistician and the MTRP TRUST coordinator have access to the study data. Completed CRFs for chart reviews were stored in a secure locked cabinet within MTRP.

All data were anonymized. A patient identification number was assigned to each patient, so patient names and chart numbers were not recorded on the CRFs. Completed CRFs were stored in a secure locked cabinet within MTRP. No patients were contacted. When accessing clinic charts there were no ethical issues encountered. The charts were reviewed in the clinic space and did not leave the premises ensuring the safety and confidentiality of patient information. The HSCT list was stored in a password encrypted Excel file and kept in a secure location that only the biostatistician and MTRP coordinator can access.

9. Ethical Considerations

There were no ethical concerns with this study for the following reasons. Research Ethics Board (REB) approval was obtained from all institutions providing data and from the privacy office. MTRP has REB approval to collect the relevant data monthly and include it in the TRUST database; however, whenever the data are to be used for a project such as this one, a separate REB submission is made requesting approval to do so. REB approval for this study was obtained from HHS. The REB confirmation number is 11-583-C. In order to use the patient list obtained from the provincial transplant centre, REB approval was obtained after an amendment.

Personnel accessing charts met the institutions' requirements for having access to charts and all had signed confidentially agreements. No patients were contacted directly. Data

were stored securely and did not contain direct patient identifiers. All results were presented as aggregate data. Privacy issues related to using the database are discussed in the next chapter.

CHAPTER III: Methodological Challenges

1. Privacy Issues for Database Research

Databases are a fundamental technology in clinical research and a catalyst for intellectual achievement. Due to recent developments in computing hardware and software capabilities, there has been an explosion of data leading to great potential for the discovery of influential trends and results (Agrawal 2008, The Claremont Report on Database Research). This constant expansion of data necessitates a perpetual evolution in the development of data management systems (DMS), which are software applications that interact with the database user in order to capture and analyze data (Anhoj 2003). DMS technology provides advantages such as effectual data capture instruments, high data processing speed, data backups, data error checking, and reliability to produce replicable results. Data management systems have evolved from being a business cost to now being an efficient and cost-effective method to conduct clinical research. In parallel to these benefits, there are major risks for the violation of patient privacy.

The TRUST database is a relational database comprised of several tables and files. The biostatistician is able to link the data accurately and on demand by merging these tables and files using variables such as the Hospital Identification Number (HIN) and the encounter number. A complete list of variables found in the TRUST database is included in Appendix VI.

Upon review of the study's research ethics application, the hospital privacy office inquired about the procedures and technologies implemented by the TRUST database users to protect personal health information (PHI) such as the patient's HIN. Several privacy procedures were already in place and previously accepted by the research ethics board. For example, if the data were removed from the database, the data were being protected using the TrueCrypt encryption software, as well as other security technologies such as SCP (Special Containment Procedures) and SSL (Secure Sockets Layer), which help establish an encrypted link between a web server and a browser. The server was firewalled, isolated, and locked in a secure area with swipe card access, password protection, and security camera monitoring. Due to recent technological advances involving the way data are protected, the Computer Services Unit (CSU) was asked by the hospital privacy office to upgrade the server to Microsoft SQL (Structured Query Language) Server Enterprise, and the entire database was encrypted including the PHI. The upgrade to the SQL server resulted in a delay in data collection for several months - a necessary delay in order to ensure the data will be stored and collected in compliance with the most current privacy standards.

2. Challenges with the Sampling Strategy

Twenty eligible patients were ICD-10 coded for both the study and control groups. Instead of excluding these patients, further examination of the medical records was performed. It was determined that 15 of these 20 patients were in fact study patients as they had aplastic anemia as a pre-cancer condition. The remaining 5 patients were

suitable control patients as they were diagnosed with aplastic anemia and did not subsequently develop cancer. All 20 patients were determined to be eligible and were not excluded from the sample which eliminated a possible selection bias.

3. LIS Antibody Specificity Data

The data regarding antibody specificity were not entirely within TRUST, as only the last positive antibody screen was retained in TRUST, and other negative screens were absent. For a comprehensive list of antibody screen results, data were retrieved directly from the LIS database. This process allowed us to ensure that all alloimmunized patients were captured even if their antibody disappeared over time. Positive screen results in LIS were available as a comment in text format, which was not in a suitable format for a nominal variable data point. These text-based data were considered noisy data, and they required manual review by a research assistant in order to distill out the antibody specificity and assign it a code (e.g. anti-Kell is 1, anti-D is 2, etc.). The Claremont Report on Database Research states that there is much effort in the database research community to develop software that can manage and structure text-based data (Agrawal 2008).

4. Case Report Forms

During the chart review process, there were a few patients with exceptional events that could not be documented directly on the CRF because these events were unforeseen during CRF design. These events were recorded separately as a comment that is linked to the patient using the PID. Examples of such exceptional events are patients who

underwent HSCT at a different transplant centre at which a chart review was not permitted by the REB. In these cases, the transplant data were recorded as a comment to ensure the capture of this relevant information for alloimmunized patients.

5. Excluding CNS Tumors

A concerted effort was made to avoid excluding patients who were eligible for the study in order to optimize the sample size. Although patients with CNS tumors such as low grade glioma are generally considered to be treated with chemotherapeutic regimens that do not suppress the bone marrow or the immune system, they were excluded from the control group for these two reasons. First, it is impossible to objectively ascertain that the drugs they receive impart no immunosuppressive effect. Second, these patients are often diagnosed using imaging alone without tissue pathology, leading to possible changes in diagnosis and management upon follow-up due to either malignant transformation or misdiagnosis. A retrospective chart review in such cases becomes quite complex and may lead to inaccurate patient grouping. Other patients with CNS tumors such as medulloblastoma and atypical teratoid rhabdoid tumor do receive very intense chemotherapeutic regimens with definite immunosuppressive effect. In theory, they would be appropriate as study group patients. Because they often share the same ICD-10 code ('CNS tumor') as benign tumors, their differentiation by ICD-10 code is rendered problematic. Their chart review in the absence of input from a neuro-oncologist may be complex for the same reasons stated above (limitations of diagnostic imaging and the availability of tissue). Since CNS tumors as a whole represent approximately a fifth of

childhood cancer diagnoses (Ontario Cancer Registry 2012), it can be estimated that approximately 80 CNS patients were excluded from this study (whether treated with chemotherapy or not).

In summary, patients with CNS tumors were excluded from the study due to a multitude of limitations found with the documentation of their diagnoses. Their exclusion may have been a limitation to the study. Future studies may be facilitated by the involvement of a neuro-oncology expert who would advise regarding optimal chart review strategies.

6. Data Outside of Study Period

The study period was dictated by the availability of data in the TRUST database, which was from April 1, 2002 until November 30, 2011. When data that influences the alloimmunization rate was found outside the study period, a chart review was done in order to actively seek these data. This occurred in only 3 cases: one patient had undergone chemotherapy prior to April 2002 and relapsed during the study period; 2 patients had undergone HSCT prior to April 2002. The data were documented as comments which were linked to each PID.

7. Medication Documentation

Medications administered to inpatients are normally documented in the electronic medical record. Those administered in the outpatient setting (such as rituximab and asparaginase) are only documented in the clinic chart. Patients with oncologic diagnoses that may have

received asparaginase (ALL, AML and NHL) or rituximab (ALL and NHL) were addressed with an additional review of the clinic chart, along with the standard review of the electronic medical record.

8. Outpatient Transfusions

Patient diagnostic codes are imported into TRUST from the Discharge Abstract Database. There were 36 eligible patients whose diagnoses were not available in TRUST because they had received red cell transfusions in an outpatient setting only. These 36 patients required a chart review to ascertain their diagnoses and subsequently determine if they belonged in the control, study or exclusion groups. It was subsequently determined that 25 patients met the criteria to be included in the control group, 6 were study patients, 4 were excluded based on the pre-specified criteria, and one was a previously accounted for control patient (i.e. two distinct hospital identification numbers for the same patient).

CHAPTER IV: Statistical Analysis Approach

1. Approach Overview

An analysis plan was prepared before the data collection. One of the pre-specified analytical objectives was to examine the association between two binary categorical variables (chemotherapy: yes/no; red cell alloimmunization: yes/no). After the data were collected and the results were reviewed, it became apparent that modifications to the original analysis plan were required. In this chapter, we discuss the original analysis plan and the rationale for revising it.

2. Plan for Descriptive Statistics (unchanged despite revised plan)

The original analysis plan contained a summary of the descriptive statistics that would be performed. This part of the analysis plan did not change in the final analysis. Continuous variables will be described using means and standard deviations if normally distributed. Medians, interquartile ranges, min and max values will be used if the data are skewed. Categorical variables will be summarized as proportions with 95% confidence intervals calculated around estimates where appropriate.

3. Original Analysis Plan

In the analysis plan specified *a priori* the frequency of alloimmunization would be determined in the study group and the control group (excluding hemoglobinopathy patients) using proportions (# alloimmunized patients / total # patients). Due to their

known high rate of alloimmunization, patients with sickle cell disease or thalassemia (i.e. hemoglobinopathy) would be analyzed separately. Both the exposure and outcome variables are binary (two categories), so a 2 x 2 contingency table would be constructed to summarize the distribution of the outcome between the exposed and non-exposed groups.

The original statistical plan was to compare outcome frequencies using the chi-square test. Logistic regression would then be used as a secondary analysis to control for covariates during this comparison. See chapter II for a complete description of the rationale used to choose the following covariates: age, sex, blood group, diagnosis, asparaginase use, rituximab use, quantity of blood transfused, and plasma blood product use. The covariate expected to affect the outcome the most is the quantity of RC transfused. Nominal variables would be dummy-coded (example: blood product type would be coded as Red Cells = 0; Platelets = 1; Plasma = 2). The regression analysis may not be possible in addressing all the covariates due to the small number of patients expected to be alloimmunized based on the described literature. In order to explore the potential power of the study, a preliminary analysis of pilot data was performed in order to predict the overall alloimmunization rate and modify the analysis plan if needed. Refer to part 4 of this chapter for the results of this preliminary analysis.

In summary, the original analysis plan specified *a priori* required revision because of the small number of alloimmunization events which made the use of chi square and regression problematic.

4. Preliminary Analysis

A preliminary analysis of data in TRUST (not utilizing all the eligibility criteria) indicated that a total of 3833 patients under 18 years of age were transfused between April 2002 and May 2011, and 93 (2.4%; 95% CI 1.9% to 2.9%) of them had a positive antibody screen result. Two alloimmunized patients were transfused at Hamilton General Hospital and 91 patients were transfused at the McMaster Site of Hamilton Health Sciences. This preliminary analysis finding was consistent with the anticipated frequency of 1-6% reported in the literature (Aygün 2002, Harm 2014); however, during the process of extracting the preliminary alloimmunization data from TRUST we observed that not all antibody screen results were available in TRUST when multiple screening tests were performed on a patient. These missing data could bias the results towards underestimating the frequency of alloimmunization. Applying the eligibility criteria may also change the alloimmunization rate. It was conceivable from this pilot data that the frequency of alloimmunization could be higher than 2.4% and that the chi-square test and the regression analysis would be feasible.

5. Rationale for the Revised Analysis Plan

After applying the eligibility criteria, it became evident that the 2 x 2 contingency table included a cell with a very low count of 2. When confronted with very low outcome event rates (i.e. when at least one cell has a value equal to or less than 5), the chi-square test does not provide a good approximation of statistical significance. As such, the chi-square test was no longer an appropriate nonparametric test to examine whether the difference of

proportions that we observe between groups was significant. A variant of the chi-square test, Fisher's exact test, is a superior test in this situation. It measures the probability of getting the particular contingency table observed or one that is more extreme (cell count of 1 or 0), given the same row and column totals. Fisher's exact test enables the calculation of the exact significance instead of the approximate significance.

We used the rule of thumb that a logistic regression model should be used with a minimum of 10 outcome events per covariate. Because the outcome was an infrequent event, the effect of covariates on alloimmunization could not be estimated by conventional logistic regression. Statisticians have proposed that rare events result in a high risk of small sample bias (King and Zeng 2001). Some strategies have been developed to deal with small sample bias and rare events. These strategies are beyond the scope of this thesis, but they include: penalized likelihood, the Firth method, and exact logistic regression (computationally intensive).

As a measure of association between chemotherapy and alloimmunization, the odds ratio (OR) will be calculated and the 95% CI will be reported. The OR describes the ratio of outcome odds given exposure status. It will provide an appropriate approximation of the Relative Risk because the outcome in this study is expected to be rare (Schmidt 2007).

6. Summary of the Revised Analysis Plan

The final analysis plan contained:

1. Descriptive statistics (as defined in the original analysis plan)
2. Fishers exact test (2x2 contingency table)
3. Odds ratio with 95% CI

NOTE: Logistic regression was removed from the plan.

7. Handling Missing Data

If there are any missing data during the chart review, they will be reported as ‘unknown’ on the CRF. If the missing data are in critical elements such as ‘diagnosis’, listwise deletion will be performed. Listwise deletion has the advantage of simplicity and leads to unbiased estimates if missingness is rare and at random (i.e. not due to bias). Although in theory listwise deletion affects sample size, we do not expect large numbers of patients to have missing data because much effort was made in the sample selection plan and the data collection plan to avoid non-at-random missing data.

CHAPTER V: Results

1. Patient Demographics

There were 1273 patients in the study: 949 in the control group and 324 in the study group. There were slightly more females in the control group (48%) than the study group (44%). Mean age was 8.6 years (SD 6.4) in the control group which was slightly higher than 7.2 years (SD 5.5) in the study group. ABO and Rh blood group distribution was similar in both groups. Table 1 below summarizes the demographic characteristics of the study sample.

Table 1: Patient Demographics

		Control Group					Study Group				
		Thal	SCD	AA	Other	Total	HSCT	Non-HSCT	Hem Malign	ST	Total
# Patients		17	23	42	867	949	48	276	237	87	324
Mean Age (SD)		6.6 (5.8)	8.5 (5.7)	8.1 (5.0)	8.7 (6.5)	8.6 (6.4)	8.6 (5.5)	6.9 (5.5)	7.9 (5.4)	5.2 (5.3)	7.2 (5.5)
Sex # (%)	Male	13 76	12 52	20 48	444 51	485 52	28 58	152 55	133 56	47 54	180 56
	Female	4 24	11 48	22 52	423 49	460 48	20 42	124 45	104 44	40 46	144 44
ABO Group # (%)	A	4 24	7 30	18 43	325 37.5	354 37.3	21 44	109 40	92 38.8	38 43.7	130 40
	AB	0 0	1 4	1 2.4	36 4.2	38 4	0 0	12 4	10 4.2	2 2.3	12 3.7
	B	4 24	6 26	7 16.6	114 13.1	131 13.8	9 19	45 16	42 17.7	12 13.8	54 16.7
	O	9 52	9 40	16 38	387 44.6	421 44.4	18 37	110 40	93 39.3	35 40.2	128 40
	N/A*	0 0	0 0	0 0	5 0.6	5 0.5	0 0	0 0	0 0	0 0	0 0
Rh Type # (%)	-	1 6	2 9	5 12	129 14.9	137 14.5	6 12.5	38 14	31 13	13 15	44 14
	+	16 94	21 91	37 88	733 84.5	807 85	42 87.5	238 86	206 87	74 85	280 86
	N/A*	0 0	0 0	0 0	5 0.6	5 0.5	0 0	0 0	0 0	0 0	0 0

* ABO and Rh group for 5 patients were unknown because these patients were admitted under emergent events and cross matches were not done.

Thal = Thalassemia; SCD = Sickle Cell Disease; AA = Aplastic Anemia; HSCT = Hematopoietic Stem Cell Transplant; Hem Malign = Hematologic Malignancy; ST = Solid Tumor; N/A = Not Available

2. Red Cell Utilization

The RC utilization in the control and study groups is presented by diagnosis in Table 2

below. Overall, patients in the study group required more RC units (median 7) than

control patients (median 2); hence, the potential for alloimmunization based on exposure would appear to be greater in the study group.

Table 2: Red Cell Utilization by Diagnosis

Diagnostic Group	# patients	# RC units transfused	Median # RC units/ patient (Q1-Q3)	Median # days with a transfusion episode/ patient (Q1-Q3)
CONTROL GROUP				
Thalassemia	17	1600	39 (5-132)	38 (3-90)
SCD	23	238	3 (1-11)	2 (1-9)
Aplastic Anemia	42	591	5.5 (3-12)	4 (2-8)
Other*	867	3603	2 (1-4)	1 (1-2)
Overall	949	6032	2 (1-5)	1 (1-3)
Overall excluding hemoglobinopathy patients	909	4194	2 (1-5)	1 (1-2)
STUDY GROUP				
Hematologic Malignancies (leukemia, lymphoma)	237	2490	7 (4-14)	6 (3-10)
Solid Tumors	87	1184	9 (4-20)	9 (4-16)
HSCT	48	789	16 (6.5-24.5)	10 (6-18)
Non-HSCT	276	2885	7 (4-13.5)	5 (3-11)
Overall	324	3674	7 (4-16)	6 (4-11)

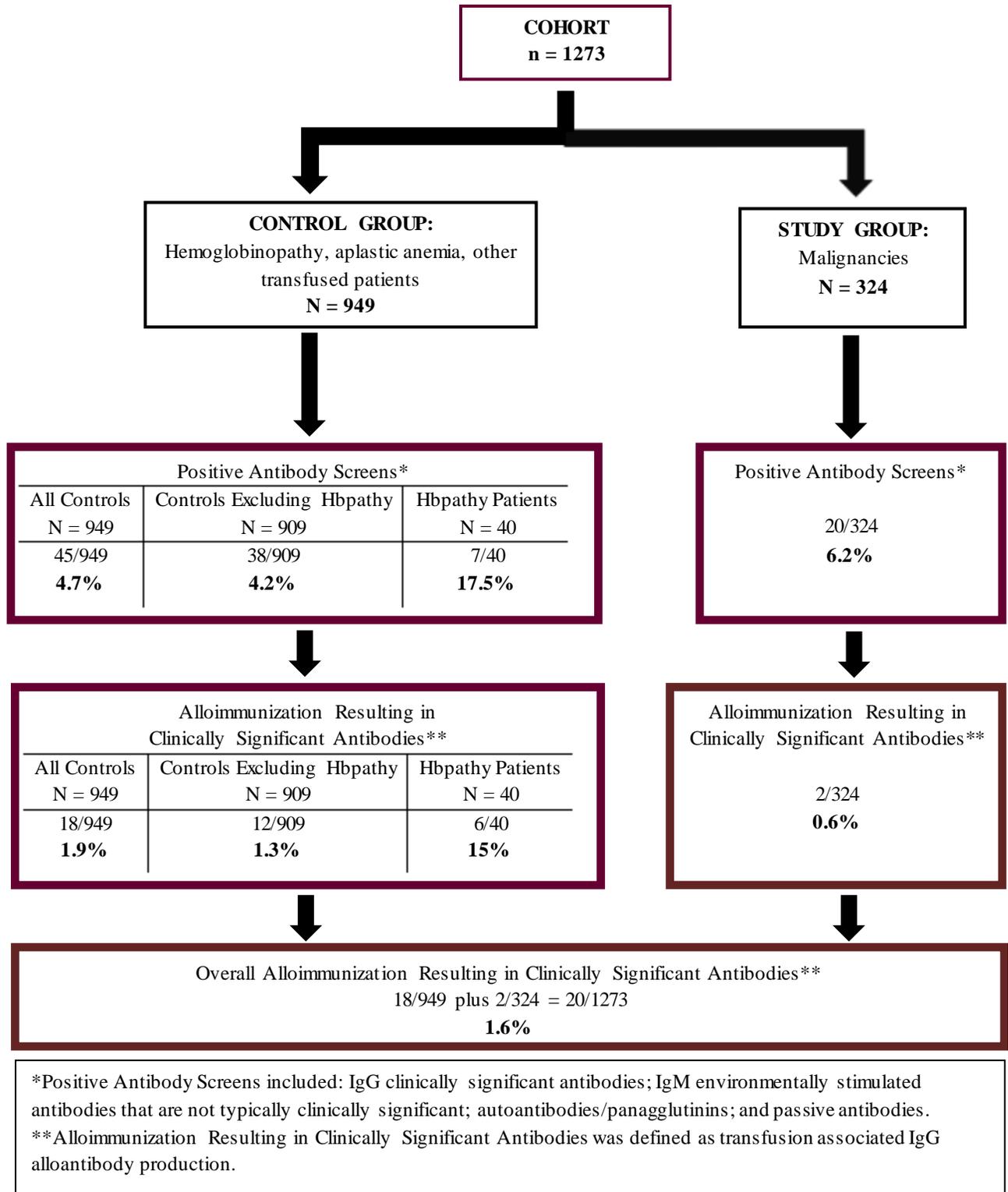
*patients receiving red cell transfusion for surgical, medical or trauma situations
 RC = Red Cell; SCD = Sickle Cell Disease; HSCT = Hematopoietic Stem Cell Transplant

3. Overall Study Results

Patients typically have an antibody screen performed before receiving a red cell transfusion; hence, many of the patients in this study had multiple antibody screens over the course of the study period. Not all positive antibody screens (PAS) are equal in terms of clinical relevance. Positive antibody screens can be divided in four categories: 1) some

antibodies are naturally stimulated from environmental exposures; 2) some are passively acquired from plasma transfusions; 3) others are autoantibodies/panagglutinins; and 4) some are IgG antibodies stimulated by red cell transfusion and usually clinically significant antibodies (CSA; true alloimmunization). In this study, PAS were defined as all antibodies detected by doing an antibody screen, regardless of specificity, and CSA were considered as active alloimmunization resulting in an IgG antibody that is known to have the potential to cause red cell destruction. The overall results are summarized in a flowchart in Figure 2 and described in more detail in the sections below.

Figure 2: Results Flowchart



3.1 Positive Antibody Screens

One or more PAS were detected in 65/1273 patients: 45 control patients; and 20 study patients. Overall, the proportion of patients with a PAS (all antibodies regardless of specificity and source i.e. passive or active alloimmunization) was not statistically different between control patients (4.7%; 95% CI: 3.4, 6.1) and study patients (6.2%; 95% CI: 3.6, 8.8), $p=0.30$.

The high frequency of PAS in the hemoglobinopathy patients (17.5%) is consistent with what has been published in the literature. When hemoglobinopathy patients were removed from the control group (Aygun 2002, Saied 2011), the percentage of control patients with a positive antibody screen decreased to 4.2% compared to 6.2% in the study patients ($p=0.14$). HSCT recipients had a higher rate of positive antibody screens (14.6%; 95% CI: 4.6, 24.6) than non-HSCT (4.7%; 95% CI: 2.2, 7.2) and this difference appeared to be statistically significant ($p=0.02$). This finding is likely explained by passive antibodies resulting from the infusion of plasma products during transplant such as IVIg, but IVIg data were not available from the transplant centre to confirm this hypothesis. Positive antibody screens occurred in 5.5% (95% CI: 2.6, 8.4) of patients with hematologic malignancies and 8.0% (95% CI: 2.3, 13.8) of patients with solid tumors ($p=0.40$).

3.2 Clinically Significant Antibodies

When the specificities of the antibodies responsible for the positive antibody screens were scrutinized, it was noted that some of these positives detected antibodies that were

naturally (environmentally) stimulated IgM rather than transfusion-induced IgG antibodies. Other antibodies were shown to be passively acquired from transfusion of plasma-containing blood products, and in some cases the antibodies were auto (panagglutinins) not alloantibodies. It was determined that 18 of the 45 patients with a PAS in the control group and 2 of the 20 patients with a PAS in the study group were due to a clinically significant IgG antibody (CSA) arising from transfusion associated alloimmunization (Tables 3, 4 and 5 below). The frequency of alloimmunization resulting in CSA was 1.6% overall (20/1273): 0.6% (95% CI: 0, 1.47) in the study group (2/324) and 1.3% (95% CI: 0.58, 2.06) in the control group (12/909). Knowing that Fisher's exact test is superior to the chi-square test when an outcome frequency is below 5, the Fisher's exact test was performed showing a p value of 0.38. The contingency table is presented in Table 6 below. The odds ratio of alloimmunization in the study group compared to the control group is 0.46 (95% CI: 0.10, 2.09). In other words, the study group is about half as likely to form CSA as the control group, but the 95% CI for the OR includes 1, so the true OR could be between 0.10 and 2.09. Figure 3 represents a bar plot of patients with positive antibody screens and clinically significant alloimmunization in control and study groups. Table 3 presents a list of CSA.

Table 3: Antibody Specificities in Control and Study Groups

Antibody	Control Group		Study Group	
	# antibodies by specificity	Total # of patients	# antibodies by specificity	Total # of patients
Clinically significant (IgG) alloantibodies	9 anti-E 5 anti-Jk ^a 4 anti-Kell 4 anti-C 2 anti-D 1 anti-c 1 anti-e	18	1 anti-D 1 anti-E	2
Others (Not considered active alloimmunization)		27		18
Passive	11 anti-D 1 anti-C 1 anti-e		6 anti-D 1 anti-E	
Naturally occurring IgM	3 anti-M 2 anti-A ₁ 1 anti-Le ^a		1 anti-A ₁ 2 anti-M	
Autoantibody/ Panagglutinin	4		1 Rouleaux 1 Cold autoantibody	
Unidentified (specificity undetermined)	13		5	

Table 4: Summary of the number (%) of patients with a Positive Antibody Screen (PAS) in Control and Study Groups by diagnostic category

	Control Group					Study Group				
	Thal	SCD	AA	Other*	Total	HSCT	Non-HSCT	Hem Malig	ST	Total
# of Patients	17	23	42	867	949	48	276	237	87	324
# of patients with an antibody screen result after their first study period transfusion	16	13	33	389	451	47	258	225	80	305
# Patients with PAS**	3	4	2	36	45	7	13	13	7	20
Percentage	17.6	17.4	4.8	4.2	4.7	14.6	4.7	5.5	8.0	6.2
95% CI	0, 35.8	1.9, 32.9	0, 11.2	3.0, 5.4	3.4, 6.1	4.6, 24.6	2.2, 7.2	2.6, 8.4	2.3, 13.8	3.6, 8.8

PAS = Positive Antibody Screen; Thal = Thalassemia; SCD = Sickle Cell Disease; AA = Aplastic Anemia; HSCT = Hematopoietic Stem Cell Transplant; Hem Malig = Hematologic Malignancy; ST = Solid Tumor

* Patients receiving red cell transfusion for surgical, medical or trauma situations.

** Positive antibody screen (PAS) defined as any positive result including IgG, naturally stimulated IgM or passive antibody.

NOTE: The data in the shaded columns are further categorized in Table 5

Table 5: Summary of the number (%) of patients in the study group with a Positive Antibody Screen (PAS) categorized by type of hematologic malignancy and type of solid tumor

	Study Group														
	Hematologic Malignancies					Solid Tumors									
Type	AML	ALL	HL	NHL	Total	NBL	WT	OS	GCT	RMS	HCC	ESFT	RCC	Other	Total
# patients	26	161	23	27	237	22	15	14	6	9	1	4	7	9	87
# Patients with PAS	3	8	1	1	13	3	0	1	0	1	1	0	0	1	7
% Patients with PAS	11.5	5.0	4.3	3.7	5.5	13.6	0	7.1	0	11.1	100	0	0	11.1	8.0

PAS = Positive Antibody Screen; AML = Acute Myeloblastic Leukemia; ALL = Acute Lymphoblastic Leukemia; HL = Hodgkin Lymphoma; NHL = Non-Hodgkin Lymphoma; NBL = Neuroblastoma; WT = Wilms Tumor; OS = Osteogenic Sarcoma; GCT = Germ Cell Tumor; RMS = Rhabdomyosarcoma; HCC = Hepatocellular Carcinoma; ESFT = Ewing Sarcoma Family of Tumors; RCC = Renal Cell Carcinoma

Table 6: 2x2 Contingency Table for Clinically Significant Alloimmunization (CSA)

	CSA		Total	%	95% CI
	Yes	No			
Study Group	2	322	324	0.6	0, 1.47
Control Group (Excluding Hemoglobinopathy Patients)	12	897	909	1.3	0.58, 2.06
Total	14	1219	1233		
OR: (2/322) / (12/897) = 0.6 / 1.3	0.46				0.10, 2.09

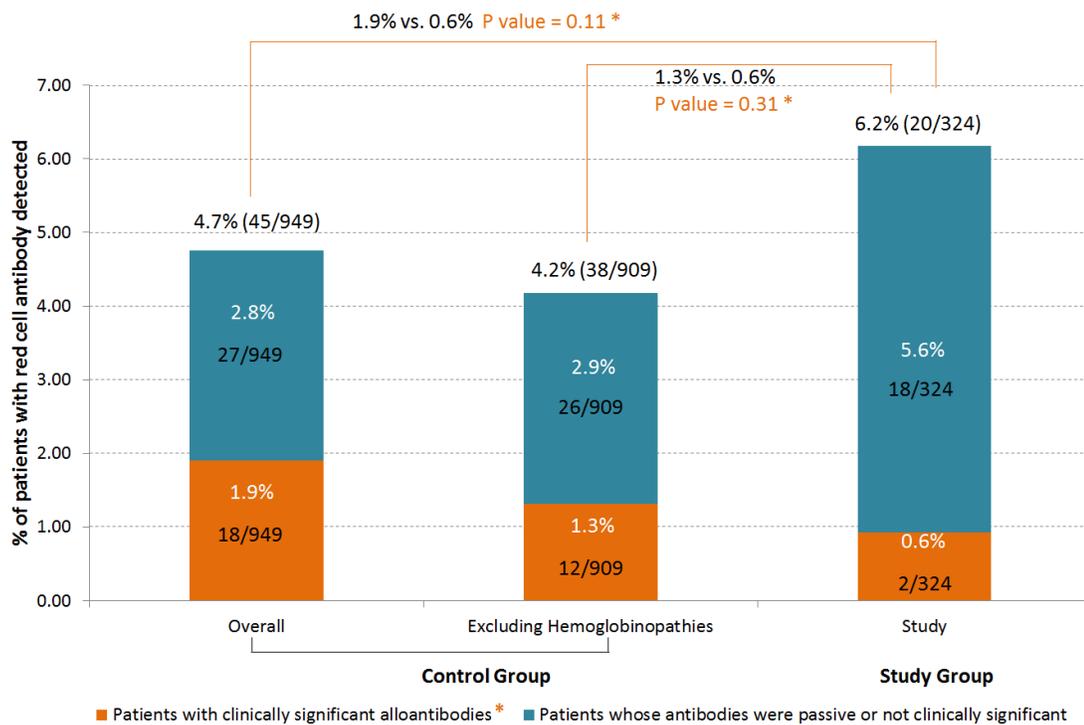


Figure 3: Bar plot of % patients with positive antibody screens and the proportion that represented clinically significant alloimmunization in control and study groups

3.3 Alloimmunized Cases in the Study Group

There were only two patients in the study group who became alloimmunized. The clinical details for these two patients are summarized below. A timeline diagram for these two alloimmunized study patients is illustrated in Figure 4.

Study ID H0208: This patient was diagnosed with AML/MDS in July 2000 at the age of 10 years. A matched unrelated donor (MUD; donor age and sex not available) HSCT was performed on January 19, 2001 (before study period). The patient relapsed in Nov 2003 and underwent a second MUD HSCT (donor age and sex not available) in February 2004 and was discharged from the transplant centre on June 11, 2004 with follow up at our oncology centre. The patient passed away in January 2005 from respiratory failure and shock. The ABO and Rh groups of the donors for each HSCT were not available. The patient's blood type was A+. Transient anti-D was detected (2 months after discharge from the transplant centre) on August 9, August 16 and September 10, 2004 and screens at our hospital were negative before and after these dates. The last transfusion at our hospital before the positive antibody screen on August 9, 2004 was a RC transfusion on January 25, 2004, but we were unable to obtain transfusion records from the transplant centre. She received IVIg on May 8, 2002 and July 31, 2002 and Varicella Zoster Immunoglobulin on January 21, 2002. Her HSCT regimen would have included IVIg infusions as well. The cause of her anti-D is unknown and it is unlikely that she developed anti-D because her Rh antigen status is positive (blood type A+). One possibility may be that the stem cell donor's Rh status was negative, resulting in transient donor anti-D against the recipient's Rh antigen or passive antibody from the administration of RhIg. Unfortunately transfusion data and donor information cannot be obtained; hence, it is impossible to make a definitive conclusion around alloimmunization.

Study ID H1258: This patient was diagnosed with high risk pre-B cell ALL at the age of 17 years and began chemotherapy on August 15 2008 which included asparaginase but not rituximab. She completed chemotherapy on September 3 of 2010 (no HSCT) and is doing well. Transfusions prior to alloimmunization included two RC units on August 9, two RC units on Aug 12, one Single Donor Platelet (SDP) unit on Aug 15, and another SDP on August 19, 2008. No IVIg was administered to this patient. Transient anti-E was detected on August 18 and Aug 29, 2008 and screens were negative before and after these dates. Both SDP units tested negative for antibodies suggesting that this was not passive antibody transfer from the transfused platelets.

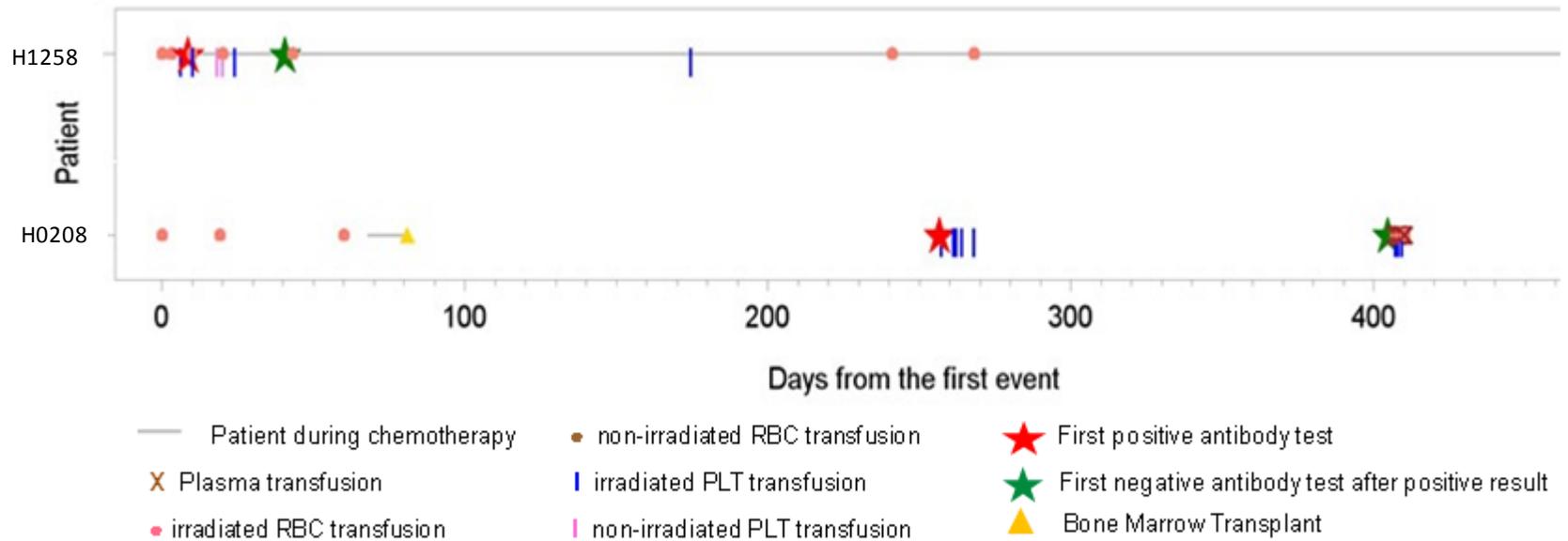


Figure 4: Timeline diagram of the 2 alloimmunized study group patients. Day 0 is the beginning of the study observation period. Patient H1258 was not transfused before Day 0. Patient H0208 was transfused before Day 0 because therapy (including HSCT) was initiated before the study period.

4. Effect Modification by Other Medications

It was determined *a priori* that the use of medications such as asparaginase and rituximab would be evaluated as effect modifiers of alloimmunization because of their immunosuppressive effects. As stated in chapter 4, it was no longer possible to perform a logistic regression to evaluate this potential effect modification due to the low number of outcome events as shown in Table 7. Hence, this question remains unresolved.

Table 7: Alloimmunization in Study Group Patients who Received Asparaginase or Rituximab

Chemotherapeutic agents	Study Patients			
	# patients	# patients with PAS	# patients with CSA	Antibody Specificity
Asparaginase	180	9	1	* Anti-E
No Asparaginase	144	9	1	**Anti-D
Rituximab	11	0	0	-
No Rituximab	313	20	1	**Anti-D

* Patient H1258 only received asparaginase.

** Patient H0208 did not receive asparaginase or rituximab.

PAS = positive antibody screen, CSA = clinically significant alloimmunization

5. Data Validation Prior to Analysis

At the end of the chart review process, thirty charts (10 per CRF user) were randomly chosen (by a computer) and the chart reviews were redone by a different person in order to validate data collection results. For example, 10 of observer A's charts were reviewed by observer B; 10 of observer B's charts were reviewed by observer C; and 10 of observer C's charts were reviewed by observer A. There was excellent inter-observer agreement. In total, two discrepancies involved only minor elements such as the date of stem cell infusion, and none involved critical elements such as diagnosis.

Not all antibody screen results were in TRUST, so these data were obtained from the LIS which contains comprehensive screen results. The LIS data were available only in text format, so in order to make them amenable to statistical analysis each text-based data entry was translated into a code that identified the antibody specificity. One study patient's (H0616) antibody screen result was miscoded, which made a "passive anti-D" result appear as "anti-e". Upon data cleaning, this data entry error was detected and corrected. Therefore, initially it appeared that three of the 20 study patients had a CSA, but after data cleaning two patients were determined to have a CSA, highlighting the importance of data cleaning.

CHAPTER VI: Discussion and Conclusions

1. Alloimmunization

How is it detected?

Red cell alloimmunization is detected by performing an antibody screen using a method that has good sensitivity for detecting clinically significant IgG antibodies. However, some antibody screening techniques lack specificity and detect IgM antibodies which are environmentally stimulated and usually of no clinical significance.

How were clinically significant antibodies defined?

In this study, the specificities of the antibodies were captured from the laboratory transfusion records, and using this information it was possible to classify the antibodies as: clinically significant IgG; environmentally stimulated IgM; passively acquired antibodies from a blood product; or autoantibody. Only patients who developed clinically significant IgG alloantibodies were categorized as alloimmunized in this study. This definition of alloimmunization avoided overestimating the true frequency of alloimmunization in these cohorts and focused only on clinically significant alloantibodies.

2. Main Study Findings and Interpretation

There were two objectives for this study: 1) to describe RC alloimmunization frequency and alloantibody specificities in transfused pediatric patients and their diagnostic

subgroups; and 2) to determine if immunosuppressive chemotherapy affects the frequency of red cell alloimmunization.

2.1 The Frequency of Alloimmunization in Pediatric Patients

The study was able to address the primary objective of measuring the frequency of active alloimmunization in:

- All transfused pediatric patients: control and study groups combined (1.6%);
- Control group: all transfused children who did not receive chemotherapy (1.9%);
- Control group: defined above and excluding hemoglobinopathy patients (1.3%);
- Study group: children treated with chemotherapy (0.6%); and
- Pediatric patients with hemoglobinopathies (15%).

These findings are significant as they represent the first report of red cell alloimmunization in pediatric patients from a variety of diagnostic entities. Determining these different rates of alloimmunization has identified specific patient groups who were likely or unlikely to become alloimmunized.

There was some variability in the frequency of alloimmunization between patient subgroups in the study cohort (patients receiving chemotherapy); however, the differences were not statistically significant.

- Pediatric HSCT (2.1%)
- Pediatric Non-HSCT (0.4%)

- Pediatric Hematologic Malignancies (0.8%)
- Pediatric Solid Tumors (0%)

HSCT versus non-HSCT patients

The frequency of alloimmunization was 2.1% (95% CI: 0, 6.1%) in HSCT patients, and 0.4% (95% CI: 0, 1.1%) in non-HSCT patients; $p = 0.41$. Each of the two alloimmunized patients in the study group had a different HSCT status: one underwent HSCT, and one did not. The alloimmunization event rate was low and the number of patients in each of these groups was relatively low; hence, the study was not powered to find a significant result. Our finding is similar to a report in the literature by Perseghin et al., who showed that 1% of patients produced new RC alloantibodies after HSCT (Perseghin 2003). HSCT patients in our cohort utilized more RC units than non-HSCT patients, so one might expect a higher frequency of alloimmunization because of increased RC exposure. The effect of RC utilization on alloimmunization in HSCT patients may warrant further study.

Hematologic Malignancies vs. Solid Tumors

Alloimmunization may be affected by B lymphocyte activity, so cancers affecting B cell function such as pre-B cell ALL may in theory have lower alloimmunization rates. In our study group, one alloimmunized patient had pre-B ALL and the other had AML/MDS, both hematologic malignancies. These small numbers precluded any conclusions about alloimmunization in pediatric patients with malignancies affecting B cells.

Cancers that suppress the bone marrow such as hematologic malignancies (leukemia, lymphoma) could cause reduced alloimmunization rates compared to solid tumors that do not involve the bone marrow. A difference in the frequency of alloimmunization could not be observed between patients with hematologic malignancies (0.8%; 95% CI: 0, 2.0) and solid tumors (0%; 95% CI: 0, 4.2); $p = 0.16$. Once again this may be due to a lack of power as there were only 237 and 87 patients in these groups respectively and the frequency of alloimmunization was low.

Pediatric Cancers by Diagnosis

The data presented in Table 5 shows the distribution of positive alloantibody screens among different diagnostic categories of pediatric cancers. Once the specificities of alloantibodies are scrutinized, only one of 26 AML patients (3.8%) and one of 161 ALL patients (0.6%) had active alloimmunization with clinically significant antibodies. The rest of the pediatric cancers did not show active alloimmunization. Further research with a much larger sample size would be needed to make any definitive conclusion about differences in alloimmunization between different pediatric cancers especially those who undergo HSCT. However, it is interesting that although the frequency of alloimmunization is extremely low, the laboratory routinely performs antibody screens on these patients which utilize technical resources and comes at a cost. The results raise the question as to whether the frequency of testing could be reduced with minimal additional risk.

Patients with Hemoglobinopathies

Our results confirm high alloimmunization rates in hemoglobinopathy patients (15%) as reported in the literature (Aygun 2002, Saied 2011). This finding provides reassurance that the methods of sample identification and data collection in the study were sound, and suggests that our results could be generalizable to other centres with similar patient populations.

2.2 What can we learn from the positive antibody screen data?

As mentioned previously the antibody screen is not specific for IgG clinically significant alloimmunization. The screening technique also detected autoantibodies, passive antibodies and some IgM environmentally stimulated antibodies that are not typically clinically significant. It is also important to note that some patients had more antibody screens performed than others as this test is typically done when a patient requires a transfusion. So a patient who requires more transfusions will have greater antigen exposure and presumably an increased risk alloimmunization, and will have more antibody screens performed increasing the likelihood that an antibody would be detected.

Some of the patients in our study were given plasma products or IVIG which may result in positive antibody screens because of passive antibody transfer. The majority of patients with passive anti-D were in the “Other” subgroup of the control group, which included patients transfused for surgical, medical or trauma situations (non-hemoglobinopathy, non-aplastic anemia patients). These patients may acquire anti-D from plasma containing

products. There were 9 patients with IgM antibodies and 6 patients with autoantibodies. These patients came from a variety of diagnostic categories.

When the frequency of positive antibody screens were considered regardless of antibody specificity or passively/actively acquired, more HSCT patients had positive antibody screens (14.6%; 95% CI: 4.6, 24.6) than non-HSCT patients (4.7%; 95% CI: 2.2, 7.2) and this difference appeared to be statistically significant ($p=0.02$). This potential difference in patients with positive antibody screens associated with HSCT status may have been related to a greater number of infusions of plasma-containing products such as IVIg during HSCT.

Positive screens occurred in 5.5% (95% CI: 2.6, 8.4) of patients with hematologic malignancies and 8.0% (95% CI: 2.3, 13.8) of patients with solid tumors ($p=0.40$). This rate in pediatric hematologic malignancies appears to be significantly lower than adult hematology/oncology patients (15% to 20%) (Sanz 2013, Schonewille 2009). This difference could be related to the fact that children have had less antigen exposures than adults, or that children receive less plasma products or IVIg, resulting in a lower rate of positive antibody screens in children.

The high frequency of positive antibody screens - many of which were due to passive antibody or IgM - illustrates a potential specificity issue with the screening technique. Detecting IgM and/or passive antibodies results in additional work and investigation for

the laboratory to identify and characterize antibodies that have no impact on clinical care. Like the low rate of alloimmunization, the detection of antibodies that have no clinical relevance raises the issue as to whether the frequency of screening could be reduced to improve the cost/safety balance.

2.3 The Effect of Chemotherapy on Alloimmunization

If chemotherapy impacted alloimmunization, it might be possible to identify individuals who were unlikely to become alloimmunized due to immune suppression and this in turn could guide clinical and laboratory practices and lead to streamlined laboratory testing. The study was not able to explore the association between chemotherapy and alloimmunization due to the low outcome frequency and limited sample size. Fisher's exact test showed a p value of 0.38, and the odds ratio (OR) of alloimmunization in the study group compared to the control group was 0.46, but the 95% CI showed that the true OR could be between 0.10 and 2.09. The study would require a larger sample of transfused children in order to attain sufficient power to ascertain the effect of chemotherapy on alloimmunization. Multiple pediatric centres would need to collaborate on such a study to achieve adequate power.

In our study, only one patient (H1258) was confirmed to have an alloantibody (anti-E). This patient received platelets raising the possibility that the antibody was passively acquired; however, follow up with Canadian Blood Services confirmed that the antibody screens on the plasma from the donors were negative, ruling out passively acquired anti-

E. Patient H0208 underwent a HSCT; however, the transplant and transfusion data were not available from the transplant centre. It is possible that the patient received plasma products and/or IVIG and her anti-D could have been passively acquired, or the anti-D could have been from a donor-recipient D antigen mismatch. To be on the conservative side this patient was categorized as alloimmunized; however, if this was a passively acquired antibody the alloimmunization frequency in the chemotherapy group would have decreased to 0.3% (1 alloimmunized patient) instead of 0.6% (2 alloimmunized patients).

Asparaginase and Rituximab

Asparaginase is a drug used in therapy for ALL, AML, and Non-Hodgkin Lymphoma (NHL). It is an enzyme that hydrolyzes and depletes the amino acid asparagine (required for DNA synthesis) which inhibits survival of cancer cells. This enzyme may also deplete the quantities of other proteins such as alloantibodies. Rituximab is a drug used in therapy for ALL and NHL. It is a monoclonal antibody against CD20 which is a protein found on the surface of the immune system's B cells. When it inhibits B cells (essential in antibody production) alloantibody quantities may be reduced. For this study, data for these two drugs were available and collected; however, because of the low frequency of alloimmunization in this study, no definitive conclusions could be drawn.

Only 1 of 180 patients who received asparaginase (0.6%) and 1 of 144 who did not receive asparaginase (0.7%) developed active alloimmunization. There are no published studies of red cell alloimmunization in the setting of asparaginase therapy. None of the 11

patients who received rituximab, and 1 of the 313 who did not receive it (0.3%) developed active alloimmunization. There is one case report in the literature of rituximab used successfully in conjunction with systemic steroids to treat red cell anti-E alloimmunization in a 15 year old girl with sickle cell disease (Cattoni 2013).

3. Other Factors That Could Affect Alloimmunization

It is important to acknowledge that there are other factors which could also affect alloimmunization. These include:

Intensity of chemotherapy: Certain phases of chemotherapy regimens are more toxic and more immunosuppressive than others, which has led to their classification into induction, intensification or consolidation, and maintenance phases. Not all chemotherapy regimens are equal in intensity. For example, acute myeloid leukemia (AML) regimens lead to greater toxicity and immunosuppression than standard risk acute lymphoblastic leukemia (ALL) regimens. Grading chemotherapy regimens by intensity is difficult. Regimens were often modified based on the patient's cancer's response to the drugs, and new protocols were coming into effect during the study period of 2002 - 2011. The literature does not identify an objective measurement of chemotherapy intensity. Potential measurements would include the absolute neutrophil or lymphocyte count but these are often affected by other factors such as infections and medications. Hence, for this retrospective study, it was not possible to objectively measure the intensity of chemotherapy for each patient.

Ethnicity: Red cell alloimmunization may increase due to antigenic differences between blood donors and recipients from different ethnic backgrounds. This is exemplified by the high alloimmunization rates in sickle cell disease patients (African ethnicity) whose blood donors in Canada are predominantly of North American Caucasian ethnicity. For this study, ethnicity data were not available within the hospital or research databases.

Radiation therapy (RT): Radiating the bone marrow may weaken the immune system which may lead to reduced alloimmunization and increase the need for transfusion due to bone marrow suppression. For this study, it was not possible to obtain exact RT data (see the Study Limitations section below).

4. Potential Biases in Retrospective Studies

Information and Selection Bias

Information bias results from incorrect determination of the exposure and/or the outcome or unequal searching for the outcome in the two groups. Because this study is observational in nature, a secondary data collection method has been implemented meaning that the data were collected for purposes other than the study (i.e. for clinical care), so it is not possible to control some potential sources of bias. Secondary data collection carries a high risk of information bias. For this reason, we see in Table 4 that some patients had no follow-up antibody screen after a transfusion (i.e. missing outcome measurement). In fact, control patients only had a follow up screen in 47.5% of cases (451 / 949), while the study group were followed up with a screen in 94.1% of cases (305

/ 324). But this bias was unavoidable in this retrospective cohort study as it was due to the fact that patients undergo antibody screening before a red cell transfusion to ensure donor-recipient antigen matching, and most control patients did not require a subsequent transfusion. Bias was minimized because data were collected in the same way for both control and study groups (Grimes 2002).

Selection bias results from poor comparability between the control and study group.

Table 1 shows that both groups were similar in demographic and blood type characteristics; however, there may have been other characteristics that we were not able to capture which could have varied between the control and study patients.

Internal and External Validity

Systematic error was minimized by precisely and clearly defining the outcome measurement: initially capturing information on any positive antibody screen, then narrowing the measurement down to clinically significant antibodies only. In other words, the measurement of alloimmunization in our study truly measures what it purports to measure. This measurement accuracy strengthens the validity of the results and minimizes false-positive reports.

Random error was minimized by ensuring that the same result was obtained for a given subject regardless of who was doing the data collection. This was achieved by training research personnel in the standardized approach to data collection and performing a

triplicate chart review of a randomly selected patient sample. The reproducibility of the data reinforces the reliability of the results. Data cleaning was performed at multiple points in the study, ensuring that data entry errors are detected within Case Report Forms, antibody screening results, and study groups assignments. It is conceivable that antibody testing errors had occurred at the time of lab sample collection and the research investigators would not have been able to detect such errors.

Control for Confounding

Threats to study validity were met with strategies to control for confounding as explained in Chapter II. Confounders included: neonatal age, immunodeficiency, other immunosuppressive drugs, CNS tumors. They were treated with restriction (exclusion criteria). However, it is acknowledged that confounding is always an issue with observational studies as there may be confounders that are imbalanced and/or not identified between groups.

Judgement of Significance

The p value reflects the likelihood of getting the results we obtained given that the null hypothesis is true (Null hypothesis: There is no association between chemotherapy and alloimmunization). If the p value is equal to or less than the significance level (5%), then the null hypothesis must be rejected and the alternative hypothesis is accepted. Knowing that this study may be underpowered to find a clinically important difference, the author did not use only the p value. All results were reported using the 95% confidence intervals

(CI) which offer more information about the plausible range within which the estimate falls.

5. Study Limitations

The study was limited by low power due to a low frequency of alloimmunization making it difficult to detect a statistically significant difference even with a sample as large as 1273 patients. The sampling strategy was one of convenience dictated by the number of children transfused at our centre. The study highlights the importance of multi-centre collaboration in pediatric transfusion and pediatric oncology research when dealing with extremely low outcome event rates.

Information about radiation therapy (RT) was not specifically collected and some may consider this a limitation of the study. Radiation therapy may suppress the bone marrow weakening the immune response and increasing the need for transfusion, but in this study RT was considered part of the overall therapeutic regimen along with chemotherapy so RT data were not collected. Also, the effects of RT on the immune system may vary between negligible and important depending on the area of the body being radiated, and data on location of radiation were not possible to obtain. Pediatric patients treated with surgery and radiation therapy alone without chemotherapy are quite rare, so this was not expected to cause sample bias.

Patients with CNS tumors were excluded, and some may suggest that they would be appropriate control patients as they may not receive chemotherapy. For example, patients with glioma do not receive immunosuppressive chemotherapy while those with medulloblastoma do, and frequently these patients fall under the same ICD-10 code as ‘CNS tumor’ which renders their differentiation problematic with a retrospective study design. In order to include CNS tumor patients, future studies should involve a neuro-oncologist who may be able to advise on the chemotherapeutic heterogeneity of this group in order to properly assign patients to the control and study arms.

Immunosuppressive drugs used after HSCT (such as cyclosporine and tacrolimus) were not recorded because their effect on alloimmunization is unclear in the literature, and these drugs are dosed by patient response causing their effect, if any, to be fluctuant and difficult to track by retrospective chart review.

Some patients had no subsequent transfusion and, therefore, no follow-up antibody screen. This may have led to underestimation of the number of positive antibody screens and active alloimmunization in both the control and study groups.

6. Post Hoc Sample Size Derivation

An *a priori* sample size calculation was not possible due to the lack of information about the frequency of the outcome, exposure and confounders. The actual power of this study to detect the observed difference in alloimmunization between the chemotherapy and the

control group was calculated post hoc to be 23%. Based on the information in Table 8 below, a sample size of 6016 patients would have been required to have 80% power ($\alpha=0.05$, and $\beta=0.8$) to make a definitive conclusion that the observed frequencies between groups were in fact statistically different.

Table 8: Sample sizes for a variety of potentially desired clinical differences. P is the proportion of patients with alloimmunization, delta is the clinical difference desired, and n is the number of patients for the study and control group (if $n=n_1=n_2$). $\alpha=0.05$; $\beta=0.8$.

P(study)	P(control)	delta	n
0.006	0.013	0.005	5895
0.006	0.013	0.007	3008
0.006	0.013	0.01	1474
0.006	0.013	0.015	655

7. Study Strengths

This is the first alloimmunization study in a transfused pediatric population with a control group lacking our exposure of interest (chemotherapy). See Appendix I for a summary of the literature regarding alloimmunization in oncology patients. This is also the first study that has looked at the frequency of alloimmunization in transfused pediatric patients by diagnostic category.

This novel research endeavor explores an area of medicine that has not been previously addressed with methodological rigor. Despite the limitations discussed in this chapter, this study presents a detailed account of methodological challenges expected in this area of medical research and proposes solutions to them. Future studies in this field may benefit from learning about our approach. The high alloimmunization rates in hemoglobinopathy patients correlate well with the literature, which is an attestation to the soundness of data collection methodology.

8. Conclusions and Future Directions

In summary, we conclude that the rate of alloimmunization in pediatric patients who receive chemotherapy and in those who do not receive chemotherapy is quite low. It appears to be significantly lower than adult patients who receive chemotherapy as summarized in Chapter I (Sanz 2013, Schonewille 2009). Pediatric hemoglobinopathy patients at our centre have a similar alloimmunization rate to those in other centres (Aygun 2002, Saied 2011). Further studies are needed to assess the effect on alloimmunization of the high RC utilization rate shown in cancer patients and HSCT recipients. HSCT data were lacking so future studies should ensure close collaboration with the transplant centre. Due to the limited sample from our single centre and the low outcome rate, our study could be considered a pilot study, and could lead to a larger collaborative study to address alloimmunization in pediatric patients. A prospective approach would ensure all antibody screens are recorded with minimal missing data, but our retrospective approach was financially advantageous.

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APPENDIX I: Studies of antibody formation in oncology patients

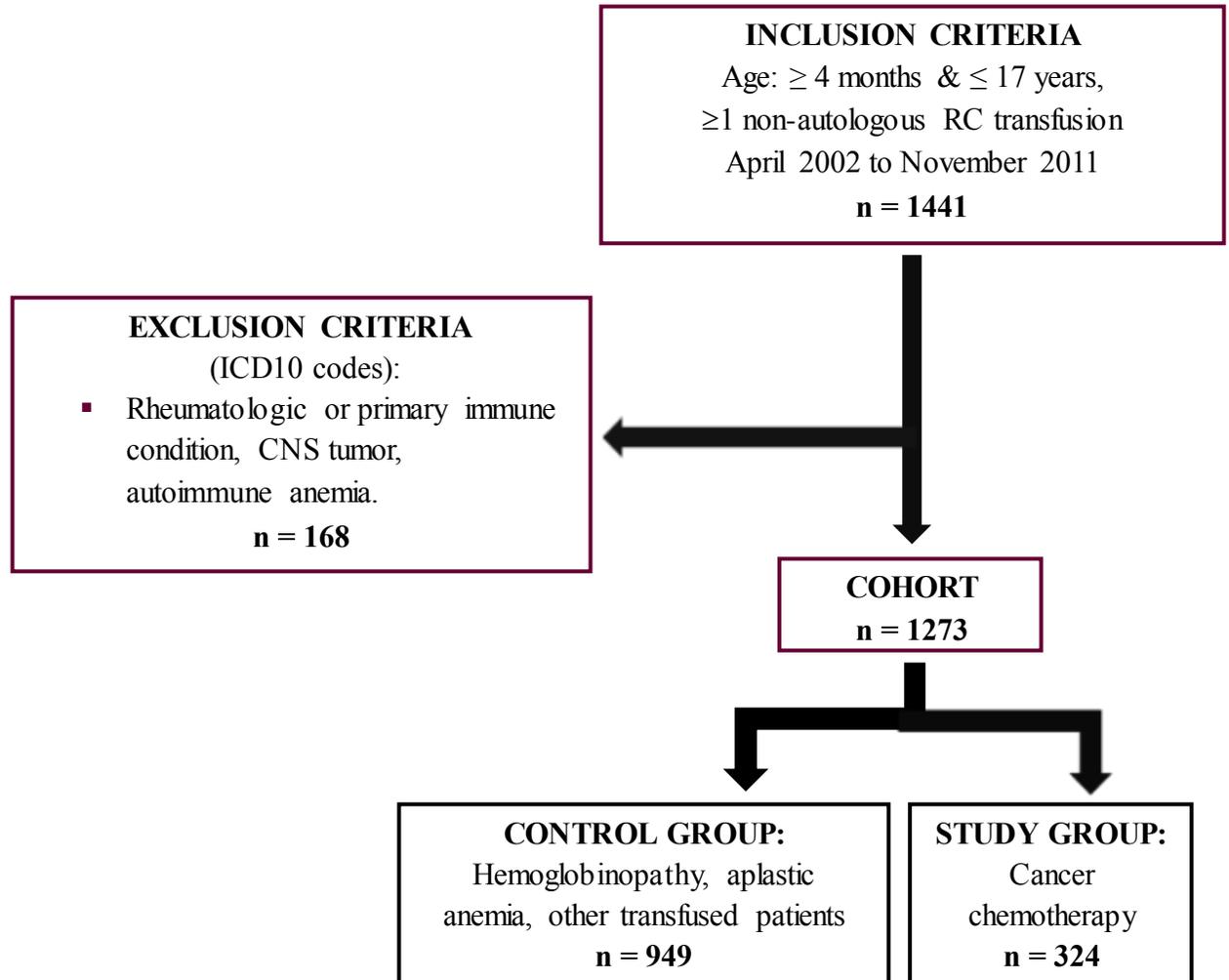
Article/Author	Design: PICOTS *	Findings	Limitations
Sanz 2013	<p>P: 272 patients with MDS/CMML</p> <p>I: Cancer treatment</p> <p>C: No comparator group</p> <p>O: Antibody screens: complex immunization defined as autoantibody or alloantibodies that decreased blood compatibility below 3%.</p> <p>T: 1990 to 2009</p> <p>S: Retrospective cohort design</p>	<p>15% of MDS/CMML patients develop RC alloimmunization.</p> <p>The majority of alloimmunization involves the Rh and Kell systems.</p> <p>Extended antigen matching would presumably reduce the RC immunization rate.</p>	<p>Restricted to MDS/CMML patients.</p> <p>Excluded HSCT patients.</p> <p>Mortality: 70%</p> <p>Lost to follow-up: 6%</p> <p>Included only patients who received at least 2 RC transfusions 1 month apart, so would miss patients who were alloimmunized after 1 RC transfusion.</p> <p>Mean age 74 years.</p>
Schonewille 2009	<p>P: 115 previously RC alloimmunized hemato-oncology patients (acute and chronic leukemia, CMPD, MDS, NHL, and MM)</p> <p>I: Additional RC transfusion</p> <p>C: No comparator group</p> <p>O: Antibody screens: primary (newly formed) and additionally formed</p>	<p>RC alloimmunization rate is 20% in hemato-oncology patients.</p> <p>Extended antigen matching may be considered.</p>	<p>Excluded patients with alloimmunization against low and high frequency antibodies and anti-M.</p> <p>Did not include patients with no previous alloimmunization.</p>

	<p>antibodies</p> <p>T: 1983 to 2007</p> <p>S: Retrospective cohort design</p>		
<p>Martín Ibáñez 2003</p>	<p>P: 50 pediatric patients with ALL</p> <p>I: ALL treatment on SHOP-LAL-94 protocol</p> <p>C: No comparator group, but 3 time points: ALL onset, during treatment, and 12 months after treatment finishes</p> <p>O: IgA, IgG and IgM levels</p> <p>T: April 1994 to August 1999</p> <p>S: Retrospective cohort design</p>	<p>Therapy for ALL is associated with a quantitative decrease in IgG and IgM, while IgA is less affected.</p> <p>Ig levels normalize before 1 year post treatment.</p>	<p>Objective was assessing ALL treatment's effect on quantitative immunoglobulin levels, not on alloimmunization.</p> <p>Only looked at ALL patients.</p> <p>Immunodeficiency patients were excluded, but other confounders were not discussed. Author mentions that IVIg is accounted for in the analysis but no explanation is provided.</p>
<p>Molnar 2002</p>	<p>P: Pediatric oncology patients who receive Rh (D) incompatible platelets</p> <p>I: Cancer therapy (7 received HSCT, 35 did not)</p> <p>C: No comparator group</p> <p>O: Presence of anti-D</p> <p>T: January 1997 to</p>	<p>Approximately 2300 D-incompatible transfusions were administered with no evidence of alloimmunization.</p>	<p>Did not address alloimmunization after RC concentrate transfusion.</p> <p>Focused on patients receiving D-antigen-incompatible platelet transfusions and the question of RhIg prophylaxis against D-alloimmunization specifically.</p>

	<p>June 2000</p> <p>S: Retrospective cohort design</p>		
Schonewille 1999	<p>P: 564 adult patients with malignant hematologic diseases</p> <p>I: RC containing transfusions</p> <p>C: No comparator group</p> <p>O: Antibody screens</p> <p>T: 1987 to 1996</p> <p>S: Retrospective cohort design</p>	<p>The rate of alloimmunization was (51/564) 9% overall.</p> <p>Patients who received more intensive chemotherapy produced alloantibodies at a much lower rate despite requiring more platelet transfusions.</p> <p>The study concluded that transfusion-related antibody formation in the adult oncology patient population was similar to reported rates in other diseases.</p>	<p>No clear definition of chemotherapy intensity.</p> <p>Only adult patients.</p> <p>133 (20%) patients were not evaluable due to lack of follow-up antibody screen.</p>

* PICOTS = Population, Intervention, Comparator, Outcomes, Timeline, Study design.
 RC = red cell; HSCT = hematopoietic stem cell transplant; MDS = myelodysplastic syndrome; CMML = chronic myelomonocytic leukemia; CMPD = chronic myeloproliferative disease; NHL = non-Hodgkin lymphoma; MM = multiple myeloma; ALL = acute lymphoblastic leukemia; IVIg = intravenous immunoglobulin; RhIg = Rh immunoglobulin

APPENDIX II: Patient Eligibility Flowchart



APPENDIX III: ICD-10 Codes

Study Group

Eligible patients were selected from the database and included in the study group if they were diagnosed with a malignancy based on these ICD-10 codes:

C22.2 Hepatoblastoma
C22.7 Other specified carcinomas of liver
C30.0 Nasal cavity (Olfactory neuroblastoma)
C41.9 Bone and articular cartilage, unspecified
C49.0 Connective and soft tissue of head, face and neck
C49.1 Connective and soft tissue of upper limb, including shoulder
C49.2 Connective and soft tissue of lower limb, including hip
C49.3 Connective and soft tissue of thorax
C49.4 Connective and soft tissue of abdomen
C49.5 Connective and soft tissue of pelvis
C49.6 Connective and soft tissue of trunk, unspecified
C49.8 Overlapping lesion of connective and soft tissue
C49.9 Connective and soft tissue, unspecified
C56.0 Malignant neoplasm of ovary, unilateral
C56.1 Malignant neoplasm of ovary, bilateral
C56.9 Malignant neoplasm of ovary, not specified whether unilateral or bilateral
C58 Malignant neoplasm of placenta
C62.0 Undescended testis
C62.1 Descended testis
C62.9 Testis, unspecified
C64 Malignant neoplasm of kidney, except renal pelvis
C69.2 Retina
C74.9 Adrenal gland, unspecified
C81.0 Nodular lymphocyte predominant Hodgkin lymphoma
C81.1 Nodular sclerosis classical Hodgkin lymphoma
C81.2 Mixed cellularity classical Hodgkin lymphoma
C81.3 Lymphocytic depletion classical Hodgkin lymphoma
C81.4 Lymphocyte-rich classical Hodgkin lymphoma
C81.7 Other classical Hodgkin lymphoma
C81.9 Hodgkin lymphoma, unspecified
C83.3 Diffuse large B-cell lymphoma
C83.5 Lymphoblastic (diffuse) lymphoma
C83.7 Burkitt Lymphoma
C84.6 Anaplastic large cell lymphoma, ALK-positive
C84.7 Anaplastic large cell lymphoma, ALK-negative
C85.1 B-cell lymphoma, unspecified
C85.2 Mediastinal (thymic) large B-cell lymphoma

C85.7 Other specified types of non-Hodgkin lymphoma
C85.9 Non-Hodgkin lymphoma, unspecified
C91.0 Acute lymphoblastic leukaemia [ALL]
C91.6 Polymorphocytic leukaemia of T-cell type
C91.7 Other lymphoid leukaemia
C91.8 Mature B-cell leukaemia Burkitt-type
C91.9 Lymphoid leukaemia, unspecified
C92.0 Acute myeloblastic leukaemia
C92.3 Myeloid sarcoma
C92.4 Acute promyelocytic leukaemia [PML]
C92.5 Acute myelomonocytic leukaemia
C92.6 Acute myeloid leukaemia with 11q23-abnormality
C92.8 Acute myeloid leukaemia with multilineage dysplasia
C92.9 Myeloid leukaemia, unspecified
C93.0 Acute monoblastic/monocytic leukaemia
C93.3 Juvenile myelomonocytic leukaemia
C93.7 Other monocytic leukaemia
C93.9 Monocytic leukaemia, unspecified
C94.0 Acute erythroid leukaemia
C94.2 Acute megakaryoblastic leukaemia
C94.6 Myelodysplastic and myeloproliferative disease, not classified
C94.7 Other specified leukaemias
C95.0 Acute leukaemia of unspecified cell type
C95.7 Other leukaemia of unspecified cell type
C95.9 Leukaemia, unspecified
C96.7 Other specified malignant neoplasms of lymphoid, haematopoietic and related tissue
C96.9 Malignant neoplasm of lymphoid, haematopoietic and related tissue, unspecified
D39.1 Ovary
D40.1 Testis
Z85.0 Personal history of malignant neoplasm of digestive organs
Z85.1 Personal history of malignant neoplasm of trachea, bronchus and lung
Z85.2 Personal history of malignant neoplasm of other respiratory and intrathoracic organs
Z85.3 Personal history of malignant neoplasm of breast
Z85.4 Personal history of malignant neoplasm of genital organs
Z85.5 Personal history of malignant neoplasm of urinary tract
Z85.6 Personal history of leukaemia
Z85.7 Personal history of other malignant neoplasms of lymphoid, haematopoietic and related tissues
Z85.8 Personal history of malignant neoplasms of other organs and systems
Z85.9 Personal history of malignant neoplasm, unspecified
Z94.8 Other transplanted organ and tissue status
Z94.9 Transplanted organ and tissue status, unspecified

Control Group

The control group was defined as patients who meet the eligibility criteria but who are not diagnosed with a malignancy and thus have not undergone the intervention of interest (chemotherapy). The following ICD-10 codes were used to divide the control group into subgroups:

THALASSEMIAS

- D56.0 Alpha thalassaemia
- D56.1 Beta thalassaemia
- D56.2 Delta-beta thalassaemia
- D56.3 Thalassaemia trait
- D56.4 Hereditary persistence of fetal haemoglobin [HPFH]
- D56.8 Other thalassaemias
- D56.9 Thalassaemia, unspecified

SICKLE CELL DISORDERS

- D57.0 Sickle-cell anaemia with crisis
- D57.1 Sickle-cell anaemia without crisis
- D57.2 Double heterozygous sickling disorders
- D57.3 Sickle-cell trait
- D57.8 Other sickle-cell disorders

APLASTIC ANEMIAS

- D60.0 Chronic acquired pure red cell aplasia
- D60.1 Transient acquired pure red cell aplasia
- D60.8 Other acquired pure red cell aplasias
- D60.9 Acquired pure red cell aplasia, unspecified
- D61.0 Constitutional aplastic anaemia
- D61.1 Drug-induced aplastic anaemia
- D61.2 Aplastic anaemia due to other external agents
- D61.3 Idiopathic aplastic anaemia
- D61.8 Other specified aplastic anaemias
- D61.9 Aplastic anaemia, unspecified

Exclusions

Patients who met the inclusion criteria were excluded if they were diagnosed with any of the following as the ‘Primary Diagnosis’ or the ‘Most Responsible Diagnosis’ by ICD-10 coding:

- C71.0 Cerebrum, except lobes and ventricles
- C71.1 Frontal lobe

C71.2 Temporal lobe
C71.3 Parietal lobe
C71.4 Occipital lobe
C71.5 Cerebral ventricle
C71.6 Cerebellum
C71.7 Brain stem
C71.8 Overlapping lesion of brain
C71.9 Brain, unspecified
C72.0 Spinal cord
C72.1 Cauda equina
C72.2 Olfactory nerve
C72.3 Optic nerve
C72.4 Acoustic nerve
C72.5 Other and unspecified cranial nerves
C72.8 Overlapping lesion of brain and other parts of central nervous system
C72.9 Central nervous system, unspecified
C96.0 Multifocal and multisystemic (disseminated) Langerhans-cell histiocytosis [Letterer-Siwe disease]
C96.5 Multifocal and unisystemic Langerhans-cell histiocytosis
C96.6 Unifocal Langerhans-cell histiocytosis
C96.8 Histiocytic sarcoma
D59.0 Drug-induced autoimmune haemolytic anaemia
D59.1 Other autoimmune haemolytic anaemias
D59.2 Drug-induced nonautoimmune haemolytic anaemia
D80.0 Hereditary hypogammaglobulinaemia
D80.1 Nonfamilial hypogammaglobulinaemia
D80.2 Selective deficiency of immunoglobulin A [IgA]
D80.3 Selective deficiency of immunoglobulin G [IgG] subclasses
D80.4 Selective deficiency of immunoglobulin M [IgM]
D80.5 Immunodeficiency with increased immunoglobulin M [IgM]
D80.6 Antibody deficiency with near-normal immunoglobulins or with hyperimmunoglobulinaemia
D80.7 Transient hypogammaglobulinaemia of infancy
D80.8 Other immunodeficiencies with predominantly antibody defects
D80.9 Immunodeficiency with predominantly antibody defects, unspecified
D81.0 Severe combined immunodeficiency [SCID] with reticular dysgenesis
D81.1 Severe combined immunodeficiency [SCID] with low T- and B-cell numbers
D81.2 Severe combined immunodeficiency [SCID] with low or normal B-cell numbers
D81.3 Adenosine deaminase [ADA] deficiency
D81.4 Nezelof's syndrome
D81.5 Purine nucleoside phosphorylase [PNP] deficiency
D81.6 Major histocompatibility complex class I deficiency
D81.7 Major histocompatibility complex class II deficiency
D81.8 Other combined immunodeficiencies

D81.9 Combined immunodeficiency, unspecified
D83.0 Common variable immunodeficiency with predominant abnormalities of B-cell numbers and function
D83.1 Common variable immunodeficiency with predominant immunoregulatory T-cell disorders
D83.2 Common variable immunodeficiency with autoantibodies to B- or T-cells
D83.8 Other common variable immunodeficiencies
D83.9 Common variable immunodeficiency, unspecified
D84.0 Lymphocyte function antigen-1 [LFA-1] defect
D84.1 Defects in the complement system
D84.8 Other specified immunodeficiencies
D84.9 Immunodeficiency, unspecified
D89.8 Other specified disorders involving the immune mechanism, not elsewhere classified
D89.9 Disorder involving the immune mechanism, unspecified
K50 Crohn disease [regional enteritis]
K51 Ulcerative colitis
L93 Lupus erythematosus
M05 Seropositive rheumatoid arthritis
M06 Other rheumatoid arthritis
M07 Psoriatic and enteropathic arthropathies
M08 Juvenile arthritis
M09 Juvenile arthritis in diseases classified elsewhere

APPENDIX IV: Variables Obtained from the TRUST Database

Hospital ID Chart Number
Age
Sex
Patient Facility
Date of Admission and Time
ABO group of patient
Rh type of patient
Presence of alloantibodies
Location identifier
Patient Status
Product Name, received from Canadian Blood Services (CBS)
Products to include:
Red cells, Platelets, Plasma, Cryoprecipitate
Product Unit Number
Final disposition of product (Transfused/ Discarded)
Status Date/ Time (Transfused, Discarded, Expired)
Receive Date and Time
Type of product transfused
Unique CBS identifier for product
Product and Patient Special considerations (i.e.,
irradiated; CMV neg., etc.)
ABO group of product
Rh type of product (if applicable)
Patient's hemoglobin values, platelet counts,
Coagulation results (PT, PTT, fibrinogen)
Creatinine
Most responsible diagnosis
Primary diagnosis
Date of admission
Admission Category
Discharge Date
Exit Status
Length of hospital stay (Total)
ICU stay during admission

APPENDIX VI: Complete list of variables available in TRUST

Data Elements Extracted from the Laboratory Information System (LIS)	Data Elements Extracted from the Medical Records Database
<p>Demographic Information (Patient) Hospital ID Chart Number Health Insurance Number (HIN) Postal Code (first 3 digits) Age Sex Patient Facility Date of Admission and Time Patients Submitting Physician ABO group of patient Rh type of patient Presence of alloantibodies Location identifier Patient Status Product Name (received from CBS) Products to include: Red cells, Plasma, Platelets, Cryoprecipitate, Albumin, Factor Concentrates, IVIG, Synagis, and Immune serum globulins Product Status name Parent Pool Name Product Unit Number Parent Collection Date Final disposition of product (Transfused/ Discarded) Status Date/ Time (Transfused, Discarded, Expired) Receive Date and Time Type of product transfused Unique CBS identifier for product Lot # CBS ID (Codabar and ISBT) Inventory Site Location Transfusion Unit Comments (Transfusion Reaction, Unit returned, etc) Product and Patient Special considerations (i.e., irradiated; CMV neg., etc.) ABO group of product Rh type of product (if applicable) Product age (calculated from date of collection and date used) Patient’s hemoglobin values, platelet counts, Coagulation results (PT, PTT, fibrinogen), Creatinine</p>	<ul style="list-style-type: none"> • Institution • Hospital ID Chart Number <ul style="list-style-type: none"> • Health Insurance Number (HIN) • Postal code(first 3 digits) • Age • Sex • Most responsible diagnosis • Primary diagnosis • Weight • Date of Birth • Doctor Type • Doctor Service • Doctor Code • Diagnosis Code • Diagnosis Type • Procedure Date • Procedure Code • Procedure Doctor • Procedure Doctor Service • Procedure Anaesthetist • Anaesthetic Technique • Unplanned return to OR • Trauma Score • Complexity Level • Complexity Age group • Resource Intensive Weight (RIW) • Date of admission • Admission Category • Entry Code • Discharge Date • Exit Status • Length of hospital stay (Total) • Acute LOS • ICU stay during admission • Blood Transfusion Data (Yes /No) • Allogeneic red cells transfused • Autologous red cells transfused • Platelets transfused • Plasma transfused • Other blood products transfused • Use of acute normovolemic hemodilution

APPENDIX VII: Applicable Aspects (**in bold**) of Observational Study Designs in Exploring an Association between Chemotherapy and Alloimmunization. Adapted from: http://www.ciphi.ca/hamilton/Content/content/resources/explore/fb_case_v_cohort.html

Study type	Strengths	Weaknesses	When best to use
Case-control study	<p>Economical and quick</p> <p>Rare or specifically defined outcomes</p> <p>Can examine multiple exposures: N/A because chemotherapy is the only exposure of interest</p> <p>Most statistical power</p> <p>Can be used if population at risk is unknown: N/A because the population at risk of alloimmunization is known as the RC transfused population</p>	<p>Cannot examine multiple outcomes: N/A because alloimmunization is the only outcome of interest</p> <p>Difficulty in selection of comparison group</p> <p>Cannot be used to calculate absolute risk</p>	<p>When disease is rare or can be defined with great specificity</p> <p>When exposure is common: N/A because chemotherapy is a rare exposure in the general pediatric population</p> <p>When the population at risk is unknown: N/A because the population at risk of alloimmunization is known as the RC transfused population</p>
Cohort study	<p>Rare or specifically defined exposures</p> <p>Can examine multiple outcomes: N/A because alloimmunization is the only outcome of interest</p> <p>Provides most direct measurement of absolute risk</p>	<p>Can study only single exposure or group of related exposures: N/A because chemotherapy is the only exposure of interest</p> <p>Population at risk must be known: N/A because the population at risk of alloimmunization is known as the RC transfused population</p>	<p>When the population at risk is a well-defined group</p> <p>When exposure is rare or can be defined with great specificity</p> <p>When disease is common: N/A because alloimmunization is rare</p>

N/A: Not Applicable in Exploring an Association between Chemotherapy and Alloimmunization