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Effectiveness of Leukoreduction in Neonates

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Abstract

As of September 1999, all donated units of blood in Canada undergo a process known as pre-storage leukoreduction. This process removes a significant proportion of white blood cells from blood at the time of donation. The decision to implement universal leukoreduction was based on fairly strong evidence that leukoreduction was beneficial in some adult populations such as cardiac and colorectal surgical patients. However, very little information exists on its effectiveness in other populations such as the neonatal population. The purpose of this thesis was three fold: 1) to conduct a systematic review of the literature to assess the effectiveness of leukoreduction; 2) to undertake a methods paper outlining the optimal design to study the effectiveness of leukoreduction given its universal nature; and 3) to conduct a study assessing the effectiveness of leukoreduction in the neonatal population.

The results of the systematic review elucidate the paucity of well-conducted, methodologically sound studies evaluating the effectiveness of leukoreduction in the neonatal population. The current evidence suggests that leukoreduction may be effective. However, further studies are needed especially with respect to clinically important outcomes. The lack of convincing data and the significant cost of leukoreduction mandate evaluations to determine its clinical and economic impact.

The methods manuscript describes important methodological issues confronted in the design of the before/after evaluation. Because of the universal application of many transfusion interventions, one has to consider, carefully, the methodological rigor with which these interventions are evaluated. The methodological considerations discussed are: 1) threats to internal validity; 2) precision; and 3) generalizability. Properly conceived, designed, conducted, and analyzed, such a before/after study

design can yield informative associations.

The final paper presents the results of the before/after study. The study included a total of 515 infants <1250 grams from three sites across Canada. The effect of leukoreduction on our primary outcome of nosocomial bacteremia was an odds ratio of 0.59 (95%CI: 0.34-1.01). Crude and adjusted rates for all major neonatal morbidities suggest that leukoreduction improved all outcomes. The adjusted odds ratio for a composite measure of any major neonatal morbidity was 0.31 (95%CI: 0.17-.56). Based on the results of this study, it is concluded that the implementation of universal pre-storage leukoreduction significantly improved clinical outcomes in premature infants requiring blood transfusions.

Résume

Au Canada, depuis septembre 1999, tous les dons sanguins sont sujet à un processus connus sous « l'entreposage de déleucocytation universelle ». Au moment de l'extraction sanguine, l'intervention retire du sang, une proportion significative des culots blancs. La décision de mettre en œuvre la déleucocytation universelle a été basée sur des faits saillants donc celle-ci est bénéfique chez certains groupes de patients adultes qui subissent une intervention chirurgicale cardiaque ou colorectale. Cependant, il existe très peu d'information sur l'efficacité de cette pratique chez d'autres groupes tels que chez la population néonatale. Le but de cette thèse porte trois volets : 1) effectuer une évaluation systématique de la litérature qui détermine les effets bénéfiques de la déleucocytation ; 2) étant donné la nature mondiale portée à ce sujet, elle permet de procéder à la mise en œuvre d'un manuscrit méthodique qui dresse une vue d'ensemble optimale de l'étude des effets bénéfiques de la déleucocytation ; et 3) mener une étude qui vise l'évaluation des effets bénéfiques chez la population néonatale.

Les résultats de l'évaluation systématique explique la pénurie d'études méthodiques approfondies qui évalue les effets bénéfiques de la déleucocytation chez la population néonatale. Les résultats actuels suggèrent que la déleucocytation peut être véritable. Cependant, il faudra effectuer des études supplémentaires afin d'obtenir des résultats cliniques significatifs. Le manque de données ainsi que le coût considérable attribués à la déleucocytation oblige la mise en marche d'évaluations additionelles afin de déterminer l'impacte économique ainsi que clinique.

Le manuscrit méthodique décrit l'importance des résultats méthodologiques affrontés dans la conception d'une évaluation avant/après. À cause des différentes pratiques transfusionnelles mondiales, il faut porter une attention particulière à la qualité méthodologique des évaluations des

pratiques transfusionnelles. Les considérations méthodologiques discutées sont : 1) les menaces de validité interne 2) la précision ; et 3) la généralisation. Adéquatement conçu, mené et analysé, une évaluation avant/après rapporte des résultats informatifs et significatifs.

Le manuscrit représente des résultats d'une étude avant/après. L'étude inclua un nombre total de 515 enfants <1250 grammes dans trois sites participants canadiens. Le résultat de déleucocytation sur la bactériémie nosocomiale résultat à un *odds ratio* de 0.59 (95%CI: 0.34-1.01). Les taux univarié et ajusté pour toutes les morbidités néonatales majeures suggèrent que la déleucocytation favorise les résultats. L'ajustement du *odds ratio* de l'indice composé des morbidités néonatales majeures était 0.31 (95%CI: 0.17-.56). Basés sur les résultats de l'étude, il est conclu que la mise en œuvre de pratiquer l'entreposage de déleucocytation universelle favorise de façon importante et significative les résultats cliniques chez les enfants prématurés nécessitant une transfusion sanguine.

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I consider myself extremely fortunate to be mentored under thesis supervisors of the caliber of Professors Stan Shapiro and Paul Hébert. Besides being accomplished academics and researchers, they are wonderful people. Their substantial commitment to me in terms of time, support, and teaching is deeply appreciated. To my thesis committee, I thank you for your assistance and support throughout the development, conduct and preparation of the thesis and manuscripts.

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I would also like to acknowledge my co-investigators on the effectiveness of leukoreduction in neonates study. Being neither a neonatologist nor a transfusion medicine specialist, I am extremely grateful for their expertise and support. On behalf of the study investigators, we are thankful to the Canadian Neonatal Network, Dr. Bernard Fernandes and Dr. Louis Wadsworth for providing clinical and transfusion data. We also thank Sarka Lisonkova, Jennifer Claydon, Shawn Stewart, Lyle Unrau and Tara Routh for their research assistance.

Pursuing my doctorate would not have been possible if it were not for the support of my family. Growing up, my Mom and Dad taught me the values of hard work, pursuit of knowledge and simply enjoying life and the world around us. I am privileged and proud to have such parents. To my wife Natalie, and our sons, Nicholas, Nathan, and James, I thank you for your unwavering support, encouragement and patience. I dedicate this work to you.

Contributions of Authors

I conceived, drafted and prepared all manuscripts included in this thesis. The contribution of the other authors listed on the manuscripts was the provision of clinical, methodological, and statistical guidance in the manuscript formulation and review process.

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Chapters 1.0 and 5.0 have been written exclusively for this thesis.

Chapter 2.0 has been published as Fergusson D, Hébert PC, Barrington, KJ, Shapiro SH. Effectiveness of WBC reduction in neonates: what is the evidence of benefit? *Transfusion.* 2002; 42(2): 159-65.

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Chapter 4.0 has been submitted for publication as Fergusson D, Hébert PC, Lee SK, Walker CR, Barrington KJ, Joseph L, Blajchman MA, Shapiro S. Effectiveness of Universal Leukoreduction of Blood Transfusions in Premature Infants, *New England Journal of Medicine* (submitted 2002)

Statement of Originality

This PhD thesis contains original contributions to both the clinical and methodological understanding of the effectiveness of leukoreduction in the neonatal population. To my knowledge, I conducted the first published systematic review on the effectiveness of leukoreduction in the neonatal population. Also to my knowledge, I conducted the first study evaluating the effect of leukoreduction on clinical important outcomes in neonates. In addition, I conceived, prepared and published a manuscript outlining methodological considerations of the before and after study design in transfusion medicine.

I declare that the intellectual content of this thesis is the product of my own work, even though I may have received clinical, methodological, or statistical expertise and guidance from others.

Manuscripts and Authorship

As an alternative to the traditional thesis format, a dissertation can consist of a collection of papers that have a cohesive, unitary character making them a report of a single program of research. The structure for the manuscript-based thesis must conform to the following (cited from the "Guidelines for Thesis Preparation" of the Faculty of Graduate Studies and Research):

"Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.)

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.

The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts. The thesis must include the following: A table of contents, an abstract in English and French, an introduction which clearly states the rational and objectives of the research, a comprehensive review of the literature (in addition to that covered in the introduction to each paper),

and a final conclusion and summary.

As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defence. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers."

Glossary of Major Terms

Methodological

Before and After Study: A before and after study design measures an outcome in a specified population during a period of time when the exposure is absent followed by a measurement in the same population during a period of time when exposure is present. There are a variety of before and after designs depending on whether an individual or a population is being measured pre and post-intervention and/or whether a control group is used.

External Validity*: "A study is externally valid or generalizable if it can produce unbiased inferences regarding a target population (beyond the subjects in the study)."

Information Bias*: "A flaw in measuring exposure or outcome data that results in different quality (accuracy) of information between comparison groups."

Internal Validity*: "The index and comparison groups are selected and compared in such a manner that the observed differences between them on the dependent variables under study may, apart from sampling error, be attributed only to the hypothesized effect under investigation ."

Multivariate Logistic Regression: For a binary response variable Y, the logistic regression has the form:

$$\text{Logit}(p)=\log(p/1-p)= \beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots+\beta_iX_i$$

or equivalently,

$$p= \frac{\exp(\beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots+\beta_iX_i)}{1+\exp(\beta_1X_1+\beta_2X_2+\beta_3X_3+\ldots+\beta_iX_i)}$$

The logistic regression models the logit transformation of the *i*th observation's event probability, p_i, as a linear function of the explanatory variables in the vector $X_1+X_2+X_3+X_i$.

Secular Trends*: "Changes over a long period of time, generally years or decades."

Selection Bias*: "Error due to systematic differences in characteristics between those who are selected for study and those who are not."

Systematic Review: A systematic review is an overview of the literature conducted in a systematic, organized and well-defined manner be it quantitative or qualitative.

Clinical

BPD (Bronchopulmonary Dysplasia) or "Chronic Lung Disease":** Constant and recurring lung injury with ongoing repair and healing. BPD occurs in 5-30% of infants treated with oxygen and/or mechanical ventilation, for example, prolonged treatment of IRDS or RDS (Idiopathic Respiratory Distress Syndrome). BPD is a disease that is spawned in the NICU. The primary injury (i.e. IRDS) is aggravated by continually high-inspired oxygen tensions and by barotraumas from mechanical ventilation and by infection. BPD is treated by decreasing the factors that produce the injury and by allowing the lung to heal. The condition may last for weeks or months.

Cytomegalovirus (CMV): CMV is a virus related to the herpes virus group. Although inactive at times, it is incurable and is a life-time infection. CMV may be passed from a mother to her baby during pregnancy and is the most common congenital viral infection. CMV without symptoms is common in babies and young children. It is found in saliva, urine, semen, and other body fluids. It can be transmitted to the fetus during pregnancy and to the baby during delivery or in breast milk.

HLA: Human leukocyte antigens

Immunomodulatory: the modification, by an agent, of the immune response or the functioning of

the immune system (e.g. decreased antibody formation or the inhibition of white blood cell activity)

IVH (Intraventricular Hemorrhage):** Intracranial bleeding or blood in the ventricular system in the centre of the brain. IVH has received more attention in recent years due to the high incidence in premature infants and the ease of detection with a CT scan or cranial ultrasound. Classification schemes have been provided to assess the degree of bleeding or amount of blood present as seen on a CT scan: 0= no bleeding; I = germinal matrix only; II = germinal matrix with blood in the ventricles; III = germinal matrix with blood in the ventricles and dilation of the ventricles; IV = intraventricular and parenchymal bleeding (other than germinal matrix). IVH often clears up spontaneously but may require treatment. It may also result in subsequent neurological sequelae, such as mental retardation and developmental delay.

Leukoreduction: the process used to filter white blood cells from whole blood

Necrotizing Enterocolitis (NEC) :** The occurrence of impaired blood supply to portions of the bowel. This leads to small perforations with air dissecting in the bowel wall or even entering the peritoneal cavity. NEC is usually a disease of premature infants. The exact cause is unknown but mucosal injury can occur due to a variety of things such as shock, perinatal asphyxia, gastrointestinal infection, severe cardiopulmonary disease, and hyperosmolar feedings. Once mucosal injury occurs, bacterial invasion occurs and gas is formed. The incidence of NEC is lower in breast-fed infants but the reason for this is unknown. Treatment may require surgery.

Nocosomial bacteremia (also nosocomial infection or bacterial sepsis):** Infection acquired during hospitalization. Premature babies are highly susceptible to infection due to premature stress, immature immune systems, complicated medical and surgical problems, and the invasive techniques that are required for monitoring and treatment. Infection can be prevented by sanitation practices

such as hand washing, skin and cord care, use of sterilized instruments, and by ensuring overall employee health. Treatment of infection in neonates can be achieved by the administration of antibiotics which have significantly reduced mortality rates.

Red Blood Cells (RBCs): Red blood cells, also referred to as erythrocytes, are responsible for delivering oxygen throughout the body.

ROP (Retinopathy of Prematurity – also called Retrolental Fibroplasia):** Injury to the retina of the eye. Retinal damage occurs due to prematurity in association with oxygen administration. The result is an abnormal mass of scar tissue that forms in the eyes of premature infants. It may be very mild and of little significance, or it may be of such an extent that it displaces the retina of the eye and causes blindness.

White Blood Cells (WBCs): White blood cells, also referred to as leukocytes, are produced by the immune system to help defend the body against infection. They are formed in the bone marrow and either enter the blood or migrate to key organs, such as the spleen, lymph nodes, or gut.

* Last, JM, ed. (1995). A Dictionary of Epidemiology, 3rd ed. New York, Oxford University Press.
** Canadian Neonatal Network database Procedures Manual

Chapter 1

1.1 Introduction and Background

In response to the HIV crisis of the 1980's, a number of testing modifications have been introduced during the preparation of allogeneic red blood cells (donated blood) such as p24 antigen and third generation hepatitis C testing to provide the safest blood products possible. To further maximize the safety and benefits of those in need of blood transfusions, Canadian Blood Services and Héma Québec, Canada's sole suppliers of blood products, implemented a universal pre-storage leukoreduction program in mid-1999. The current leukoreduction process makes it possible to manufacture red blood cell products with less than 2.5×10^5 residual leukocytes present in each product. Thus, all red blood cell and platelet products supplied in Canada are leukoreduced without exception.

Greater than 50% of infants born under 1250 grams admitted to neonatal intensive care units (NICUs) require red blood cell (RBC) transfusions.[1] Despite recent trends in increasing the transfusion threshold and the development of technologies designed to avoid RBC exposure such as erythropoietin, allogeneic RBC transfusions remain an important supportive and life-saving measure for neonatal intensive care patients experiencing illness and anemia of prematurity.

While there is conflicting evidence regarding the immunosuppressive effects of leukoreduced RBCs versus non-leukoreduced RBCs in adult populations, leukoreduction has been clearly shown to be beneficial in three situations:1) prevention of cytomegalovirus (CMV) transmission in CMV-negative bone marrow transplant recipients; 2) prevention of HLA alloimmunization and platelet refractoriness in patients with hematological malignancies who are thrombocytopenic; and, 3) prevention of febrile non-hemolytic transfusion reactions. While evidence

exists for adult populations, there is a paucity of studies examining the benefits of leukoreduction in the neonatal population.

In consideration of their immature immune system and extreme frailty, the question is raised whether it is sensible to extrapolate the results of studies in adult populations to the neonatal population. It is the same undeveloped immune system and frailty that makes neonates a unique population to examine the immunosuppressive effects of RBCs. Indeed, if RBCs have clinically important immunosuppressive effects, then this effect should be observed in the neonatal population. It is also conceivable that the leukoreduction process increases red cell hemolysis or results in other unexpected clinical consequences.[2] Based upon available research, the benefits and harms of leukoreduction remain unclear in this vulnerable population.

1.2 Utilization of RBC transfusion in Neonates

Despite concerns regarding transfusion-transmitted viruses, allogeneic RBC transfusions clearly save lives in the neonatal critical care setting. RBCs are transfused in neonates to restore circulating blood volume, to increase oxygen-carrying capacity or to replace blood removed for laboratory tests.[3] While studies exist in the United States regarding transfusion practices in the neonatal setting, only one study has been published with Canadian data.[4] One study appearing only as an abstract, indicated that 59% of all neonates were transfused at the Toronto Sick Kids Hospital during1994-1995.[5] Data from the United States suggests that 78% of infants <1500 g received RBCs in 1989 which was reduced to 61% in 1993.[6,7] Studies, using survey methods, have also shown that the volume of RBCs transfused has decreased from an average of 82 mL in 1982 to 50 mL in 1993.[6] Additionally, studies have reported wide practice variation among and between NICUs. Reasons for

disperse practice variation include differing transfusion thresholds, mean number of phlebotomies performed, and mean illness severity scores of admitted patients.[8,9] Individual patient predictors include number of phlebotomies, birthweight, gestational age, ventilation requirements, and severity score. [9,10]

NICU patients are among the most frequently transfused patients in tertiary care hospitals. They also consume a significant amount of health care resources both in the short term and long term. If the removal of leukocytes from red blood cells does indeed decrease sequalae, then this should translate into long term beneficial consequences. For these reasons, transfusion practice in the neonatal intensive care unit setting was chosen for examination.

1.3 Potential Benefits of Leukoreduction

The presence of leukocytes in cellular blood components including platelet concentrates and red cell concentrate (RBC) are thought to be associated with a number of significant adverse effects in recipients. Leukocytes in both platelets and RBCs can: 1) result in immunomodulation in recipients, 2) induce febrile non-hemolytic transfusion reactions, 3) transmit viral, bacterial and protozoal infections and most importantly, 4) evoke HLA or platelet antigen alloimmunization resulting in refractoriness to future platelet transfusions. Consequently, in adult populations, leukocyte reduction has been shown to reduce the frequency of HLA-alloimmunization [11], cytomegalovirus virus and human T cell leukemia virus infections [12] and reduce febrile non-hemolytic transfusion reactions. [13] It has also been suggested that leukoreduction might reduce the risk of transfusion transmission of new variant CJD, if transmission does indeed occur through blood transfusion. [14,15]

In assessing the effectiveness of universal pre-storage leukoreduction, an evaluation of the neonatal population is particularly important. There are conflicting clinical studies, conducted almost exclusively in the adult patient population, supporting the use of pre-storage leukoreduction in patients at risk of adverse consequences from immune suppression induced by allogeneic RBC transfusions. Neonates are unique given that they have an immature immune system, are frequently transfused with RBCs, and seem to respond differently than adults regarding the creation of antibodies, development of transfusion reactions, and response to infectious agents such as cytomegalovirus. The neonatal population <1250 grams is one group which, if leukoreduction indeed has an impact, we should see a benefit. They are at an extremely high risk of nosocomial infection and RBC transfusion, making them an attractive population for the evaluation of universal leukoreduction. Although all of the benefits of leukoreduction are important to recipients, it is likely that the most significant potential benefit is a decrease in transfusion-related immune suppression. Transfused leukocytes in allogeneic RBCs have a number of poorly characterized effects on immunologic responses.[16-20] Several studies in adults have investigated the role of allogeneic RBC transfusions in depressing cell mediated immunity and altering lymphocyte subsets. In dialysis patients receiving allogeneic blood, lymphocyte reactivity measured using mitogen, antigens and homologous lymphocytes was noted to be decreased by 50%.[21] While studying lymphocyte subsets, Kaplan found a moderate decrease in T4/T8 lymphocyte ratios and decreased natural killer activity following repeated allogeneic transfusions.[17]

Quite clearly, if truly present and clinically significant, leukocyte-associated immune suppression may result in an increased frequency of nosocomial infections that, in turn, could result in increased mortality, organ failure and longer neonatal intensive care unit and hospital stays. An

alternative explanation for the deleterious effects of leukocytes is that inflammation rather than infection could mediate tissue injury and subsequent organ failure.

1.4 Description of Universal Pre-storage Leukoreduction

All RBC products are supplied by Canadian Blood Services or Héma Quebec. In Canada, allogeneic RBCs are collected into CP2D anticoagulant solution and stored in 100 mls of Nutricel additive. Characteristics of an RBC unit include a volume ranging from 175 to 225 mls, a hematocrit of 55%, and a storage time averaging 18 days (ranges from region to region). As smaller volumes are required by infants, RBC units are typically divided into smaller units called aliquots. Prior to the implementation of leukoreduction, approximately 60% of RBCs were buffy coat depleted as a consequence of leukoreduction of platelet products.

Universal pre-storage leukoreduction, instituted by Canadian Blood Services and Héma-Quebec, involves one of two in-line systems.(Appendix I) A Leukotrap-RC leukocyte reduction filtration system is used for red blood cells (Pall Corporation) while the Leukotrap WB is used for filtration of products prior to centrifugation. The bag receiving the filtered blood is placed in a horizontal position. The bag containing the unfiltered RBCs is placed 1 metre in height above the bag receiving the filtered material. Once all RBCs have been filtered the tubing is heat-sealed and the filter discarded. Leukofiltration in this manner reduces white blood cell content of a unit of RBCs from an average $3.0x10^9$ /unit to $2.5x10^5$ unit (a decrease of 4 logs).

1.5 Goal and Objectives of Thesis

The goal of my dissertation was to study the effect of leukoreduction on the neonatal

population. Three objectives were undertaken to achieve this goal. They were: 1) to conduct a systematic literature review of the effectiveness of leukoreduction in the neonatal population; 2) to rigorously explore the methodological options and considerations in developing an optimal study design to assess the effectiveness of leukoreduction; and 3) to undertake and conduct such a study with the primary objective of determining the effect of leukoreduction on serious nosocomial infection in neonates admitted to the neonatal intensive care unit (NICU). Additional aims of the study were to determine the effect of leukoreduction on mortality and major NICU morbidities including: bronchopulmonary dysplasia; retinopathy of prematurity; necrotizing enterocolitis; and intraventricular hemorrhage.

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Chapter 2

2.1 Preface to Manuscript #1: Systematic Review of the Literature

Freedman argues that for clinical research to be ethical it must be valuable.[1] To be of value, a study must add to current knowledge. Knowledge, curiosity and intuition were the creative drivers of the hypothesis that leukoreduction would beneficially effect a neonatal population. However, in order to justify a hypothesis, it is important to incorporate published and unpublished research, as well as expert and community opinion. Conducting a systematic review of the literature ensured the originality of this dissertation. The value of a systematic review extends beyond justifying a research question. A systematic review also: 1) forces researchers to become aware of the published literature that extends beyond their current collection; 2) provides a transparent and reproducible process for evaluating the literature for research ethics boards, journal editors, and readership; and 3) provides researchers a comprehensive list of clinical, methodological, and statistical issues that may not have been previously considered or addressed. It is for these aforementioned reasons a systematic review of leukoreduction in the neonatal population was conducted.

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2.3 Manuscript #1: Systematic Review of the Literature

Fergusson D, Hébert PC, Barrington, KJ, Shapiro SH, Effectiveness of WBC reduction in neonates: what is the evidence of benefit? Transfusion. 2002 Feb;42(2):159-65.

Abstract

Background: The presence of leukocytes in red blood cells (RBCs) is thought to be associated with a number of significant adverse effects in recipients. In adults, leukocyte reduction has been shown to reduce the frequency of HLA-alloimmunization, cytomegalovirus virus (CMV) and human T cell leukemia virus infections and reduce febrile non-hemolytic transfusion reactions. Neonates are unique given that they have an immature immune system and are frequently transfused with RBCs. This population is clearly very different from other populations making it difficult, and imprudent, to extrapolate findings from adult studies to neonates. Therefore, in order to summarize and evaluate the benefits of leukoreduction in neonates, a systematic review was undertaken.

Objective: The objectives of this systematic review were to determine whether leukoreduction of RBCs transfused to neonates decreases the transmission of CMV, reduces the ability to develop anti-HLA antibodies, or reduces the risk of immunomodulation. In addition, nosocomial infection, mortality, and length of stay, all possible manifestations of these outcomes were identified and analyzed.

Methods: All studies of leukoreduction were identified by a systematic review of the literature. Studies meeting the inclusion criteria were grouped based on study outcome. Where appropriate, studies were pooled to obtain an overall measure of effect.

Results: Nine eligible studies were identified from the systematic literature search including five randomized controlled trials, three non-randomized controlled studies, and one before/after study. Of the nine studies identified, only six were deemed evaluable. Two studies evaluated leukoreduction and the development of CMV with each study giving different results. The pooled odds ratio was 0.19 (95% CI 0.01 – 3.41) suggesting a clinical but non-statistically significant effect. Two studies

evaluated leukoreduction and the development of aHLA antibodies. As with CMV, the two studies were not congruent in terms of their results. The pooled odds ratio was 0.17 (95% CI 0.01 – 2.43). As for immunomodulation, two small studies presented evidence of a statistical change in lymphocyte subsets. No studies were identified with a primary objective of evaluating the impact of leukoreduction on nosocomial infection, mortality, or length of stay.

Conclusion: The primary finding of this systematic review is that there is a paucity of well-conducted, methodologically sound studies evaluating the effectiveness of leukoreduction in the neonatal population. The current evidence suggests that leukoreduction may be effective, however further studies are needed. The lack of convincing data and the significant cost of leukoreduction mandate evaluations to determine the clinical and economic impact.

Introduction

Preterm neonates are a heavily transfused population. [1] Red blood cells (RBCs) are transfused in neonates to restore circulating blood volume, to increase oxygen-carrying capacity or to replace blood removed for laboratory tests.[2] Despite small blood volumes required during transfusion, this patient population can be exposed to numerous donors.[3,4,5] Thus, neonates are potentially at an increased risk of acquiring deleterious biological effects from blood products. The presence of leukocytes in RBCs is thought to be associated with a number of significant adverse effects in recipients. In adults, leukocyte reduction has been shown to reduce the frequency of HLA-alloimmunization [6], cytomegalovirus virus (CMV) and human T cell leukemia virus infections [7] and reduce febrile non-hemolytic transfusion reactions. [8] CMV infection acquired from CMV-seropositive blood transfusions is usually asymptomatic. However, such infections can cause hepatitis, thrombocytopenia, hemolytic anemia and pneumonia.[9,10] Anti-HLA antibodies present in transfused whole blood have been shown to be responsible for complications such as non-hemolytic febrile transfusion reactions and platelet refractoriness.[11] Transfused leukocytes in allogeneic RBCs have a number of poorly characterized effects on immunologic responses including an increase in serious nosocomial infection. [12-16] It has also been suggested that leukoreduction might reduce the risk of transfusion transmission of new variant Creutzfeld-Jakob Disease (nvCJD), if transmission does indeed occur through blood transfusion. [17,18]

Leukoreduction can be achieved by filtering RBC units through a filter either during the production process (pre-storage) or at the bedside (post-storage). Leukocyte reduction filters differ from manufacturer to manufacturer in white blood cell reduction efficiency and different leukocyte reduction technologies produce different residual white blood cell subsets. A number of countries

such as Canada, France, and United Kingdom have directed their respective blood systems to implement universal pre-storage leukoreduction. The term universal refers to all RBC units and, by extension, those prepared and transfused to neonates. While a number of studies and systematic reviews examining the effectiveness of leukoreduction have been conducted in the adult population, far fewer studies evaluating the benefits of leukoreduction in the neonatal population have been performed. Therefore, it is possible that the neonatal population is receiving leukoreduced RBCs that may provide very little or no incremental benefit compared to standard RBCs.

Neonates are unique given that they have an immature immune system and are frequently transfused with RBCs.[1] Given this uniqueness, the neonatal population is one group where if leukoreduction indeed has an impact, we should see a benefit. This population is clearly very different from other populations making it difficult and imprudent to extrapolate findings from adult studies to neonates. Therefore, in order to summarize and evaluate the benefits of leukoreduction in neonates, a systematic review was undertaken. The objectives of this systematic review were to determine whether leukoreduction of RBCs transfused to neonates decreases the transmission of CMV infection or disease, reduces the ability to develop aHLA antibodies, or reduces the risk of immunomodulation. In addition, all possible manifestations of these outcomes including nosocomial infection, mortality, and length of stay, will be examined.

Methods

Literature Search and Study Selection

A MEDLINE search for the years 1966 to September 2000 was performed to identify all articles with any of the terms *leukoreduction, leucoreduction, leucocyte removal, leukocyte removal,*

white cell removal, removal of leukocytes, removal of leucocytes, white cell filtration,leukocyte filtration, leucocyte filtration, reduction of white cells, leucocyte-reduced, leukocyte-reduced, leukocyte depletion, leucocyte depletion combined with any of the terms *neonates, neonatal pediatric, paediatric, newborns, infants, preterm.* Additional databases searched were Current Contents/All Editions 1993 Week 26 to 2000 Week 41 and HealthSTAR 1975 to October 2000.

Initially, a search strategy with two published filters (Haynes, Cochrane) were used to identify randomized controlled trials.[19,20] However, due to the small number of RCTs identified, the search was expanded to include all studies by conducting the search strategies without the two RCT filters. The abstracts from the resulting citations were reviewed. All prospective studies and review articles evaluating leukoreduction compared to at least one of the primary outcomes were retrieved. References of all identified studies and review articles were searched to identify further prospective studies. Studies were included regardless of whether they were full publications, abstracts, or letters to the editor; or were published in languages other than English. Duplicate publications and retrospective studies were excluded.

Data Collection

Data from the studies were independently abstracted onto data forms by two individuals (DF, PH). Any disagreements were resolved by discussion. No attempt was made to conceal the author or the medium of publication. Authors of the studies were not contacted to clarify published information or provide additional information.

The primary outcomes were CMV infection or disease, aHLA antibodies, generic immunomodulatory parameters and information on nosocomial infection, mortality, and length of

stay. Other variables collected from the studies were study methodology, patient population, type of transfusions (blood exchange transfusion or RBC aliquot units), type of leukoreduction device, method of filtration (pre- or post-storage), inclusion/exclusion criteria, technique to identify outcome(s), time(s) of blood draws, whether mother's serum was tested, whether infants received mother's breast milk/ frozen donor breast milk, mean residual white blood cell count in filtered group, number of males/females, mean gestational age, mean weight, mean number of transfusions, and the number of term/preterm subjects in each group.

Analysis

The purpose of this systematic review is to provide the best available evidence on the effectiveness of leukoreduction in neonates. Thus, homogenous studies, in terms of patient population, outcome, and study methodology were statistically pooled. Dependent on the number of evaluable studies, subgroup analyses were performed on important clinical parameters and laboratory parameters. Discrete data (e.g. proportions of subjects acquiring CMV infection) were analyzed using a random-effects model with effect sizes presented as Der Simonian-Laird odds ratios (OR) with 95% confidence intervals (Meta-Analyst[977], J.Lau & T. Chalmers). An OR of 1 suggests no difference between intervention and control, an OR less than 1 suggests that fewer subjects in the intervention group developed the outcome while an OR greater than 1 suggests that fewer subjects in the control group developed the outcome. Studies with a zero cell, meaning that no outcomes were experienced in either of the two arms, had 0.5 added to that cell to allow for computation. Studies that could not be statistically pooled are presented descriptively.

Results

Of 476 references identified by the systematic literature search, nine studies meeting the inclusion criteria were identified[21-29] including five randomized controlled trials, three non-randomized controlled studies, and one before/after study. An additional duplicate publication[30] and an evaluation of cardiac perioperative parameters[31] were identified and not included. Four studies evaluated the effectiveness of leukoreduction on acquiring CMV infection[21-24], three studied the effect on developing aHLA antibodies[25-27], and two on immunomodulatory effects[28,29]. No studies were identified with a stated primary objective of examining the effect of leukoreduction on either CMV disease, nosocomial infection, mortality or length of stay. However, three studies provided information on pneumonia [21,24,28] while one collected information on sepsis.[28]

Cytomegalovirus

A total of four studies including two randomized controlled studies, one before/after study, and one non-randomized controlled study were identified (Table 1). All four studies had a primary outcome of CMV infection and no studies evaluated CMV disease. The largest study[21] included 72 patients and the smallest 48.[22] For purposes of this evaluation, the before/after study was divided into two evaluations, one of blood exchange transfusion (BET) and one of standard RBC aliquot units. Four studies compared leukoreduced RBCs with non- leukoreduced RBCs. In three of the four studies all patients received at least one unit of CMV+ blood. The one study that did not specifically and knowingly transfuse at least one unit of CMV+ blood per patient was conducted in a highly endemic area.[24] One study compared irradiated leukoreduced RBCs with irradiated non-leukoreduced RBCs.[24]

In two of the studies, pre-transfusion test results were either not conducted or not reported [22,24] (Table 3). This could have an impact on the results observed. In one study, only infants whose mothers were CMV- were enrolled in the study.[22] Thus, it is highly likely that CMV could only be acquired through CMV+ blood transfusion. The other study[24] included CMV+ mothers and therefore the observed outcomes could be confounded by neonates acquiring CMV through maternal secretion via the birth canal or breast milk. Therefore, this study was excluded from further analysis. Like the Ohto study, the before/after study of Xu did not measure CMV secretion in the mother's breast milk and thus the potential for a biased measure of effect is high. Therefore, this study was excluded from further analysis. The studies by Gilbert and Eisenfeld enrolled only subjects born to CMV- mothers.

The estimates of effect for the two remaining studies (Eisenfeld & Gilbert) were divergent with one trial having a null result (OR 1.18, 95% CI 0.02-61.78) and the other a non-statistical significant positive effect in favour of the treatment (OR 0.06, 95% CI 0.001-1.04) (Figure 1). The pooled odds ratio of the two studies was 0.19 (95% CI 0.01 – 3.41) suggesting a clinical but non-statistically significant effect.

aHLA- Antibodies

Three studies, all with small sample sizes, evaluated the effect of leukoreduction on the development of aHLA antibodies.[25,26,27] (Table 1) Two randomized controlled trials[26,27] and one non-randomized controlled trial[25] were conducted. All three studies evaluated leukoreduced versus non-leukoreduced RBCs. In one study[26], stored units of leukoreduced RBCs were compared with fresh units or non-leukoreduced RBCs. Although stored units of RBCs could be biologically

compromised compared to fresh RBCs, it was determined that this could only bias the observed effect towards the null. One study included term and preterm neonates[25] whereas the other two studies included only preterm subjects.[26,27] The Kurul study included infants that required blood exchange transfusion.

All three studies tested for aHLA antibodies before the first transfusion and either 3-4 weeks post-transfusion (Kurul) or at monthly intervals up to six months after birth or until discharge. In two studies where aHLA antibodies were detected in pre-transfusion testing[25,27], only one study accounted for the impact.[27] The study by Bedford-Russell stated that of the 4 subjects who developed specific aHLA antibodies, the antibodies initially detected became undetectable in all four subjects. [27]One subject did eventually develop a different antibody. The study by Kurul did not completely account for all of the subjects initially detected with aHLA antibodies. Of 17 infants who had detectable antibodies pre-transfusion, antibodies could not be subsequently detected in 10 subjects post-transfusion. This leaves 7 infants for whom the route of transmission was unknown. Furthermore, the treatment arm to which these 7 infants belonged was not provided. For this reason the study by Kurul was excluded from further analysis (Table 3)

The two remaining studies give conflicting results (Figure 1). Strauss reported that by the end of the study, zero infants developed detectable antibodies. They did report that an infant in each study group did develop antibodies but these antibodies were characterized as transient because they became undetectable at subsequent testing intervals. After statistical adjustment for zero cells, the measure of effect in the Strauss study was an OR of 0.95 (95%CI 0.02 to 50.32) suggesting no clinical benefit of leukoreduction (Figure 1). Bedford-Russell reported that 7 of 23 infants developed antibodies in the control group compared to 0 of 19 infants in the treatment group (OR

0.06, 95%CI 0.01 to 1.06). The pooled odds ratio was 0.17 (95% CI 0.01 – 2.43).

Immunomodulatory Effects

Two studies that examined the effect of leukoreduction on immunomodulation were identified. [28,29] A recently published randomized controlled study[28] evaluated a variety of surface markers in six preterm neonates receiving non-leukoreduced RBC transfusions compared to eight receiving leukoreduced RBCs. All RBCs were CMV- and irradiated. At post-transfusion days 10-14, six of the 12 lymphocyte subsets were statistically significantly different (CD45RA, Cd80, CD25, CD8, CD3-/16+56, CD3-/DR+).

In the non-randomized controlled study[29], a cohort of three newborns receiving total blood exchange with leukoreduced blood experienced only a very slight enhancement (16%) of Ia+ T lymphocytes 5 days after total blood exchange transfusion compared to a zero percent change in nine control newborns receiving no transfusions. Baseline data at the group or individual level was not provided to calculate a measure of effect. The authors note that all changes were transient and no clinical symptoms persisted after 15 days.

Nosocomial Infection

Three studies presented information on nosocomial infection.[21,24,28] None of the studies cited nosocomial infection as a primary outcome. The study by Ohto was exluded because of the aforementioned methodological weaknesses while the study by Gilbert was excluded as pneumonia was not reported for neonates not infected with CMV. Wang-Rodriquez reported that pneumonia developed in 2 of 8 infants in the leukoreduction arm versus 3 of 6 infants in the control arm. The study by Wang-Rodriguez reported that sepsis developed in 2 of 8 infants in the control arm versus 2

of 6 infants in the treatment arm.

Discussion

The primary finding of this systematic review is that there is a paucity of well-conducted, methodologically sound studies evaluating the effectiveness of leukoreduction in the neonatal population. The majority of studies in this review were small (median 39, range 12 to 85), and of poor methodological quality.

In our systematic review, no studies were identified that looked at transfusion acquired CMV disease. For the prevention of CMV infection, two studies that were not methodologically flawed gave different results. We do not believe that the difference in results was due to the different methodological designs. Instead we attribute the difference to the control group used in the non-randomized controlled study. In this study, the control group received blood that was leukoreduced by the spin-cool-filter technique. This technique reportedly removes a mean of 94 to 95% of the original WBC and thus could conceivably account for the zero cases of CMV infection that were detected in the control group. The generalizabilty of the results is limited given that the spin-cool-filter technique is not routinely used. Thus, of the five evaluations, only the results provided by Gilbert seem robust in terms of study methodology, control of selection bias, and applicability. This study concluded that pre-storage leukoreduction with a filter is highly effective, although not statistically significant, in preventing CMV transmission via RBC transfusions (OR 0.06, 95%CI 0.01 to 1.04). The observed effect was not statistically significant due to small sample size. The clinical consequences of the infected infants were well documented including deterioration in respiratory status, episodes of apnea and bradycardia and pneumonia. Based on a single study of 72

patients, it is clear that further research is needed to elucidate the effect of leukoreduction on transfusion-acquired CMV.

The transient nature of aHLA antibodies was a primary finding of the two studies examining the effect of leukoreduction and alloimmunization with HLA antibodies. The results of these two studies are consistent with previous studies evaluating antibody development in neonates receiving non-filtered RBCs.[32,33] One probable explanation is the "transient" nature of the aHLA antibodies. While the authors of both studies state that multiply transfused infants can and do develop aHLA antibodies, their presence in serum may be only transient. Of the 7 neonates who developed antibodies in the Bedford-Russell study, three had subsequent negative test results. Furthermore, it is uncertain whether the "transient" nature of the antibodies has a significant impact on the health of the transfused infant or whether these results are simply false positives. The clinical impact of the study infants was not provided. The conflicting results suggest that further studies are needed.

While the physiological effect of lymphocyte subset changes has been shown, the clinical effect of immune suppression mediated by non-leukoreduced RBCs is still unknown. Wang-Rodriguez reported an increased proportion of clinical consequences in the non-leukoreduced study arm, however, sample size did not permit proper evaluation.[28] Thus, the clinical consequences remain unclear.

Unfortunately, there were no studies with a primary objective of assessing the impact of leukoreduction on nosocomial infection, mortality and length of stay. Although three studies reported such outcomes, only one study was eligible for interpretation. Clearly, further studies are warranted.

In order to provide meaningful results, a well-developed research question needs a well-defined study methodology and analysis. Serious methodological flaws were identified in a few

studies included in this review. Baseline testing and screening of the outcome variable is critical to avoid a bias in the estimate of effect. Furthermore, if the outcome can be acquired through a variety of mechanisms, then those mechanisms must be identified and incorporated into the design of the study. These two methodological oversights eliminated three studies from further evaluation.[23,24,25] The method of randomization was only well described in two studies. Furthermore, none of the studies explicitly stated that their analysis would be based on the intention to treat principle. That is, the denominator in each arm of the study is comprised of all randomized subjects regardless of reason for exclusion. Of the randomized studies included in this systematic review, only one analyzed all patients randomized.[28]

Given the recent tragedies related to transfusion-transmitted diseases such as HIV/AIDS and hepatitis C infection, regulatory agencies and providers of blood products are under intense public pressure to guarantee the safety of the blood system. However, the lack of convincing data and the significant cost of leukoreduction mandate evaluations to determine the clinical and economic impact. Large randomized controlled trials with sufficient sample size to detect a clinically important difference in outcomes are the ideal evaluations. However, national implementation of universal leukoreduction is making it increasingly difficult to practically and ethically conduct randomized trials. There exists an ever-diminishing window of opportunity for randomized controlled trials to be performed in countries where universal implementation has not been introduced such as the United States and Japan. Once the window of opportunity is closed, alternative inferior designs will need to be considered. Independent of opportunity, large definitive studies are needed to further evaluate the benefits of leukoreduction in the neonatal setting.

Table 1: Study Methods

Author	Year	Methodology	Comparison	Outcome
Kurul[25]	1998	non-RCT	LR vs non-LR	aHLA-antibodies
Bedford-Russell[27]	1993	RCT	LR vs. non-LR	aHLA-antibodies
Strauss[26]	1999	RCT	stored LR vs. fresh non-LR*	aHLA-antibodies
Xu[23]	1995	Before/After	LR vs. non-LR	CMV
		Before/After	LR vs. non-LR	CMV
Eisenfeld[22]	1992	non-RCT	LR vs. non-LR(SCF)	CMV
Gilbert[21]	1989	RCT	LR vs. non-LR	CMV
Ohto[24]	1999	RCT	LR vs. non-LR*	CMV
Wang-Rodriguez[28]	2000	RCT	LR + IRR vs. non-LR+ IRR	lymphocyte subsets
Romano[29]	1987	single cohort	LR vs. non-LR	lymphocyte subsets

RCT: randomized controlled trial, LR: leukoreduction with filter, IRR: irradiated, CMV: cytomegelovirus
*both study arms received irradiated RBCs

Table 2: Study Characteristics

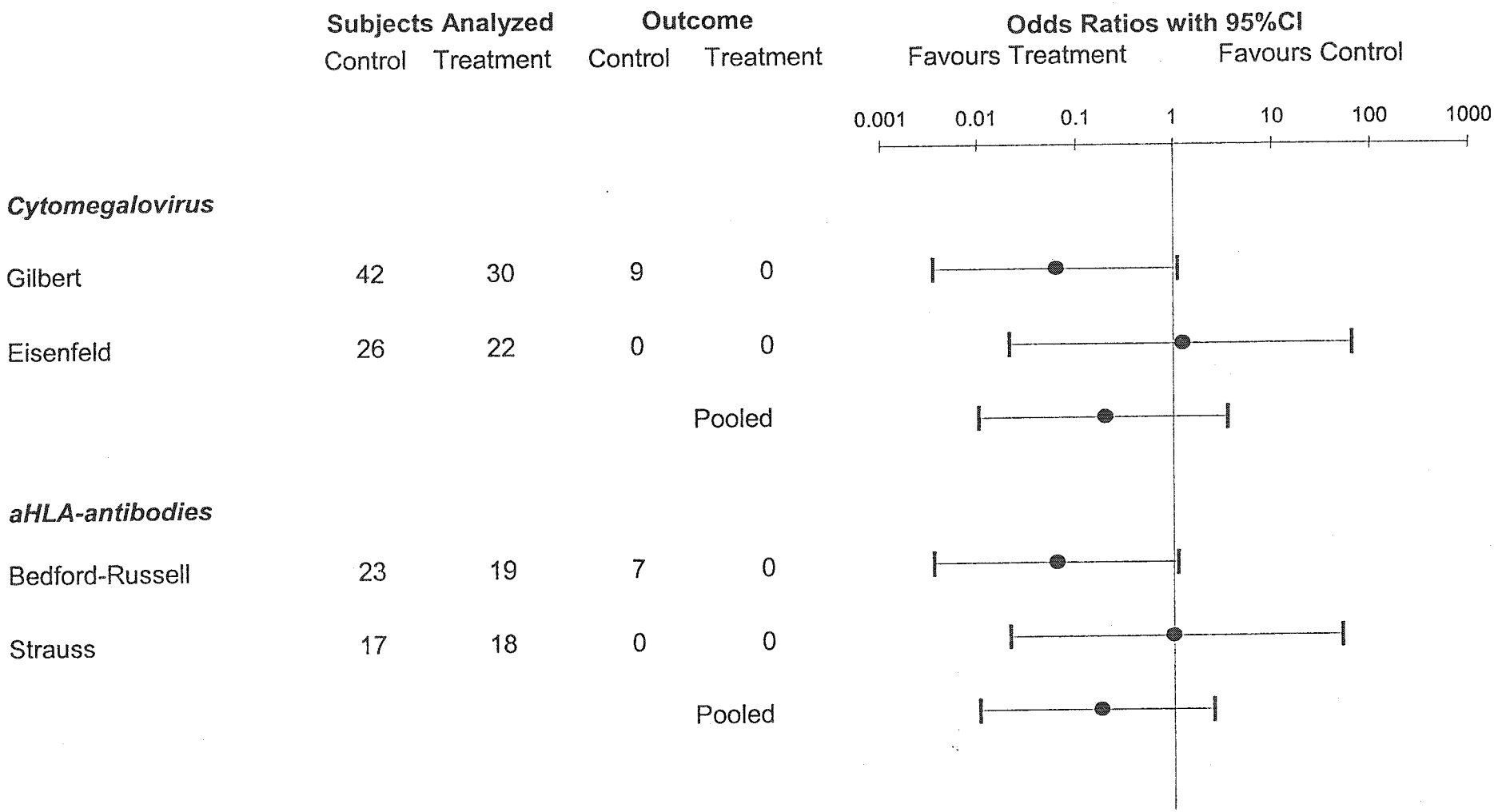
Author	Type of Filter	Type of Transfusion	Population
Kurul[25]	Leukostop-4 LT, Miramed, Italy	BET & RBCs	term & preterm
Bedford-Russell[27]	"in-line "Sepacell, Kimal Scientific Products Ltd.	RBCs	preterm neonates
Strauss[26]	BPF-4 Filter, Pall CORP, NY	RBCs	preterm neonates
Xu[23]	Pall RC100, Pall Biomed Prod Corp, NY	BET	NICU preterm infants<2000 g
	Pall RC100, Pall Biomed Prod Corp, NY	RBCs	NICU preterm infants<2000 g
Eisenfeld[22]	Erypur, Organon Teknika, NC or Sepacell,Asahi Medical Co,IL	RBCs	preterm neonates<1250 g
Gilbert[21]	Imugard 500, Teruma	RBCs	NICU admissions
Ohto[24]	Pall RC50, Pall Biomed Prod Corp, NY	RBCs	newborns
Wang-Rodriguez[28]	BDF-4 Filter,Pall Corp, NY	RBCs	preterm neonates
Romano[29]	unknown	BET	preterm neonates

RBC: red blood cells,NICU: neonatal intensive care unit
BET: blood exchange transfusion

Table 3: Justification for excluded studies

Author	Study Design	Important Methodological Weaknesses	Consequence of Weaknesses on Observed Outcome
Kurul[25]	non-RCT	Included infants with pre-transfusion aHLA antibodies	Unpredictable as the route of transmission and study arm of 7 infants with pre-transfusion aHLA antibodies was not provided
Xu[23]	Before/After	CMV testing of breast milk not conducted	Unpredicatable as CMV route of transmission remains unknown
Ohto[24]	RCT	Pre-transfusion CMV status of RBCs not known	Study conducted in an endemic area thus pre-transfusion CMV status of patients and mothers can be assumed to be very high
		Pre-transfusion CMV testing of mothers and infants not conducted	Unpredicatable as CMV route of transmission remains unknown
		Inclusion of CMV+ mothers CMV testing of breast milk not conducted	

Figure 1: Pooled analyses of eligible studies



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Chapter 3

3.1 Preface to Manuscript #2: Methods Paper

The results of the systematic review clearly illustrate the lack of evidence on the clinical effectiveness of leukoreduction. Indeed, no study evaluating clinically important outcomes had been published. This ensured the originality of the dissertation. Thus, a study to assess clinically important outcomes in the neonatal population is justified. The attention now focuses on the best study design available to answer the proposed hypothesis. While a randomized controlled clinical trial would potentially provide the most unbiased estimate of effect, the universal implementation of pre-storage leukoreduction in 1999 precludes such an approach. A randomized controlled trial was not possible due to ethical and regulatory concerns related to the perceived provision of a superior blood product to only half of patients. As with any health intervention that is uniformly introduced, the universal implementation of leukoreduction, severely limits available study designs to evaluate its effectiveness. With these restrictions, a before/after study was considered the optimal study design. The purpose of Manuscript #2 is to present the study protocol and address important threats to internal and external validity.

3.2 Manuscript#2: Methods Paper

Fergusson D, Hebert, PC, Shapiro, S, The before and after study design in transfusion medicine: methodological considerations. Transfusion Medicine Reviews. 2002; 16(4): 296-303

Abstract

To ascertain the effectiveness of an intervention, a randomized controlled trial (RCT) is considered the gold standard. An obstacle to conducting an RCT only rarely discussed is the universal implementation of an intervention. Universal implementation clearly precludes the feasibility of conducting an RCT. Thus, the most attractive alternative study design in such instances becomes the before/after study. This article describes important methodological considerations in undertaking a before/after evaluation. The methodological considerations to be discussed are: 1) threats to internal validity; 2) precision; and 3) generalizability. Two before/after studies evaluating the potential effectiveness of universal leukoreduction serve as examples. Because of the universal application of many transfusion interventions, one has to consider carefully the methodological rigor as to which of these interventions are evaluated. We have outlined the major methodological issues one must consider when undertaking a before/after study design. Properly conceived, conducted, and analyzed, such a before/after study design can yield informative associations.

Introduction

Leukoreduction is the process of filtering red blood cells (RBCs) in order to remove leukocytes (white blood cells). In 1999, Health Canada, the regulator, directed both providers of all blood products in Canada, Canadian Blood Services (CBS) and Héma Quebec, the manufacturers, to implement a program of universal pre-storage leukoreduction. A similar decision was made in France, the United Kingdom, and Portugal. The United States is still evaluating the need to implement universal leukoreduction. Unfortunately, the requirement for a universal program in Canada precluded the availability of non-leukoreduced red blood cells and limited our ability to evaluate its impact. The benefits of leukoreduction have been the source of much debate with many experts suggesting that there was no conclusive evidence to support universal leukoreduction.[1-3] Indeed, there are populations where leukoreduction has not been proven to be effective.[4,5] Proponents of a universal leukoreduction program argue that since leukoreduction has not been shown to be harmful and intuitively increases safety it should be universally implemented.[6] However, to justify universal adoption of leukoreduction, it is important that its clinical and cost-effectiveness be demonstrated across a range of populations.

To ascertain the effectiveness of an intervention, a randomized controlled clinical trial (RCT) is considered the preferred study design as it minimizes most important biases if properly conceived and executed. Despite being the gold standard, there are sometimes practical, legal, financial and ethical limitations to the use of this experimental design; such as exposing subjects to undesirable and dangerous interventions (e.g. known carcinogens such as cigarettes and toxins). While these limitations have been well described, there is one obstacle to conducting an RCT that has been rarely

discussed: the universal implementation of an intervention across an entire population. By universally implementing an intervention across an entire population, a RCT becomes impossible within that population. Thus, if the effectiveness of the technology in question is not known, any form of study with concurrent controls including the optimal design choice, an RCT, is no longer feasible. In order to evaluate the effectiveness of a universally implemented technology, researchers have no choice but to consider an observational design. There are also limitations in the choice of observational designs. Usual choices would include either a case-control or cohort study. Both study design choices require that subjects from the same population be sampled over a period of time during which they are characterized as either exposed or unexposed; in this context, exposure would refer to pre-storage leukoreduction. In a case-control study, patients are selected as diseased (cases with an outcome such as a nosocomial infection) or non-diseased (controls without such an outcome) and exposure (leukoreduction) status is ascertained after the fact or retrospectively. In a cohort study, patients are selected based on exposure or treatment while outcomes are ascertained over time or prospectively. In this instance all patients are either given leukoreduced or non-leukoreduced RBCs and therefore an association between treatment and outcome is not possible.

The only observational designs available are the standardized incidence and before/after designs. In a standardized incidence study, a standardized incidence ratio is calculated by comparing the incidence of an outcome in a defined exposed population with that of another population. In the standardization procedure, care is taken to adjust for important confounders. In the case of universal leukoreduction in Canada, the incidence of nosocomial infection after implementation would be compared to non-universal leukoreduced populations such as might be available in the United States or Japan. A before/after study design measures the frequency of an outcome in a specified

population during a period of time when the exposure is absent followed by a measurement in the same population during a period of time where exposure is present. Consecutive periods before and after the implementation of a treatment are often compared. One type of before/after study, is the interrupted time series design that proposes to make multiple determinations of an outcome, rather than only one, before and after the implementation of an intervention.

In order to evaluate the effectiveness of leukoreduction, a double-blind RCT would potentially provide the least biased estimate of treatment effect. Despite minimizing selection and information biases, an RCT would not be possible because of ethical and regulatory concerns related to the perceived provision of a superior blood product to only half of patients coupled with the fact that non-modified blood products would no longer be produced. For our evaluation of leukoreduction, we chose a before/after design given the inability to perform an RCT. This evaluative strategy could be very important in transfusion medicine as there are a number of interventions and policies that have been or will be universally implemented (e.g. leukoreduction, nucleic acid testing, donor restrictions). This article will describe important methodological considerations in undertaking a before/after study design.

Description Of Neonatal And Adult Pre-Storage Leukoreduction Studies Being Undertaken In Canada

In this article, two studies will be outlined, one in a neonatal intensive care and an adult perioperative evaluation, that will make use of observations before and after the implementation of universal leukoreduction in order to determine the effectiveness of a universal leukoreduction

program.

In the neonatal setting, we will ascertain rates of clinically important nosocomial infections in intensive care newborns weighing less than 1250 grams at birth who receive at least one aliquot of RBCs. The neonatal population was chosen as it is at high risk both for receiving blood products and for adverse infectious outcomes. In addition, pre-storage leukoreduction may have adverse health consequences in this vulnerable population. Using an existing database of all admissions to 3 Canadian neonatal intensive care units taking part in a long-term follow-up evaluation (Illness Severity, Practice Variations, and Resource Consumption in NICUs study, Principal Investigator, Dr. Shoo Lee), we will compare rates of infection one year preceding and one year following the introduction of universal pre-storage leukoreduction program.

A second major study will enroll 16,000 adult patients undergoing operative repair of a hip fracture, cardiac surgical procedures requiring cardiopulmonary bypass, and critically ill patients admitted to ICU postoperatively or following major trauma, that were administered at least one RBC transfusion. Patients will be identified in a retrospective manner from the clinical records of 20 participating centres in the one-year period preceding and one-year period following the universal introduction of leukoreduction. All outcomes, including serious nosocomial infections, will be ascertained at 30 days and one year using clinical records and provincial vital statistics registries.

The Before/After Design

If one cannot control the allocation of the intervention to an individual or group as in an experimental design, an observational or quasi-experimental research design must be used. There are

a variety of before/after designs depending on whether an individual or a population is being measured pre and post-intervention and/or whether a control group is used. In the case of the universal leukoreduction studies, the outcomes will be measured within groups of adult perioperative patients and neonates before the intervention was implemented compared to different groups of patients after the intervention was implemented.

One calendar year periods before and after the implementation of leukoreduction have been chosen as the observation periods. In both studies, the rate of nosocomial infections in the one-year period prior to universal implementation of leukoreduction will be compared to the rate of nosocomial infection in the one-year period after its implementation. In both studies, other a priori defined secondary outcomes, such as length of hospital stay, will be compared in a similar fashion. Part of the analysis of both studies will involve measuring outcomes within shorter periods of observation such as seasons and months.

Methodological Considerations Of Before/After Design: Internal Validity Or Lack Of Systematic Error

The validity of a study is separated into two components: the validity of the inferences drawn as they pertain to the members of the source population (internal validity) and the validity of the inferences as they pertain to the population outside the study sample. As with any study design and conduct, attention must be paid to both internal and external validity threats. Campbell and Stanley present the following seven threats to the internal validity of a study: 1) maturation; 2) statistical regression; 3) testing; 4) history; 5) selection; 6) instrumentation; and 7) dropout.[7] Each of these threats will be defined and discussed in the context of both the adult and neonatal leukoreduction

studies.

Maturation, statistical regression, and testing

Of the seven threats to internal validity, maturation, statistical regression, and testing are not applicable to the proposed before/after study designs. Maturation refers to changes in the outcome variable due to normal developmental processes. This would be a major source of bias in a neonatal study that compared the same patient before and after an intervention because of the significant developments in newborns over short time intervals. The second threat, statistical regression (regression artifact or regression to the mean) is the statistical tendency for extreme scores on the first measurement to move closer to the mean on the second measurement. Again, this phenomenon occurs when repeated measurements are made in the same individual at two different times. As an example, extreme laboratory values known to vary significantly are much more likely to be closer to the mean values upon repeated measurements. The final threat in this category is "testing". This threat refers to the influence of a pre-intervention measurement on post-intervention measurements. Specifically, patients may become familiar with the testing procedure and in turn have improved score on subsequent evaluations using a comparable study instrument. Fortunately, maturation, statistical regression and testing are not a concern in the proposed before/after study designs because all measurements and outcomes are ascertained only once. Patients will either be included in the pre-intervention period or in the post-intervention period. Thus, three of the seven threats to the validity are not a concern for the proposed before/after studies.

The four remaining threats to the internal validity of a before/after design include: history;

selection; dropout; and instrumentation, are potentially serious sources of bias. These are discussed in the following:

History

History refers to any secular trend or extraneous event(s) occurring before or after the implementation of an intervention that could account for some or all of the resulting change in the measured outcome. As an example, a novel prophylactic treatment for the prevention of infections given to expectant mothers could have a significant impact on infectious outcomes in the newborn population. If such treatment were only introduced in the period following the implementation of universal pre-storage leukoreduction, then any inference made regarding the effectiveness of leukoreduction would be seriously jeopardized. History is potentially the greatest threat to the validity of before/after studies. Therefore, it is necessary to identify, measure, and control for any potential secular or extraneous events that may occur during the two observation periods.

There are a number of strategies investigators may choose to implement to minimize or understand the influence of secular trends in a study. In both the adult and neonatal studies, one of the first steps is to identify important variables that may affect the study outcome. Each of these variables must be included in the data collection instrument. In the proposed studies, it is imperative to monitor changes in transfusion practice, co-interventions and rates of nosocomial infection as well as other outcomes over the two years of observation. To do so, essential data will be gathered from patients who meet all eligibility criteria but who were not transfused. Patients who are not transfused (therefore not exposed) in both the pre and post intervention period will act as concurrent controls for identifying potential confounding variables (e.g. outbreaks). By including these patients in the

analysis of the study, we will be able to examine rates of transfusion and infections in the entire population of patients (exposed and unexposed to RBCs). If these rates change during the study periods, appropriate adjustments can be explored in the analysis phase (providing the reasons for change are identified and collected).

Another important "historical" concern is that of baseline characteristics of patient populations pre and post universal leukoreduction. Since the overall use of RBCs has decreased since the early 1990s[8], a continued decrease following the introduction of universal leukoreduction may result in a time dependent selection bias. A decrease in RBC usage might result in less healthy patients being enrolled in the second phase of the study. By monitoring rates of transfusion and transfusion thresholds, we can comment on this possibility in the reporting of the results. Performing the study in multiple study populations during the same time period will add another layer of "control" in the study. It would be difficult to conceive of a situation that alters overall rates of infection in neonates, in adults who are critically ill and in many perioperative patient populations in the same short time frame. This would also be true of mortality rates as well as other important outcomes.

Similarly, an examination of the rates of infection in the transfused and non-transfused patient populations will also help identify and control for time-dependent trends such as epidemics or outbreaks within specific patient populations under study. To minimize the potential impact of such a possibility, the study design should include comparable periods of observation. This is especially true for outcomes for which there are known seasonal variations. For instance, there may be seasonal patterns to rates of infection caused by viruses such as influenza. There may also be seasonal fluctuations in the disease mix of patients admitted to the intensive care unit (more

exacerbations of chronic lung diseases during influenza season and more blunt trauma in the summer months). If the periods of observation are not seasonally balanced, the measure of effect could be biased by the under or over representation of seasons. Because of this concern both the neonatal and adult studies will collect information on one complete year of observation, both before and after the implementation of the intervention.

In addition to equal periods of observation, the length of the observation period should be an important consideration. There is a much greater possibility of changes in patient care and therefore outcomes over prolonged periods of observation as compared to shorter periods. For instance, there may be significant historical trends in a study that requires 10 years of observation in both the pre and post intervention periods as compared to a study that will be completed using two consecutive years of observation.

A number of steps can also be introduced into the analysis phase. The analysis can be stratified by procedures and major disease groups as well as centers. This should enable us to identify important sources of variability and better understand its influence on study results. Multivariate procedures can be used to adjust for the influence of multiple variables at one time and to describe trends that explain the effect over time in both periods of observation.

Selection

Selection bias occurs occur whenever the inclusion of cases (transfused with leukoreduced RBCs) or controls (not transfused with leukoreduced RBCs) into the study depends in some way on the intervention (or in this case on the period) of interest. The bias is due to the systematic

differences in characteristics between those who are selected for the study and those who are eligible but are not selected. It is of a particular concern for retrospective study designs because both the treatment and outcome status of subjects have both occurred. To minimize selection bias in the proposed before/after studies, a number of measures were integrated into the study designs.

First, the process for identifying intervention and outcome status is important. By the very nature of the study design itself, a before/after study initiated after the universal implementation prohibited the use of prospective patient identification and data collection. The "before" cohort and their data must be identified and collected retrospectively. The implication is that retrospective identification of subjects is prone to selection bias. If knowledge of disease or exposure status is known and influences the selection of individuals then selection bias may result.

Second, while not always feasible, every effort should be made to identify all subjects meeting the study inclusion criteria. This often necessitates the need for a well-defined population that is perhaps narrower than originally intended. Furthermore, if the study is multi-centre, then emphasis should be placed on ascertaining all eligible subjects at a specific number of sites rather than simply increasing the sample size by increasing the number of sites without full identification of all eligible subjects. While all eligible patients may not be included in the study, it is nevertheless important to identify the sample frame. From this sample frame, a study sample can be taken. It is preferable to include all identified eligible patients, but if this is not feasible due to resource constraints, a random sample from this sampling frame should be taken to minimize the risk of selection bias. The use of population-based, administrative, or clinical databases, either wholly or partially, is particularly ideal for such retrospective designs. They can be much more efficient in their ability to provide a complete sample frame compared to other methods of identifying all eligible

subjects such as medical chart review. This, of course, is dependent on how well the intervention, outcome, and important confounding variables are classified and documented.

Third, comparable time frames should be chosen for each of the before and after cohorts. While the approaches for avoiding selection bias in before/after studies are largely harmonious with those of any observational study, there is one issue unique to before/after studies. In order to help avoid selection bias due to temporal trends, the time periods for both the control and intervention must be comparable. They must not only be comparable in terms of length of time (e.g. one year periods) but, more importantly, with respect to months included. That is, if the intervention period comprises a one-year period from October to October, then the control period should comprise a one-year period from October to October. If the study outcome and/or exposure were associated with a particular time period (e.g. asthma), then selection bias may be introduced if the control and intervention periods were not synchronized between consecutive years. Intrinsically related to the time period is the definition of the study population. By choosing a population with a high event rate and high exposure rate one can dramatically reduce the before/after time periods. For example, in the proposed study design, neonates <1250 g were chosen based on an expected high risk of transfusion ranging from 50-75% and nosocomial infection rates as high as 50%. These rates were based on a review of the literature. One must also consider that focusing on those that are highly susceptible will reduce the number of subjects available. As previously mentioned, it is ideal to have comparable before/after time periods. Thus, it is necessary to balance both the potential population at risk and the study time frame.

Fourth, if the implementation of an intervention is not immediate and occurs over a period of days, weeks, or months, the use of a washout period is an option. For example, to manage patients

with prolonged hospital stays receiving both non-leukoreduced (pre-program implementation) and leukoreduced (post-program implementation) RBCs, an adequate washout period was incorporated into the study design. Given that the current shelf life of RBCs is 42 days, patients will not be enrolled during this 42-day period following the introduction of the program. To be conservative, an additional 18 days prior to the date of the commencement of the program will be included into the washout period. This will allow 60 days to pass between the pre and post leukoreduction periods. Any remaining patients in the post leukoreduction period who were admitted to hospital and transfused prior to the introduction of leukoreduction will be excluded from the study. Exclusion of these subjects will control for bias due to contamination and will increase the ability to detect a difference between the two groups.

Instrumentation

Instrumentation or information bias refers to changes in measuring instruments, scorers, or observers that produce changes in the obtained measures. Information bias results if the manner of obtaining information on either the intervention or outcome variables differs between groups. As with selection bias, the process for identifying intervention and outcome status is an important issue. In observational studies, the retrospective identification of exposure and disease status depends on the routine availability of exposure and disease data (as well as confounders/effect modifiers) in sufficient detail from pre-existing sources (charts, databases). Even when the necessary data is available, the extent to which the data is accurate or comparable between subjects and centres remains a concern. To ensure data is comparable a number of steps can be incorporated into the

study design.

The first step relates to two aspects of data collection that significantly influence information bias 1) the development of the data collection instruments and 2) the application of these instruments by study personnel. To this end, it is important to ensure uniform data collection instruments and methods are applied to all subjects. Specifically, using the same instruments for all study subjects helps minimize differences in definitions and interpretation. Development of a single study instrument and a manual that clearly defines each of the variables minimizes information bias. There will always be some variables that are open to subjectivity; an *a priori* well-defined outcome is essential. A highly subjective outcome can produce significant information bias because ascertainment can vary systematically between and within sites and data collectors. If the outcome is assessed identically in both groups without knowledge of exposure, then any resulting bias will be non-differential. Nonetheless, this bias may preclude the study's ability to detect a clinically important difference.

As for administering the data collection instrument, prospective data collection has been shown to be superior to retrospective data collection in terms of completeness and accuracy. Unfortunately, the proposed before/after design may not allow for the prospective data collection of all subjects due to the implementation of universal leukoreduction in 1999. The before/after design does not preclude a prospective cohort. A cohort identified after the implementation of an intervention can be prospectively followed. If this approach were adopted, the pre-intervention data collection would be done retrospectively while the majority of data collected in the post-intervention phase would be done prospectively. This approach could be subject to information bias as the manner in which outcomes are identified could be differential. For example, one can imagine that

prospective documentation of infections would greatly increase the number of confirmed infections as compared to retrospective data with incomplete medical records. As with ascertainment of exposure, ascertainment of outcome is also a concern in retrospective studies. Very few observational studies are likely to correctly identify all subjects experiencing an event. Therefore, the process for ascertaining the outcome in both the exposed and nonexposed needs to be as comparable as possible. This is another justification for having data collection wholly retrospective.

In the neonates study, the decision was also made to minimize information bias resulting from the inclusion of both retrospective and prospective data by collecting all data retrospectively. The case report forms used in the data collection have undergone rigorous reliability and validity checks. Furthermore, objective demographic, clinical and outcome data will be collected. For example, our primary outcome, serious nosocomial infection, is defined as sepsis (clinical signs and symptoms of sepsis and positive blood culture for viruses, bacteria or fungi) or bloodstream infection (positive blood culture for viruses, bacteria, or fungi). Nosocomial pneumonia was not included in the definition of a serious nosocomial infection due to extreme subjectivity and possible misclassification. Blood stream infections require that a blood culture be positive. For a new infection, there must be 1) a new organism or 2) a blood culture drawn seven days after the initial positive draw comes up positive. If the same organism is found in both a blood and cerebrospinal fluid culture, they are scored separately. All positive blood cultures are to be recorded, even if they are noted or thought to be contaminants. Infants transferred into NICU with a positive culture will also be noted. While we will exclude any infections that occur before the first transfusion, we will not determine the time to event (infection) after the first and subsequent transfusions. This information would be highly susceptible to information bias as the ability to determine exactly when

an infection occurred is unworkable. The time the infection is diagnosed is possible to capture but could easily be biased by logistical parameters such as laboratory operating hours. We also believe that whether a subject experienced an event is clinically more relevant than when it occurred. Nosocomial infections were chosen as the primary outcome because infections are a direct consequences of immune suppression induced by RBC transfusions and potentially decreased by universal pre-storage leukoreduction and are frequent in neonates <1250g.The use of the objective secondary outcome, length of NICU stay, will also minimize any information bias due to the inability to blind and to collect information prospectively in both the exposed and nonexposed cohorts.

Dropout

The dropout or mortality threat to internal validity refers to the bias resulting from subjects who drop out after being included in the study. The concern is that subjects who drop out of a study may have different characteristics than those who remain and thereby introduce bias. To minimize this threat requires adequate follow-up of all patients entered into the study. Reasons for dropout need to be recorded and addressed to identify possible differences between the before/after groups. If differences do exist between the two comparison groups, then explanations for those differences need to be explored. The differences are not necessarily directly related to the exposure as they could be due to an extraneous event or policy change. In the proposed before/after study, dropout has been minimized by using data sources that ensure the identification of the entire study population at each site. Therefore, intervention and outcome status will be known for all eligible study subjects.

Methodological Considerations Of Before/After Design: Precision Or Lack Of Random Error

Precision in measurement and estimation corresponds to the reduction of random error. Thus, precision can be improved in two ways: 1) increase the sample size of the study and 2) increase study efficiency. Determining sample size estimates in the context of the before/after design is no different than any other test for the comparison of two independent proportions or means. Sample size calculations depend on desired values of Type I & II errors, the proportion of the baseline population who experience the outcome under study (or baseline mean values for continuous outcomes), and the magnitude of the expected effect. Type I error occurs when a difference in outcomes is detected when there is no true difference (false positive result) and a Type II error is noted when no difference in outcomes is detected when a difference truly exists (false negative). These characteristics determine the sample size required in each time period. In establishing the sample size, both studies sought to have a sufficient number of patients to detect differences that were both clinically as well as statistically important. Baseline rates of serious infection were estimated from the literature as well as expert opinion. With evidence from the literature and clinical expertise, a consensus was reached on a minimally important difference in the rate of nosocomial infections pre and post implementation of universal leukoreduction for all study patient populations.

Methodological Considerations Of Before/After Design: Generalizability

According to *A Dictionary of Epidemiology*, a study is externally valid or generalizable if it can produce unbiased inferences regarding a target population (beyond the subjects in the study).[9] It

must be understood that study results must be internally valid before they can be generalizable. In other words, you cannot generalize an invalid result. Generalizability has implications on study design. In order to widen the scope of generalizability, studies widen the eligibility criteria of the study to include a more representative sample of the population. This temptation, however, increases the likelihood of threats to internal validity such as dropout or information bias. To increase the generalizability of results in the leukoreduction study, a number of choices were incorporated into the study design. Rather than selecting one particular study site, three study sites representing three different regions of Canada were selected. Thus, any observed result should be applicable to the other NICU populations <1250 grams in the respective regions of Canada. Generalizability is also maximized by selecting the entire population of transfused neonates <1250g at each of the three NICUs rather than taking a random sample. As mentioned this also minimizes the threat of selection on internal validity. In the adults study, patients requiring cardiac and orthopedic surgical procedures as well as those in intensive care were included in order to ensure the generalizability of our inferences to all perioperative patients.

In an effort to increase study efficiency we chose a population (NICU neonates <1250g) both with a high rate of transfusion and high rate of infection. Consequently, this choice of study population reduces the generalizability of results to the entire NICU population. For instance, it could be argued that the results of the leukoreduction study may not be applicable to infants >1250 grams which account for approximately 80% of the NICU population. Whether or not the result of a study applies to the target population and beyond can thus be highly subjective.

Conclusion

Generally, blood suppliers procure cellular blood products for whole populations. Thus, RBCs are collected and distributed to hospitals and clinics with no regard to the patients who will be receiving them. Thus, any change in the blood system for safety or clinical effectiveness purposes, is universally applied. Leukoreduction is but one example in transfusion medicine. Other examples include removal of buffy coat, PCR testing, nucleic acid testing, fractionating plasma from whole blood. Because of the universal application of many transfusion interventions, one has to carefully consider the implications for evaluation. Ideally, a randomized controlled trial should be undertaken to evaluate the effectiveness of new interventions before they are implemented. Unfortunately, many interventions are not fully evaluated before they are implemented or are implemented for reasons besides clinical effectiveness such as public safety. In this case, a before/after design becomes the most attractive, despite its historical reputation as being one of the least attractive methodological designs. Thus, in areas where universal leukoreduction has been introduced, before/after studies will undoubtedly become more common. Indeed, a recently published study conducted in France, where universal leukoreduction was introduced in 1998, used a before/after design in their examination of leukoreducton by filtration and postoperative infections in high-risk patients undergoing abdominal aortic surgery.[10] We have attempted to outline the major methodological issues one must consider when undertaking a before/after design. Properly conceived, conducted, and analyzed, a before/after design represents a useful mechanism for addressing important policy issues.

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Chapter 4

4.1 Preface to Manuscript#3: Study Results

The protocol "Effectiveness of Leukoreduction in the Neonatal Population" was a successful submission to the *Canadian Blood Services/ Canadian Institutes of Health Research (CIHR) Partnership in Transfusion Science 2000* grant competition and received funding in March 2001. This was the second submission for peer-reviewed funding to the CIHR. The first submission to the March 2000 CIHR competition was not funded for the following reasons: 1) insufficient and superficial discussion of the limitations of the design; 2) confusing data analysis; and 3) insufficient discussion of the potential impact of the study on practice. Each of these reasons was addressed in detail and the subsequent application was successful. Study data collection commenced in April 2001 and analysis was completed in July 2002. The study received Ethics approval from all participating sites (Appendix II).

During the conduct of the trial, there were two deviations form the original protocol. First, the number of original study sites was six instead of three. Second, the number of study months expanded from 24 to 36. The second deviation is a consequence of the first. The decision to include six sites in the original protocol was based on their ongoing participation in the Canadian Neonatal Network database. However, while all six sites did contribute data to the Canadian Neonatal Network database, three sites stopped collecting data well before the end of the proposed study period. In contrast, if the study period was expanded from 24 months to 36 months for the three sites with consistent data collection, there was a manageable amount of data to be collected (Children's and Women's Health Centre of British Columbia from January 1998 to July 1998 and Royal University Hospital from January 2000 to December 2000). In addition, the three sites in this study

represented approximately 66% of admissions from the six sites combined. The decision to expand the study period to 36 months was based on having two chronologically symmetrical 18-month study periods. Thus, each 18-month period had identical types of months. By restricting the study to three sites and expanding the study period to 36 months, it was estimated that 85% of the original sample size estimate would be achieved.

Manuscript #3 provides the results of the study. Because of word restrictions required by Journal editors, greater detailed information concerning variable definitions and data analyses are not incorporated into the manuscript. Therefore, greater detail is provided in appendices III- VIII.

All data, except transfusion data for Children's and Women's Health Centre of British Columbia and Mount Sinai Hospital, were acquired through the Canadian Neonatal Network database. Additional data collection was undertaken at two sites to secure complete 36-month sets of data. For resource reasons, the Canadian Neonatal Network database did not contain Children's and Women's Health Centre of British Columbia data for the period January 1998 to July 1998 inclusive and Royal University Hospital data from January 2000 to December 2000 inclusive. Data collection for these two sites was undertaken using Canadian Neonatal Network data collection forms and personnel. The additional data collection was entered into the Canadian Neonatal Network database. The Canadian Neonatal Network database instituted detailed and comprehensive policies regarding data quality and data management. An overview of these Methods can be found in Appendix III as well as an overview of the data collection protocol. Appendix IV provides the variable definitions for data abstracted from the Canadian Neonatal Network database for the period January 1998 to December 2000 (36 months). Any transformed or renamed variable for the "Effectiveness of Leukoreduction in the Neonatal Population" study is duly noted.

The study database consisted of data transferred electronically from the Canadian Neonatal Network database and transfusion data abstracted onto standard forms from the clinical chart. All data was merged into a single NCSS (Utah, 2000 Version) database consisting of all patient data relevant to this evaluation. Patient and maternal identifiers were removed from the database and kept in a separate secured file. For the transfusion data, appropriate range and logic checks were integrated into the NCSS database and double data entry was used.

Descriptive statistics for each of the study variables included in the final model including measures of central tendency and dispersion are presented in Appendix V. The principal analysis used multivariate logistic regression modeling techniques. Logistic regression was conducted to describe the relationship between the dependent variable and clinically important and biologically relevant covariates. A list of the multivariate logistic regression models for each of the dichotomous outcomes as well as the multivariate linear regression model used to compare the continuous variable neonatal length of stay are presented in Appendix VI. The comparison of imputed versus non-imputed regression models are presented in Appendix VII. The odds ratios provided in Appendix VI are the reciprocals of the odds ratios provided in the thesis abstract and Manuscript #3. Traditionally, odds ratios less than 1 suggest benefit in favour of the intervention and therefore the calculated odds ratios provided by the statistical software had to be inversed. Data from the non-transfused cohort are provided in Appendix VIII.

4.2 Manuscript # 3: Study Results

Fergusson D, Hébert PC, Lee SK, Walker CR, Barrington KJ, Joseph L, Blajchman MA, Shapiro S, Effectiveness of Universal Leukoreduction of Blood Transfusions in Premature Infants, *New England Journal of Medicine* (slightly modified version of manuscript submitted in 2002)

Abstract

Background: Leukocytes present in blood transfusions may depress immune function thereby increasing nosocomial infections and possibly resulting in organ failure and death. Given their immature immune system, neonates may be uniquely predisposed to the effects of transfused leukocytes. Therefore, we evaluated the clinical consequences of a universal pre-storage leukoreduction program in premature infants weighing less than 1250 grams upon admission to the neonatal intensive care unit (NICU).

Methods: A retrospective before and after study comprising three Canadian NICUs was conducted. The intervention group consisted of premature infants admitted to the NICU in the 18-month period following the introduction of leukoreduction and the control group consisted of premature infants admitted to the NICU prior to the introduction of universal leukoreduction. Clinical information was gathered from the Canadian Neonatal Network database and transfusion data was collected from hospital blood banks. The primary outcomes in this study were nosocomial bacteremia and death. Major secondary outcomes included bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhages. Comparison of the groups before and after the implementation of universal leukoreduction was conducted using multivariate regression.

Results: A total of 515 infants <1250 grams were included. For nosocomial bacteremia, the odds ratio was 0.59 (95%CI: 0.34-1.01). Crude and adjusted rates for all secondary outcomes suggest that leukoreduction improved all outcomes. The adjusted odds ratio for a composite measure of any major neonatal morbidity was 0.31 (95%CI: 0.17-0.56).

Conclusion: The implementation of universal pre-storage leukoreduction significantly improved clinical outcomes in premature infants requiring blood transfusions.

Introduction

Greater than 50 percent of infants born under 1250 grams admitted to neonatal intensive care units require blood transfusions.[1] Despite recent trends in decreasing transfusion thresholds and the development of technologies designed to avoid exposure to blood such as erythropoietin, transfusions remain an important life-saving measure in the care of premature infants.

Leukocytes present in blood transfusions may depress immune function thereby increasing nosocomial infections and possibly resulting in organ failure and death.[2-3] However, randomized trials, conducted exclusively in adults in a variety of surgical settings, have not all observed increased rates of postoperative nosocomial infections.[4-5] In addition, there is a paucity of studies examining the possible risks and benefits of the leukoreduction of blood products in premature infants.[6] Given their immature immune system, neonates may be uniquely predisposed to the effects of transfused leukocytes. Leukocytes from frequent transfusions may depress the immune response, generate alloantibodies and perhaps cause widespread microvascular injury through the enhanced generation of free radicals in susceptible tissue beds such as the lungs and retina.[2] Further, it is conceivable that the leukoreduction process increases red cell hemolysis or results in unexpected clinical consequences.[7] Based upon available research, the benefits of leukoreduction remain unclear in this vulnerable population.

We therefore evaluated the clinical consequences of the institution of a national universal pre-storage leukoreduction program to premature infants weighing less than 1250 grams upon admission to the neonatal intensive care unit.

Methods

Study Design and Participants

The implementation of a Canada-wide leukoreduction program for blood products in 1999 enabled the conduct of a before and after study using three study sites from the Canadian Neonatal Network. The study was conducted at three tertiary care neonatal intensive care units: Children and Women's Health Centre in Vancouver; Royal University Hospital in Saskatoon; and Mt. Sinai Hospital in Toronto. The intervention group consisted of patients admitted to one of the participating neonatal units in the 18-month period following the introduction of leukoreduction. The control group, the non-leukoreduction cohort, consisted of all eligible infants admitted to an neonatal intensive care unit in the 18-month period preceding the introduction of universal leukoreduction. All premature infants from the three neonatal intensive care units who weighed less than 1,250 grams, received at least one allogeneic blood transfusion and survived more than 48 hours were included. Infants surviving less than 48 hours were excluded to remove those with an extremely poor prognosis, such as overwhelming infections acquired from their mothers. Infants were also excluded if they were admitted with a birth weight exceeding 1250 grams at the time of admission, were previously admitted to the neonatal intensive care unit or received both leukoreduced and non-leukoreduced blood. The Research Ethics Committees of all participating institutions approved the study protocol.

Data Collection

Information gathered on each admission from the Canadian Neonatal Network database

included the patient's gestational age and sex, illness severity scores including the SNAP II (Score for Neonatal Acute Physiology) on day 1 and day 3; as well as the APGAR scores at 1 and 5 minutes. Important interventions were recorded including: the use of supplemental oxygen; continuous positive airway pressure and mechanical ventilation; central and peripheral venous access; red cell and platelet transfusions; the use of IVIG; cardio-pulmonary resuscitation; and the number of blood draws. The use of medications such as vasoactive drugs, antibiotics, surfactant, and corticosteroids was also recorded. Maternal risk factors recorded included the type of delivery (cesarean versus vaginal) and the use of any antenatal steroids. Transfusion data was collected from the Canadian Neonatal Network database for one site (Royal University Hospital) and from the respective blood banks for the two remaining sites.

Data abstractors for the Canadian Neonatal Network database were responsible for collecting standardized information from every eligible admission to the neonatal intensive care unit. Entry into the Canadian Neonatal Network database required infants to stay in the neonatal intensive care unit for more than 24 hours, die once admitted, or be transferred to another neonatal units within 24 hours. Moribund infants at admission were not included. Data were gathered from the medical record during the admission to the neonatal intensive care unit and transcribed directly into computerized case report forms. Standardized definitions were instituted to ensure consistency among sites. Any data items not available were scored as missing.

Description of Intervention and Transfusion Parameters

Canadian Blood Services introduced universal pre-storage leukoreduction throughout Canada during 1999. Once collected, red cells are passed through a Leukotrap-RC leukocyte reduction

filtration system (Pall Corporation). Leukoreduction with this technology reduces white blood cell content of a unit of blood from an average $3.0x10^9$ per unit to $2.5x10^5$ per unit, a decrease of 4 logs. CPD-2 anticoagulant solution is then added to each unit with 100 mls of Nutricel additive. All blood products were produced by one agency who conducts national quality control measures of the leukofiltration program.[8] Prior to transfusion, each unit is divided into aliquots suitable for the neonatal population. One site (Children's and Women's Health Centre of British Columbia) washed blood designated for premature infants during the entire non-leukoreduced period and during the first four months of the leukoreduction period. Washing of RBCs reduces the proportion of leukocytes by 85% compared to greater than 99.9% for pre-storage leukoreduction.

Study Outcomes

The primary outcomes in this study were nosocomial bacteremia and death. Nosocomial bacteremia was defined as a positive blood culture for bacteria. For a new infection, there had to be a new organism or a second positive blood culture drawn at least seven days after the initial positive test. Survival status during the index hospitalization was ascertained from the medical record. Major secondary outcomes included bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhage. Bronchopulmonary dysplasia was defined as the ongoing need for assisted ventilation or supplemental oxygen on day 28 of life. The presence of any grade of retinopathy was recorded as an outcome in this study. Without an eye examination, retinopathy was defined as absent. A diagnosis of necrotizing enterocolitis was based upon a grading of stage 2 or greater of Bell's criteria.[9] A diagnosis of an intraventricular hemorrhage required the

presence of intraventricular blood on a routine imaging study such as an ultrasound, computerized tomography or magnetic imaging study of the brain. Intraventricular hemorrhage was graded based upon standard criteria developed by Papile.[10] For the purposes of this study, a composite of grade III and IV intraventricular hemorrhage was included as an outcome. All secondary outcomes were compared both separately and as a composite measure, as a means of evaluating the overall effect of leukoreduction on multiple organs simultaneously.

Length of stay in the neonatal intensive care unit as well as minor and major interventions received while in the intensive care unit were recorded as tertiary outcomes. Major interventions included all major surgical procedures such as laparatomies and thoracotomies. Minor interventions included cryogenic or laser therapy for the retinopathy of prematurity, tracheostomy, endoscopic procedures such as bronchoscopy and all transcutaneous procedures such as nephrostomy and cardiac catheterizations. The use of umbilical vein and artery lines, peripheral arterial lines, venous cutdowns and needle aspiration of body fluids were excluded from this category.

Information was also recorded that reflected the intensity of care on day 1 provided to each premature infant. On day 1, the use of supplemental oxygen, conventional and high frequency mechanical ventilation, arterial and central venous access as well as the use of medications such as vasopressors, glucocorticoids, antibiotics and muscle relaxants were recorded. Supplemental oxygen was defined as the administration of continuous enriched oxygen in concentrations exceeding 21 percent via oxyhood, nasal cannula, nasal catheter, facemask or other forms of respiratory support. The use of "Blow-by" oxygen alone was not sufficient to meet the definition nor was oxygen administered for a hyperoxia test. Mechanical ventilation was defined as use of conventional mechanical ventilation regardless of the respiratory rate. We also recorded the use of high frequency

ventilation using a jet ventilator or oscillator. Peripheral intravenous access was defined as the presence of one or more intravenous catheters, including heparin locks used for drug administration. An arterial line was defined as the presence of a central line including an umbilical venous line, a Broviac line, or a percutaneous catheter placed centrally. Unsuccessful attempts at line placement were not reported. The use of vasopressors was defined as the administration of vasoactive medications administered through intravenous, intramuscular or aerosol routes. Glucocorticoid and antibiotic use was documented if an intravenous, oral or nebulized preparation were used. Finally, muscle relaxant use was recorded daily if at least one dose of the medication was administered during the time interval in question.

Statistical Analysis

Baseline characteristics of premature infants receiving at least one blood transfusion before and after the introduction of leukoreduction were evaluated using measures of central tendency and dispersion. Absolute differences between periods were calculated for each characteristic with appropriate 95 percent confidence intervals. All patients not transfused were described using a similar approach.

A priori, we decided to compare all primary outcomes using crude and adjusted odds ratios with 95 percent confidence intervals. Crude and adjusted odds ratio were also calculated for secondary outcomes including bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhage independently. As a second step, we incorporated all secondary outcomes associated with prematurity into a composite measure and subsequently

compared groups using crude and adjusted odds ratios. Lengths of neonatal intensive care unit stay were compared using multivariate regression analysis. Based upon *a priori* input of experts in neonatology and transfusion medicine, all multivariate models incorporated clinically important variables including gestational age, sex, centre, type of delivery, antenatal use of glucocorticoids, APGAR @ 5 minutes, the SNAP II score as a measure of illness severity, number of days on CPAP, interventions on Day 1 including the use of supplemental oxygen and mechanical ventilation, medications used on day one including glucocorticoid or surfactant, vasopressors, muscle relaxants and antibiotics use.

In order to assess the possibility of secular trends, the crude and adjusted odds ratios for major outcomes were also calculated for premature infants less than 1250 grams who were not exposed to blood transfusions. Adjusted odds ratios were also calculated for infants who received surfactant on Day 1 versus those who did not, as well as for infants who were administered CPAP and mechanical ventilation on Day 1.

In reporting our results, an odds ratio less than 1 suggests that fewer infants in the leukoreduced group acquired the outcome in question; while an odds ratio greater than 1 suggests that fewer infants in the non-leukoreduced group acquired the outcome under scrutiny. Measures of effect for multivariate linear regression were expressed as number of days of NICU stay saved with 95 percent confidence limits. Missing data for three variables: birthweight (3 imputations), APGAR at 5 minutes (14 imputations) and SNAP II on Day 1 (48 imputations), were estimated using the multivariate normal procedure. For the multiple imputations, a regression analysis was conducted using the variable containing the missing value as the dependent variable and all variables with nonmissing data for this patient as independent variables. The values of the nonmissing variables

from the patients containing the missing value and those patients with complete data were used in the regression equation to compute a predicted value for the missing value. This process was iterated by using the imputed missing values from one run during the estimation phase of the next. The imputation procedure was conducted for each of the three variables independently. The outcome estimates for the models with and without imputed data were comparable, so we report the results from the imputation model only.

Results

Baseline Characteristics

A total of 516 premature infants weighing less than 1250 grams were identified from the three sites: 237 from Children and Women's Health Centre; 54 from Royal University Hospital; and 225 from Mt. Sinai Hospital. One infant from Mt. Sinai was removed because the patient received both non-leukoreduced and leukoreduced RBCs. Thus, a total of 515 transfused neonates were included in the analysis; 268 infants received non-leukoreduced RBC transfusions and 247 received leukoreduced RBC transfusions.

All baseline characteristics, except for some respiratory interventions, were comparable in infants in the non-leukoreduced and leukoreduced periods (Table 1). More infants in the non-leukoreduced group required mechanical ventilation (89.7 percent vs 81.3 percent) while fewer patients in this group received supplemental oxygen (77.4 percent vs 84.6 percent), continuous positive pressure ventilation (16.7 percent vs 51.3 percent), high frequency ventilation (4.8 percent vs 9.2 percent) and use of surfactant (53.6 percent vs 67.5 percent) as compared to

premature infants receiving leukoreduced RBC transfusions.

Major Outcomes

Crude and adjusted rates of nosocomial bacteremia and all secondary outcomes assessed separately, except grade 3 or 4 intraventricular hemorrhage, were clinically and statistically less than 1 suggesting that leukoreduction was associated with improvement in clinical outcomes (Table 2). The proportion of infants that acquired bacteremia after an RBC transfusion was 79/267 in the non-leukoreduced period and 63/246 in the leukoreduced period. For NICU mortality, there were 45 deaths in the leukoreduced period and 44 in the leukoreduced period. The adjusted odds ratio (OR) for bacteremia was 0.59 (95%CI: 0.34-1.01) and 1.22 (95%CI:0.59-2.50) for mortality. When bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhage were considered together as a composite outcome, the adjusted odds ratio was 0.31 with 95 percent confidence intervals ranging from 0.17 to 0.56.

Results of unadjusted subgroup analyses for all major outcomes also suggest a consistent beneficial effect of leukoreduction in infants who did not receive either surfactant, mechanical ventilation, or CPAP on Day 1 except for those who did not receive mechanical ventilation and experienced bronchopulmonary dysplasia, and those who received surfactants and experienced bacteremia or grade III or IV intraventricular hemorrhage (Table 3). The confidence intervals for the former three associations all included unity.

A post-hoc analysis was conducted to examine the influence of leukoreduction on major neonatal morbidities in those neonates that had died. The average NICU length of stay was 18.5±5.3 days for non-survivors versus 85.9±3.6 for those that survived. Leukoreduction had an

apparent protective but non-statistically significant effect on bacteremia in the non-survivors (35.6% in non-leukoreduced versus 20.5% in the leukoreduced, OR=0.47, 95%CI: 0.17- 1.22). There was no apparent association between leukoreduction and bronchopulmonary dysplasia or grade III or IV intraventricular hemorrhage in infants that died (OR=1.03, 95%CI=0.38-2.78 and OR=1.06, 95%CI=0.45-2.50 respectively). There were too few cases of necrotizing enterocolitis (n=11) and retinopathy of prematurity (n=4) to comment on associations.

In the untransfused neonates weighing less than 1250 grams, unadjusted rates of bacteremia, mortality, bronchopulmonary dysplasia, retinopathy of prematurity and intraventricular hemorrhage either were higher in the leukoreduced period compared to the non-leukoreduced period (Table 4). Both the rate of necrotizing enterocolitis and the lengths of stay in the neonatal intensive care unit decreased in the leukoreduced period as compared to the non-leukoreduced period.

Discussion

We demonstrate in this study that the implementation of universal pre-storage leukoreduction of blood transfusions was associated with a significant improvement in all major secondary outcomes and did not worsen mortality in premature infants weighing less than 1250 grams. Indeed, if considered together as a composite measure, for each 10 premature infants transfused leukoreduced blood, leukoreduction was associated with the prevention of a major secondary complication of premature birth. This significant clinical benefit was accompanied by an average decrease of 11 days of NICU stay. The magnitude of this decrease represents a substantial decrease in the cost in the care of these infants.

Our study demonstrates very little about the effect of leukoreduction on mortality except for essentially ruling out differences greater than 50% in either direction. Upon post hoc examination, 50 of the 89 deaths occurred within the first 14 days and 73/89 (82%) occurred within the first 29 days. Thus, the benefit of leukoreduction, as with many interventions, in these infants is doubtful. For those infants that survive greater than 1 month, we would have required a much greater sample size to detect differences in mortality as the probability of death is very low.

To date, we are unaware of any other published study that has examined the association between leukoreduction of allogeneic blood products and major clinical outcomes in premature neonates, including: bacteremia; mortality; or other major complications of prematurity such as bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis and intraventricular hemorrhage.[6] There have been a few studies that have shown that blood transfusions correlate with an increase risk of bronchopulmonary dysplasia and retinopathy of prematurity.[11-15] However, the specific role of leukocytes and leukoreduction in the pathogenesis of these conditions were not assessed in any of the studies. If transfused leukocytes do indeed result in or increase the severity of such complications, then this may be mediated by the enhanced generation of free radicals or some other poorly defined effect either on the immune system or the microvasculature.[16-18] The beneficial effect of the leukoreduction of blood products on several organ systems simultaneously suggests that the putative mechanism of action is having a widespread effect throughout the body.

There are potential sources of bias in this study, particularly in the sampling of patients. Indeed, the greatest threat to the inferences drawn from these data is the possibility of secular trends over time. There was evidence of important changes in respiratory management in the 36-month period of study. It is clear that the use of surfactant increased over this period of time, as did the use

of CPAP as a mode of ventilatory support as compared to other modes of mechanical ventilation. These secular trends were likely to be most evident in the development of bronchopulmonary dysplasia. However, multivariate and stratified analyses consistently demonstrated that there was a beneficial decrease in the odds of bronchopulmonary dysplasia with or without the use of surfactant (Table 3). Similarly, the effects of different ventilatory modalities appeared not to affect any of the outcomes except the unadjusted association between mechanical ventilation and bronchopulmonary dysplasia. Indeed, the magnitude of the effect and the consistency of effects in all major outcomes strengthen our conclusions.

One of the major advantages of this study is that our cohort of premature infants included all consecutive admissions, both transfused and non-transfused, during both time periods. This patient sampling strategy enabled us to add an extra level of control in comparing the risks and benefits of leukoreduction in this population. Additionally, both time periods were 18 months in duration and thus, reasonably short and symmetrical. This duration minimizes seasonal variation in the patterns of admission or patient care. Finally, the use of multivariate analyses enabled us to help control for the confounding influence of a number of factors all at once

Other design choices may have limited the inferences drawn from the data. The relatively small sample size did not allow us to detect meaningful clinically important differences in the rates of mortality and bacteremia if truly present. Indeed, the 95 percent confidence intervals remained wide given the sample size of 515 transfused infants. Also, in limiting our choice of index nosocomial infections solely to the presence of bacteremia, we may have missed other important immunomodulating effects of leukoreduction. Because of resource constraints, one of the additional limitations of our study was our inability to document the dates of diagnoses of major complications

such as retinopathy of prematurity and intraventricular hemorrhage. Therefore, we remain uncertain as to the time and total exposure to blood transfusions in these infants prior to their diagnosis. However, we believe that this information would not significantly change our results as each of the complications occur after prolonged stays in neonatal intensive care unit. Indeed, more than 50% of neonates received a blood transfusion within the first five days of neonatal intensive care unit admission and greater than 75% within the first 15 days. The issue of timing was not a concern for other major outcomes including mortality, bacteremia, and bronchopulmonary dysplasia where more precise estimates of dates were available.

Despite these limitations, there are a number of strengths of the present study. First, is the methodological quality of data abstraction and entry by the Canadian Neonatal Network investigators who used trained data abstractors, a set of well-defined definitions, and rigorous data cleaning and follow-up.[19-21] Second, the study included a consecutive census of premature infants weighing less than 1250 grams admitted to three neonatal intensive care units, representing three different geographic regions of Canada.

In conclusion, we believe that the implementation of universal pre-storage leukoreduction has improved clinical outcomes in premature infants requiring blood transfusions as part of their care in the neonatal intensive care unit. Until there is evidence of harm, we would recommend the adoption of universal leukoreduction in the care of all neonates requiring blood transfusions. While we believe these data are persuasive, we would appeal for and endorse the conduct of a large randomized controlled trial to determine definitively the effectiveness of pre-storage leukoreduction in the neonatal population as well as laboratory and clinical studies to elucidate the hypothesized mechanisms of action.

le 1: Baseline Characteristics of the 515 Transfused Premature Infants Before and After Leukoreduction*

eline Characteristic	Non-Leukoreduced (N=268)	Leukoreduced (N=247)	Difference	95% CI Lower	95% CI Upper
iographics					
((Males) (%)	44.0	49.4	-5.4	-14.0	3.3
thweight (grams)	814.8	839.6	-24.8	-59.1	9.6
stational Age (weeks)	26.2	26.3	-0.2	-0.6	0.2
inatal care					
tenatal Corticosteroids (%)	68.4	72.8	-4.3	-12.3	3.7
livery (vaginal) (%)	51.1	56.6	-5.5	-14.2	3.2
PGAR @ 1 minutes	4.6	4.6	-0.0	-0.4	0.4
PGAR @ 5 minutes	7.1	7.1	-0.1	-0.4	0.3
lmission Status (outborn) (%)	14.6	12.7	1.9	-4.0	7.8
ess Severity*					
NAPII on Day 1	20.0	19.4	0.6	-1.8	3.0
NAPII on Day 3	7.8	7.6	0.1	-1.5	1.7
insfusions					
lume Transfused (mL)**	58.0	63.0	-5.0	-19.0	7.0
f Transfusions per infant	3.7	3.7	-0.0	-0.6	-0.6
spiratory Interventions***					
upplemental O2 (%)	77.4	84.6	-7.2	-14.1	-0.3
PAP (%)	16.7	51.3	-34.6	-42.4	-26.8
lechanical Ventilation (%)	89.7	81.3	8.4	2.2	14.6
entilation with relax (%)	1.2	1.7	-.5	-2.6	1.6
igh frequency ventilation (%)	4.8	9.2	-4.4	-8.9	0.1
urfactant (%)	53.6	67.5	-13.9	-22.5	-5.4
nous and Arterial Access***					
eripheral Intrvenous (%)	62.7	60.0	2.7	-5.9	11.3
rterial Line (%)	77.4	68.3	9.1	1.22	16.9
entral Venous (%)	62.3	66.7	-4.4	-12.8	4.1
edications***					
asopressor (%)	43.0	38.8	4.28	-4.4	13.0
ntibiotic or antifungal (%)	99.6	100.0	-0.4	-1.2	0.4
lucocorticoid (%)	7.1	5.0	2.14	-2.1	6.4
mmune Globulin(%)	0.4	1.7	-1.3	-3.1	0.5
ther Interventions***					
lood Draws (number)	6.7	7.4	-0.7	-1.3	-0.1
latelet Transfusions (%)	1.2	1.7	0.5	-2.6	1.6
NBC Transfusions (%)	0.0	0.0	0.00	-	-
olume Exchange (%)	0.4	0.0	0.40	-0.4	1.2
PR (%)	2.4	5.0	-2.62	-6.0	0.7

* expressed as means
** expressed as median
*** measured on Day 1 (1st 24 hours upon admission to the NICU)

Table 2: Crude and Adjusted Odds Ratios for the 515 Transfused Premature Infants

Outcome	Crude			Adjusted**		
	Odds* Ratio	95% CI Lower	Upper	Odds Ratio	95% CI Lower	Upper
Primary Outcomes						
Bacteremia	0.82	0.56	1.20	0.59	0.34	1.01
Mortality	1.09	0.68	1.72	1.22	0.59	2.50
Major NICU Morbidities						
Retinopathy of Prematurity	0.50	0.34	0.72	0.56	0.33	0.93
Bronchopulmonary Dysplasia	0.48	0.34	0.68	0.42	0.25	0.70
Necrotizing Enterocolitis	0.50	0.27	0.93	0.39	0.17	0.90
IVH Grade III or IV	0.76	0.46	1.23	0.65	0.35	1.19
Any Major NICU Morbidity***	0.39	0.26	0.61	0.31	0.17	0.56

* An Odds Ratio of less than 1.00 indicates a beneficial effect of leukoreduction

**All multivariate models included: Site, Admission Status (outborn/inborn), Gestational Age, Sex, Birthweight, Delivery, Antenatal Steroids, volume transfused, APGAR@ 5 minutes, Snap II on day 1, Any cardiovascular pressor on Day 1, CPAP Categorized (0days, 1-10 days, 11-25 days, >25 days), Fluseason (Dec-March), Mechanical Ventilation on Day 1, High Frequency ventilation on Day 1, Supplemental O2 support on Day 1, Surfactant Use on Day 1, Steroids on Day 1, Umbilical Catheter, Percutaneous Catheter

*** Either Retinopathy of Prematurity or Bronchopulmonary Dysplesia or Necrotizing Enterocolitis or IVH Grade III or IV

Table 3: Subgroup Analyses: Impact of Respiratory Interventions on Day 1 (unadjusted)

	Surfactant (n=295)			No Surfactant (n=195)		
Outcome	**Odds Ratio***	**95% CI** Lower	Upper	**Odds Ratio***	**95% CI** Lower	Upper
Bacteremia	1.01	0.62	1.65	0.38	0.18	0.81
Mortality	1.04	0.56	1.93	1.25	0.60	2.60
Retinopathy of Prematurity	0.43	0.26	0.71	0.58	0.31	1.08
Bronchopulmonary Dysplasia	0.38	0.24	0.60	0.60	0.33	1.09
Necrotizing Enterocolitis	0.53	0.24	1.19	0.49	0.17	1.42
Any IVH Grade III or IV	0.78	0.41	1.49	0.76	0.34	1.68

	Mechanical Ventilation (n=421)			No Mechanical Ventilation (n=71)		
Outcome	**Odds Ratio**	**95% CI** Lower	Upper	**Odds Ratio**	**95% CI** Lower	Upper
Bacteremia	0.88	0.58	1.35	0.53	0.14	2.00
Mortality	1.14	0.68	1.89	0.91	0.26	3.13
Retinopathy of Prematurity	0.47	0.31	0.71	0.79	0.27	2.27
Bronchopulmonary Dysplasia	0.42	0.28	0.63	2.33	0.74	7.14
Necrotizing Enterocolitis	0.59	0.30	1.16	0.24	0.04	1.45
Any IVH Grade III or IV	0.74	0.43	1.27	0.79	0.22	2.86

	CPAP (n=165)			No CPAP (n=327)		
Outcome	**Odds Ratio**	**95% CI** Lower	Upper	**Odds Ratio**	**95% CI** Lower	Upper
Bacteremia	1.45	0.67	3.13	0.40	0.22	0.71
Mortality	1.85	0.59	5.88	1.14	0.64	2.00
Retinopathy of Prematurity	0.60	0.29	1.27	0.44	0.27	0.73
Bronchopulmonary Dysplasia	0.65	0.32	1.32	0.34	0.21	0.55
Necrotizing Enterocolitis	0.47	0.13	1.75	0.66	0.31	1.43
Any IVH Grade III or IV	1.37	0.48	4.00	0.57	0.29	1.14

Abbreviations: CI = confidence interval, IVH = intraventricular hemorrhage, CPAP = continuous positive airway pressure

* An Odds Ratios less than 1.00 indicates a beneficial effect of leukoreduction

Table 4: Comparison of Outcomes in the Transfused and Non-Transfused*

	Transfused (n=515)					Non-Transfused (n=399)				
Outcome	Non-Leukoreduced Period	Leukoreduced Period	Difference	Lower	Upper	Non-Leukoreduced Period	Leukoreduced Period	Difference	Lower	Upper
Bacteremia (%)	**29.6**	**25.6**	4.0	-3.8	11.7	**23.4**	**27.2**	-3.8	-12.4	4.8
Died (%)	**16.8**	**18.0**	-1.2	-7.7	5.4	**12.1**	**17.5**	-5.5	-12.5	1.6
ROP (%)	**59.1**	**41.7**	17.4	8.3	26.5	**39.1**	**58.0**	-6.9	-18.0	4.1
BPD (%)	**52.6**	**34.8**	17.8	9.4	26.2	**4.0**	**9.8**	-5.8	-10.7	-1.0
Necro (%)	**12.5**	**6.7**	5.8	0.7	11.0	**2.4**	**2.0**	0.4	-2.6	3.4
Any IVH III or IV (%)	**17.4**	**13.7**	3.7	-2.7	10.0	**4.2**	**7.0**	-2.8	-8.0	2.4
NICU LOS (days)	**77.1**	**70.9**	6.2	-1.5	13.9	**35.5**	**32.1**	3.4	-2.0	8.8

* unadjusted differences with 95% confidence intervals

Abbreviations: ROP =retinopathy of prematurity, BPD=bronchopulmonary dysplasia, Necro= necrotizing enterocolitis, NICU=neonatal intensive care unit, LOS=length of stay

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Chapter 5

5.1 Conclusion

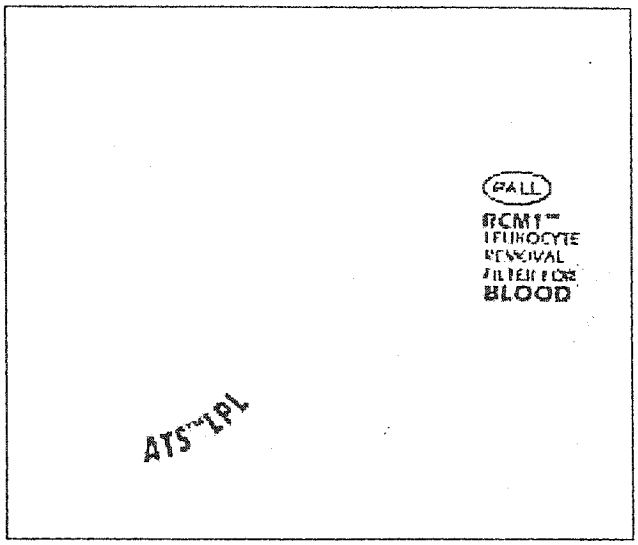
The goal of this thesis was to assess the effectiveness of leukoreduction in the neonatal population. Undertaking a systematic review of the literature and conducting an observational study accomplished this goal. The systematic review clearly indicated a lack of studies evaluating the clinical effect of leukoreduction in the neonatal population and, as such, provided justification for conducting a study. The results of the study indicate a strong association between leukoreduction and major neonatal morbidities. The findings of this thesis provide important information to policy makers, transfusion medicine specialists, and neonatologists. In addition, this study provides insights into the underlying mechanisms of action of universal leukoreduction. Transfused leukocytes appear to have both a generalized pro-inflammatory and immunosuppressive effect given the decrease in all complications of prematurity as well as decreased rates of infection. The question that remains is whether or not to leukoreduce the entire blood supply. In my opinion, additional clinical evidence in other patient populations is needed to justify the adoption of universal leukoreduction as opposed to providing leukoreduced products to those groups where benefit is established.

Appendix I: Leukoreduction Product Monograph

PALL Medical

Leukotrap®-RC PL Whole Blood Collection, Filtration and Storage Systems

In-Line Filtration System



Description

The Leukotrap-RC PL is a closed system for the collection of one unit of whole blood and prestorage leukoreduction of red cells and platelet rich plasma (with the Pall In-Process Filter System ATSLPL for Platelet Rich Plasma and Pall RCM1 Leukocyte Reduction Filter for Blood), followed by subsequent storage of the red blood cell, platelet, and plasma components.

Product Features

- The system incorporates in-line the Pall RCM1 Leukocyte Reduction Filter for Blood and the Pall In-Process Filter System ATSLPL for Platelet Rich Plasma for the leukoreduction of one unit of packed red blood cells and one unit of platelet rich plasma, respectively
- The system additive solution (AS-3, Nutricel® System) maintains red blood cell (RBC) viability throughout a 42-day storage period without the need for mannitol
- The patented CLX® platelet storage container – transparent, flexible and gas permeable – is designed to maintain acceptable pH over the component's 5-day shelf life
- The patented transfer leg closure (TLC) is designed for quick and easy opening of the fluid paths between bags
- The Diamond Protector Needle is an ultra-thinwall 16-gauge donor needle with a tamper-evident needle cover and a finger-contoured hub that incorporates a "bevel-up" indicator. Manufactured with fully automated technology, needle sharpness is 100% tested for donor safety and comfort
- The injection port caps are designed for quick and easy pull-off without excessive manipulation
- On those systems so equipped, the integral Y-Sampling System facilitates easy collection of testing samples directly from the donor while maintaining a "closed" system
- On those systems so equipped, an in-line sample pouch permits harvesting of 34 mL of undiluted blood for donor testing
- Quality controlled without compromising the safety of the closed system
- Requires no external attachments or docking
- Rapid filtration time
- High platelet and red cell recovery
- Easily fits into routine standard operating procedures

Product Specifications

- Indication: for the leukoreduction of one unit of packed red blood cells and one unit of platelet rich plasma, respectively
- Shelf life: 3 years in unopened foil pouch; 30 days in an opened/resealed foil pouch
- Donor tubing: standard, i.e. straight-line, or with the in-line 34 mL sample pouch, or with the in-line Y-Sampling System, as indicated
- Crossmatch segments: 16
- Storage conditions: room temperature; avoid excessive heat; protect from freezing
- Collection capacity: 450 mL or 500 mL, as indicated
- Anticoagulant: Citrate Phosphate Double Dextrose (CP2D); 63 mL for 450 mL collections, or 70 mL for 500 mL collections, as indicated
- Additive solution: AS-3 (Nutricel System); 100 mL for 450 mL collections, or 110 mL for 500 mL collections, as indicated
- Plastic: except for the CLX platelet storage container, all bags and tubing are polyvinyl chloride (PVC) with di (2-ethylhexyl) phthalate (DEHP) plasticizer. The CLX container is PVC with tri (2-ethylhexyl) trimellitate (TEHTM) plasticizer
- Satellite bags: standard, i.e. DEHP plastic bag, or CLX platelet storage bag, as indicated

Product Specifications *(continued)*

- Blood product dating: Up to 42 days at 1-6° C for red blood cells, leukocytes reduced; up to 5 days at 20-24° C for platelet concentrate, leukocytes reduced in a CLX storage bag; up to 1 year at ≤ -18° C for fresh frozen plasma and cryoprecipitate
- Single use
- Latex free, except for systems with the Y-Sampling System

Ordering Information

Reorder Code	Description	Packaging
763-54	450 mL capacity, *standard donor tubing*, 2 standard plus 1 CLX satellite bag	2 units/pouch 12 pouches/case
123-23	500 mL capacity, *Y-Sampling System*, 2 standard plus 1 CLX satellite bag	2 units/pouch 12 pouches/case
123-13	500 mL capacity, *in-line sample pouch*, 2 standard plus 1 CLX satellite bag	2 units/pouch 12 pouches/case

PALL Medical

2200 Northern Boulevard
East Hills, NY 11548-1289 USA

Visit us on the web at www.pall.com

Select-A-FAX
for the latest information delivered to any fax machine, anywhere in the world. This automatic system operates 24 hours a day, seven days a week. Call 1.516.942.0523 from outside the USA.

Pall Corporation has offices and plants throughout the world in locations including: Argentina, Australia, Austria, Belgium, Brazil, Canada, China, France, Germany, Hong Kong, India, Indonesia, Ireland, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Puerto Rico, Russia, Singapore, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, United Kingdom, United States, and Venezuela. Distributors are located in all major industrial areas of the world.

*Filtration. Separation. Solution.*SM

S-LeukotrapRCPLData 99.0

Appendix II: Research Ethics Board Documents

McGill

Faculty of Medicine | Faculté de médecine

May 28, 2002

Dr. Stan Shapiro
McGill University
Epidemiology, Biostatistics & Occupational Health
1020 Pine Avenue West
Montreal, Quebec
H3A 1A2

Dear Dr. Shapiro:

We are writing in response to your request for continuing review for the study A05-M38-01B entitled *"Effectiveness of Leukoreduction in the Neonatal Population"*.

The progress report was reviewed and we are pleased to inform you that full board re-approval for the study was provided on *May 27, 2002*, valid until *May 2003*. The certification of annual review has been enclosed.

We ask you to take note of the investigator's responsibility to assure that the current protocol and consent document are deposited on an annual basis with the Research Ethics Board of each hospital where patient enrollment or data collection is conducted.

Should any modification or unanticipated development occur prior to the next review, please advise the IRB promptly.

Yours sincerely,

J. Lawrence Hutchison, M.D.
Chair
Institutional Review Board

cc: A05-M38-01B
Dean Fergusson

McGill

Faculty of Medicine | Faculté de médecine

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

LAWRENCE HUTCHISON, MD

FRANCES ABOUD, PHD

GEOFFREY BLAKE, MD

MARK S. GOLDBERG, PHD

GEORGE HOUSTON, BCL

MARIGOLD HYDE, BSC

HARVEY SIGMAN, MD

has examined the research project A05-M38-01B entitled "A Effectiveness of Leukoreduction in the Neonatal Population"

as proposed by: Dr. Stanley Shapiro (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects:

May 29, 2001
Date

Chair, IRB

Dean of Faculty

Institutional Review Board Assurance Number: M-1458

Thursday, April 05, 2001

Dr. PaulC. Hebert
Department of Critical Care
4th Floor, Room 4137
Ottawa Hospital - General Campus
501 Smyth Road
Ottawa, ON K1H 8L6

Dear Dr. Hebert:

Re: Protocol # 2000380-01H Effectiveness of Leukoreduction in the Neonatal Population

Protocol approval valid until - Thursday, April 04, 2002

I am pleased to inform you that your study (listed above) was given expedited review by the Ottawa Hospital Research Ethics Board (OHREB) and is approved. No changes, amendments or addenda may be made in the protocol without the OHREB review and approval.

Approximately two months prior to the expiration date listed above, a single renewal form should be sent to the OHREB office.

The Tri-Council Policy Statement requires a greater involvement of the OHREB in studies over the course of their execution. You must maintain as part of your records copies of the signed consent form. As well, you must inform the Board of adverse events encountered during the study, here or elsewhere, or of significant new information which becomes available after the Board review, either of which may impinge on the ethics of continuing the study. The OHREB will review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Yours sincerely

Raphael Saginur, M.D.
Chairman
Ottawa Hospital Research Ethics Board

The Ottawa Hospital | L'Hôpital d'Ottawa

Research Ethics Board
Conseil d'éthique en recherches

Thursday, May 30, 2002

Dr. Paul Hebert
Clinical Epidemiology Program
Ottawa Hospital - General Campus
Box 201
501 Smyth Road
Ottawa, ON K1H 8L6

Dear Dr. Hebert:

RE: Protocol# - 2000380-01H Effectiveness of Leukoreduction in the Neonatal Population

Renewal Expiry Date - Thursday, May 29, 2003

Thank you for the letter dated April 30, 2002. I am pleased to inform you that your Annual Renewal Request (listed above) was reviewed by the Ottawa Hospital Research Ethics Board (OHREB) and is approved. No changes, amendments or addenda may be made in the protocol or the consent form without the OHREB's review and approval.

Renewal is valid for a period of one year. Approximately one month prior to that time, a single renewal form should be sent to the OHREB office.

The Tri-Council Policy Statement requires a greater involvement of the OHREB in studies over the course of their execution. You must maintain, as part of your records, copies of the signed consent form. As well, you must inform the Board of adverse events encountered during the study, here or elsewhere, or of significant new information which becomes available after the Board review, either of which may impinge on the ethics of continuing the study. The OHREB will review the new information to determine if the protocol should be modified, discontinued, or should continue as originally approved.

Yours sincerely,

Raphael Saginur, M.D.
Chairman
Ottawa Hospital Research Ethics Board

Encl.

MAR 25 1998

The University of British Columbia
Office of Research Services and Administration
Clinical Research Ethics Board

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	[illegible]
Pendray, M.R.	Paediatrics	[illegible]

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
BC Children's Hospital

CO-INVESTIGATORS:
Lee, S.K., Paediatrics

SPONSORING AGENCIES
B.C. Children's Hospital

TITLE:
Illness severity, practice variability and resource consumption in NICUs

APPROVAL DATE	TERM (YEARS)	AMENDMENT:	AMENDMENT APPROVED:
MAR 10 1998	3	4 March 1998, agency	MAR 10 1998

CERTIFICATION:

The protocol and consent form for the above-named project have been reviewed by the Committee and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

Approval of the Clinical Research Ethics Board by one of:
Dr. B. McGillivray, Chair
Dr. A. Hannam, Associate Chair
Dr. R. D.Spratley, Director, Research Services

This Certificate of Approval is valid for the above term provided there is no change in the experimental procedures

MOUNT SINAI HOSPITAL

REQUEST FOR ACCESS TO HEALTH RECORDS FOR RESEARCH PURPOSES

In accordance with Ontario Regulation 965/93, Section 22, Subsection 5.1(d)i,ii of the Public Hospitals Act, the following policy pertains for accessing medical records for research:

A board may permit, a member of the medical staff extraordinary access to medical records for teaching purposes, or scientific research that has been approved by the medical advisory committee.

PLEASE PRINT CLEARLY

- ☑ Internal Research Funded Externally
- ☐ Internal Research Not Funded Externally
- ☒ External Research

Name(s): Dr. B. FERNANDES Extension: 4462

Department: PATHOLOGY Date: 30th APR 1999

Title of series (i.e. disease or particular procedure): Hip surgery ICU admissions + Blood Transfusions.

Specific reason(s) for use: Evaluation of Universal Leucodepletion Program

Do you require health records to produce a list / report? Yes ☒ No (list is attached) ☐

Will any of the patients be contacted? Yes ☐ No ☒

Will you be establishing a registry of the patients' names? Yes ☐ No ☒

If the patients will be contacted and/or a registry of patients' names will be established, MAC may allow access to the medical records providing that the established protocol is followed, namely:

① Each patient must be contacted by his/her doctor (and not the researcher) in writing to obtain consent to the study.

② The patient's written authorization for access to his/her medical record is to be kept with the patient's chart.

Date physician requires charts to commence study: Summer 1999 / Depending on

Estimated completion date of study: Summer 2000 } start date of

Number of years study is to cover or specific time period: 1 YEAR Universal leucodepletion.

Doctor's signature: _____

Authorized by: _____ Date: 30.4.99.

Chief of Service/Division Head

If the estimated volume of charts is greater than 50 please provide justification: This is a Canada wide study See attached protocol.

Health Record Services Resource Assessment: Total staff hours 27 Total charts 300

PLEASE FORWARD TO: Ms. Connie Lambert, Room 1540
Mrs. Janine Girard-Pearlman, Room 339 (for approval)

APPROVAL BY MEDICAL ADVISORY COUNCIL

Chairman's signature: Date: May 6/99

Vice-President's signature: Date: May 5/99

MAC WILL FORWARD TO DIRECTOR, HEALTH RECORD SERVICES FOR IMPLEMENTATION

Revised: July 7, 1998

Appendix III: Data Collection Procedures

The following data collection procedures were extracted from the Abstractor's Manual of the Canadian Neonatal Network database procedures manual (Section IV: Protocol, Chapter One: Data Collection). They were initiated as part of the SNAP Project. They apply to all data except for the extra data collection required at Royal University(Saskatoon) and Children's and Women's Health Centre of British Columbia (Vancouver). For those two intervals of data, neonates were retrospectively identified by chart review and therefore the admission tracking and methods paragraphs are not relevant.

Which babies to abstract: Abstractors are responsible for abstracting *every* eligible admission to the NICU. Eligible babies are babies who stay in the **NICU for more than 24 hours OR who die/are transferred within 24 hours**. (NOTE: *For purposes of this study, time of admission is defined as the time of the first set of recorded vital signs.*) Once a baby has been admitted to your NICU, you will have ultimate responsibility for the data collection on that baby, regardless of outcomes or transfers.

Deaths: For all babies who are admitted to the NICU and die you will need to verify the cause of death by a) asking the attending physician and b) checking the death certificate to see what is listed. You should also be sure to photocopy the death summary (discharge summary) and the autopsy report (if done). The autopsy report may not be completed for several weeks. Keep unaltered copies for your own records and mail copies to the STUDY COORDINATING CENTRE with the name and medical record number deleted. You should be sure to put the baby's study ID# and the Site ID on the STUDY COORDINATING CENTRE copy. You should also talk to your Site Investigators about getting a log of all delivery room deaths (live born babies only) from Pathology. You will need to know date and time of birth and death, and cause of death. You should also find out the birthweight and gestational age of these babies. You may be able to work out a system of checking with Pathology monthly for any delivery room deaths.

Admission tracking: Abstractors should check NICU admission log books daily for new admissions. It is crucial that every eligible baby be abstracted (see "Which babies to abstract," above). Research Assistants are responsible for keeping track of when each form is "due" to be completed. For example, a baby born 1/1/96 would theoretically have the Day 1 SNAP/NTISS forms due 2/1/96, the Day 3 SNAP/NTISS due 4/1/96, the Day 14 SNAP/NTISS due 15/1/96, and the Day 28 SNAP/NTISS due 29/1/96. This due date is to aid you in staying current in your abstraction. However, as long as the relevant time period is complete (i.e. the full 12 hours for a 12 hour SNAP score) you do not have to enter the data on that particular day if you do not have time. Please note that waiting too long can result in a baby's discharge and removal of the medical record, requiring you to track down the medical record; this can be a long process and runs the risk that records may be lost. You may try to arrange with your Site Investigator to have your NICU staff hold charts of discharged babies for an extra day, thus giving you the opportunity to obtain the discharge

data before chart removal. If this is arranged, you should check for charts of discharged babies first thing each day to minimize the delay in chart removal.

Methods: Charts should be abstracted at bedside while the baby is in the NICU. You should try to stay as up to date as possible in your abstracting. The reasons for this are: 1) If something is unclear or confusing, you can ask the NICU staff questions and they are likely to know the information. If you wait too long after the baby's discharge, they may not have accurate recall of the needed information. 2) Tracking down medical records once the baby leaves the unit can be time consuming and difficult.

Definitions: Definitions should be used freely and frequently; it is extremely important that you use the standardized definitions when abstracting to ensure consistency with the other sites. Information should be recorded as quickly and accurate as possible. NICU staff should be consulted for clarification if anything in the chart is confusing or unclear.

Data Entry: With the exception of the SNAP and NTISS screens, you should enter something for every data item. If the information, asked for is unavailable, click on the "Unknown" or "N/A" option, or enter the value "-9". *For missing dates enter 1/1/11; for missing times enter 0:00 AM.* This is a way for us to make sure that data items are not left blank accidentally (See "Error Checking: Missing Values, "Chapter 3).

Scoring Periods:

1) CRIB variables: These are scored from time of birth for 12 hours (*first 12 hours of life*).
2) SNAP: SNAP is done on day of admission for the first 12 hours of *admission* (first 12 hours of admission), day 3 from 6:00 am to 5:59 am the following day, day 14 from 6:00 am to 5:59 am the following day, and day 28 from 6:00 am to 5:59 am the following day.
3) NTISS: The NTISS is done on day of admission for the first 24 hours of *admission,* day 3 from 6:00 am to 5:59 am the following day, day 14 from 6:00 am to 5:59 am the following day, and day 28 from 6:00 am to 5:59 am the following day.
4) Day 28: Day 28 data should be recorded as the first data noted after 6:00 am on day 28 of LIFE to 5:59 am of day 29.
5) Week 36: Week 36 is 36 weeks post conception (gestational age plus weeks of life). It is computed using the obstetric gestational age UNLESS the pediatric gestational age differs by 3 weeks or more. In the latter case, calculate week 36 from the pediatric gestational age. This data should not be collected if the gestational age is 32 weeks or more. Please note that if the baby is born at 32 weeks gestational age, the week 36 data will be identical to the day 28 data. Data should be recorded by using the first value noted after 6:00 am on the first day of week 36 to 5:59 am of the next day.
6) To calculate day 3, day 14, day 28: The day of ADMISSION (not birthdate) is considered day zero. Add three to the date of admission for day 3 data, add 14 to the date of admission for day 14 data, add 28 to the date of admission for day 28 data. Therefore, a baby

born and admitted Jan 1 will have day 3 data collected on Jan 4, day 14 data collected on Jan 15, and day 28 data collected on Jan 29. Similarly, week 36 would be calculated by adding the appropriate weeks to day 0 (day of admission). On day 3/day 14/day 28, the scoring period begins at 6:00 am as explained in the #2 above.

Pleases note that SNAP and NTISS, the time of admission is defined as the time the first vital signs are recorded in the NICU.

"Missing" Scores: If you are missing information from a scoring period either because a flow sheet is missing or because the baby was transferred out/went home during the scoring period you should first examine how much of the scoring period is available to you. If 12 hours or more of the scoring period is available, you SHOULD complete the score, and note in the comments box how many hours the score is based on. If less than 12 hours of the scoring period is available, you should set the score to "Missing". There is no need to make a comment in the latter case. *An exception to this rule is made for babies who die during a scoring period* (see below).

Death During a Scoring Period: If a baby dies during a scoring period (CRIB, SNAP or NTISS) you SHOULD abstract the score regardless of how many hours of the scoring period the baby lived. Please make a note in the comments box for these cases indicating the length of time the score was based on.

SNAP* Scoring: Listed in Section II are strict definitions for each of the study variables. For the high/low variables, the abstractor should scan the NICU flow sheet and locate the most abnormal value that corresponds to the variable being abstracted. For example, the value "respiratory rate: high" in an infant with documented respiratory rates of 36, 54, and 32 would be 54. For variables which rely on the results of laboratory tests, the results should be assigned to the scoring period during which the set was drawn, not to that during which the result becomes known. For example, if a blood count is drawn during the 23rd hour of a 24 hour scoring period, but the result is not known until the 4th hour of the next, then points for that lab test should be allotted to the first time period. If a lab test was not performed, then leave the variable blank. If a lab test was sent, but the result is unavailable at the time of abstraction, enter "999". Come back to this screen when the result becomes available and enter the correct value.

NTISS Scoring:** NTISS scores the most intense level for each therapy during the scoring period. When scores are computed on multiple days, attention must be paid to whether scoring is based on initiation of a therapy, or simply the presence /continuation of the therapy.

For simplicity in data collection, each of the NTISS variables is scored as either present or absent. The NTISS requires that portions of the chart other than the NICU flow sheet be scanned. The Nursing and Physician Progress notes may contain valuable information regarding the performance of procedures.

In the final calculation of scores for NTISS, points are assigned only for the most intense intervention in a therapeutic category. For example, consider a patient who began a scoring period on supplemental oxygen by hood and then was placed on nasal CPAP, followed by endotracheal intubation and mechanical ventilation with muscle relaxation. In the final score, this patient would receive points only for mechanical ventilation with muscle relaxation because it is the most intense respiratory therapy that she received within the respiratory category. In completing the scoring period data collection, however, each of the three respiratory therapies listed above should be marked as present. In this way, maximal information is accumulated about each patient's hospital course.

Rounding: Numeric entries that need to be rounded for entry into the laptop should be rounded as follows: 2.4 and smaller should be rounded to 2, 2.5 and larger should be rounded to 3. Generally, if values are listed as "<", as in "<2", score as one less than what is written e.g <2 would become 1. There are exceptions to this rule when dealing with very small numbers, such as those associated with bili values. Check with the study coordinator, if this issue come up.

Paper Abstractions: In the event that you are temporarily unable to use your laptop computer for data entry (repair, debugging, program updates, etc.) you should continue your data entry on paper. Located in Appendix II of this manual are paper copies of each data entry screen. Photocopy these as needed, being sure to fill in the "Study Subject Name", "Study Subject ID#" and "Study Site ID Code" on EACH page. Remember that SNAP and NTISS must be filled out four times for each subject: day one, day three, day fourteen, and day 28. Refer as needed to Section II of the manual for the data definitions. When you return to computer data entry, enter the data from these paper abstractions into the computer.

Babies who are Transferred: If a baby is in your NICU for more than 24 hours and then gets transferred to a non-study hospital, fill out the discharge screen and follow up with the post-transfer screen. If a baby is in your NICU for more than 24 hours and gets transferred to a study hospital, contact the 2nd hospital's R.A.(s) and ask them to continue scoring for that baby on paper and then mail you the information. It is the abstractor's responsibility to score babies who stay in their NICU longer than 24 hours, even if they are transferred.

***SNAP (Score for Neonatal Acute Physiology)**
An illness severity scoring system which sums up the worst physiological derangement in each organ system in the first 24 hours of admission to the NICU. SNAP measures a total of 34 items, 26 of which are physiological variables. This scoring system has been shown to be highly predictive of neonatal mortality and to be correlated with other indicators of illness severity including therapeutic intensity, physician estimates of mortality risk, length of stay, and nursing workload.

****NTISS (Neonatal Therapeutic Intervention Scoring System)**

An illness severity scoring system which scores the most intense level of each therapy during a 24 hour scoring period (i.e. within 24 hours of admission). For example, if an infant started off with blow-by oxygen, progressed to nasal CPAP, then to endotracheal intubation, and then to mechanical ventilation with muscle relaxants, the final score would only receive points for ventilation with muscle relaxants because this is the most intense therapy within the respiratory category. NTISS is a 63 item scoring system which assigns scores from 1 to 4 for the various intensive care therapies. This scoring system has been found to be highly correlated with in-hospital mortality rates, mortality risk estimates by physicians, nursing acuity, predicted length of stay, and hospital charges for survivors. Therefore, NTISS allows for excellent prediction of outcome and total resource use.

Appendix IV: Database Variable Definitions

The following variable definitions are extracted from the data collection manual of the Canadian Neonatal Network database accept Site and Flu Season. Transformed or renamed variables for the "Effectiveness of Leukoreduction in Neonates" study are noted.

A) Demographic and Prenatal Variables

Name - Family name of infant as recorded on medical record. If hyphenated or double name, record both. For multiple births, use "#" followed by birth order (eg. Jones #2). If fetal death occurs at or before 20 weeks, do not count in birth order. If the chart does not specify date of fetal death, use the date the death was discovered. Do not type in "BABY", "BOY", or "GIRL" or their abbreviations. If a baby has a name change or you make a mistake when entering the baby's name, do NOT record the change in the comments box. However, you may want to note the change for yourself for future reference. This variable was removed from the database

Record number – Medical record number of the infant at the study hospital.

Study number: the study number refers to the unique number assigned to each neonate for purposes of the *"Effectiveness of Leukoreduction in Neonates" study.* Once a study number was assigned all patient identifiers (Name and Record number) were removed from the database.

Site: Site was recorded as 1= Children's and Women's Health Centre of British Columbia (Vancouver), 2 = Royal University (Saskatoon), 3 = Mount Sinai (Toronto).

Birthweight – Weight in GRAMS at birth as recorded in birth hospital. If there are discrepant values, use the birth hospital value for outborn babies. If large discrepancy between birth hospital values (more than 10%) call the STUDY COORDINATING CENTRE for advice. Otherwise select modal value. If birthweight is not available, use the first weight taken up to 24 hours of life. If birthweight is only listed as an estimate, record the estimate, but make a note in the comments box that this is an approximate birthweight.

Birthdate - Date of birth according to obstetric and/or admitting records. Enter DDMMYY

Birthtime – Write time of birth, separated by colon, in military time. If time of birth is unavailable, enter 0:00 to indicate a missing value. If a baby is born at midnight, record this as 24:00.

Admit Date - Date of admission to the study NICU. This may be different than date of birth for late admission or outborn babies. Enter as DDMMYY.

Admit time - Time of admission is defined as the time of the first vital signs (at least one

vital sign) recorded IN THE NICU. Do not include time on transport for outborn infants, or time in the delivery room for inborn infants. Write time of admission, separated by colon in military time. It time of admission is midnight, score as 24:00. If time of admission is not available, enter 0:00 to indicate missing value.

For moribund babies who have not had any vitals taken, you can use another reference to admission time listed in the chart or use the missing value (if no other reference).

Moribund on admission – Infant declared moribund on admission to the NICU, as evidence by few or no therapies administered IN THE NICU (or no treatment other than comfort care). Physician and nursing notes should indicate no attempt to treat and/or IMMEDIATE withdrawal of care on admission. This might apply to infants at the border of viability, and to infants with recognized lethal anomalies. It is vital to identify these infants, since their SNAP and NTISS scores will be unusually low, yet the patient dies. If the baby is declared moribund on admission, check off this item ONLY for Destination.

Infant Sex - Record sex of infant. If sex is listed as ambiguous, but the baby is later said to be male or female, score as ambiguous.

Infant Race – Race of infant is defined as race of the mother. If the mother is both African-American and Hispanic, choose African-American. If there are different races recorded and birth certificate is available, use the race listed on the birth certificate.

Apgar at 1 minute – One minute Apgar score. If discrepancy, select modal value.

Apgar at 5 minutes – Five minute Apgar score. If discrepancy, select modal value.

Gestational Age (Obstetric estimate) – Best obstetric estimate of gestational age in full weeks according to delivering obstetrician. If noted to have discrepant obstetric last menstrual period (LMP) and ultrasound dates, select US dates if done earlier than 25 weeks GA. Otherwise, select LMP dates. If there is no specific obstetrics GA listed, but the obstetrics records refer to the baby as a term baby, enter 40. If there is only a pediatric GA listed in the chart, record obstetric GA as the missing value.

Gestational Age (Pediatric estimate) – Best pediatric estimate of gestational age in full weeks. Preference among estimates should be:

1) attending note
2) scored Ballard/Dubowitz sheet
3) other estimate referenced in chart

If there is no specific pediatric GA listed in any of the above places, but the baby is referred to as a term baby, enter 40. If the only pediatric estimate listed in the above places seems to be a reiteration of an obstetric GA, assume that the pediatrician agrees with the obstetric GA and score this as the pediatric GA. DO NOT use autopsy estimates of gestational age. If there is only an obstetric GA listed in the chart, record pediatric GA as the missing value.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, the average of the obstetric and pediatric estimate was used for the variable Gestational Age.

Admission Status - Admission status at study hospital. Score as inborn, outborn (transferred in) or readmission to study hospital. If outborn or readmission, specify in "transferred from".

Delivery Type - Record whether the delivery was vaginal or by cesarean section. If obstetric information is noted, but delivery type is not mentioned, "vaginal" may be assumed. If vaginal can be inferred (eg. "vacuum extraction"), score vaginal. If there are no obstetric records, select "unknown".

Antenatal Corticosteroid Treatment - If dates of administration are available, score as noted in #1. If dates are not available, but completeness is discussed, score as noted in #2. If dates and completeness are not discussed, score as in #3. If the chart discusses obstetric information, but does not mention steroid administration, assume "none". If there is no obstetric data in the chart, select "unknown".

1. COMPLETE defined as receipt of at least one dose of corticosteroids (betamethasone (beta),dexamethasone (decadron), cortisone, dihydrocortisone, celestone BUT NOT PREDNISONE) 24 hours or more before delivery AND 7 days or less before delivery. PARTIAL defined as at least one dose given < 24 hours or > 7 days before delivery.

2. IF NO DATES OF ADMINISTRATION ARE GIVEN, but the chart refers to "complete" or "partial" doses, score as such.

3. IF NO DATES OF ADMINISTRATION ARE GIVEN AND THE CHART DOES NOT REFER TO "COMPLETENESS," but indicates that steroid were administered, score s "partial". If it specifies that two or more doses were administered (eg. "Weekly beta"), score as "complete."

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, complete or partial treatments were collapsed into the variable "Any Antenatal Corticosteroid"

Flu Season: *scored as present if infant was admitted between December and March*

B) NTISS SCREEN DEFINITIONS (Day 1 Interventions)

General: NTISS on day one should be scored from the time of admission (defined as the time of first vitals in the NICU) for twenty-four (24) hours. Day three is scored from admission date plus three days, and goes from 6:00 AM to 5:59 AM on the following day(24 hours). Day fourteen is scored from admission date plus fourteen days, and goes from 6:00 AM to 5:59 AM on the following day (24 hours). Therapies administered during an operation SHOULD be included.

Medications: The best strategy is to check the medication sheets to confirm that each medication was administered during the time period. Score medications(diuretics, aminophylline, narcotics, steroids) administered during the time period whether given po, pg, ng, IV, IM or aersol. Only score pressors, antibiotics, narcotic infusions, acidosis treatment drugs and "other"medications (unscheduled) if these medications were administered IV, IM, or via aerosol (inhaled, nebulized).

Supplemental 02 – Receipt of continuous enriched oxygen concentration (>.21 FiO2) by oxyhood, nasal cannula, nasal catheter, facemask or other forms of respiratory support. "Blow-by" oxygen does not count unless it is the mode of oxygen administration used in a transport situation. Do not score oxygen given as part of a hyperoxia test.

Mechanical ventilation - Use of conventional mechanical ventilation during the SCORING PERIOD, regardless of IMV rate. If pavulon\pancuronium was used then score as mechanical ventilation with muscle relaxation.

Ventilation with muscle relaxants – Mechanical Ventilation along with administration of muscle relaxants (pancuronium, Pavulon, succynyl choline (sux), vecuronium (vec). At least one dose of relaxant must be given during the SCORING PERIOD. Residual effects of drug given before the beginning of the SCORING PERIOD do not count. Score HIFI with relaxants as HIFI only. In this case, do not score Pavulon (or other muscle relaxants) under "other meds".

High frequency ventilation - Use if HIFI (high frequency ventilation, by oscillator, jet or flow-interrupter) at any time during the SCORING PERIOD. Score HIFI with relaxants as HIFI only. In this case, do not score Pavulon (or other muscle relaxants) under "other meds".

Surfactant - Receipt of exogenous surfactant replacement therapy (Exosurf, Survanta, Curosurf, Infasurf,) during the SCORING PERIOD.

ECMO - On Extra Corporeal Membrane Oxygenation (ECMO) at any time during the SCORING PERIOD. ECMO starts when the patient is cannulated. ECMO stops when the patient is removed from pump\bypass, NOT at the time of decannulation. Do not score ECMO cannulation or ECMO decannulation as an operation on this screen but do include it as a major operation on D & P. ECMO given as part of an operation SHOULD be scored

here, but a note should also be made in the comments box that ECMO was given as part of an operation and not as a procedure unto itself.

Peripheral IV – PRESENCE of one or more intravenous catheters (including heparin locks for drug administration) during that SCORING PERIOD.

Arterial line - PRESENCE of a central line (CVL) during the SCORING PERIOD, including: umbilical venous line (UVL), Broviac lines, or percutaneous ("spaghetti") lines which are placed centrally. Score lines regardless of whether central placement is achieved. Do NOT score lines that are never successfully placed. Where it is unclear whether the line was successfully placed, score based on whether the line has begun infusing solutions or not. CVP monitoring is scored separately.

Antibiotics: 1-2 agents or >2 agents – Receipt of INTRAVENOUS antibiotics during the SCORING PERIOD. Topical antibiotics SHOULD NOT BE SCORED. If ONE or TWO antibiotics are administered concurrently, select "1-2 agents." If THREE or more antibiotics are administered concurrently, select">2 agents". If three antibiotics are administered during the scoring period , but one is terminated before another is initiated (only two are administered concurrently), select "1-2 agents." Antibiotics include acyclovir, amphotericin, ampicillin, cefazolin, cefotaxime, clindamycin, fluconazole, gentamicin, kefzol, penicillin and vancomycin.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, the use of any intravenous antibiotics was scored as present or absent regardless of amount.

Steroid post-natal - Steroid use (IV, po or nebulized but NOT TOPICAL) during the SCORING PERIOD, regardless of indication. Steroids include beclamethasone, beclovent puffs, cortisol (solucortef), dexamethasone (decadron), hydrocortisone, methylprednisolone (solumedrol) and prednisone.

Erythropoietin - Administration of erythropoietin during the SCORING PERIOD.

Operations: minor or major – Operations initiated or continued during the SCORING PERIOD. Operations defined as all operations/procedures performed in the operating room and/or requiring anesthesia. If multiple operations were performed under the same anesthesia episode, classify the operation as major if at least one of the procedures was major. Major and minor operations are mutually exclusive.
Minor operations include: bronchoscopy, cytoscopy, cryo/laser treatment, balloon septostomy, cardiac catheterization, CVL placement(with anesthesia), examination under anesthesia, gastrostomy, herniorrhaphy, laryngoscopy, nephrotomy, PDA ligation, rectal biopsy, skin grafting and surgically placed catheters. Do NOT double count tracheostomy.

Major operation include laparotomy (bowel resection, ileostomy, repair of abdominal

omphalocele, NEC), thoracotomy, (ASD closure, BTS for tricuspid atresia, coarctation repair, vascular ring operation) and craniotomy (placement of a Hickman catheter, reservoir or shunt CNS, re-section of an occipital encephalocele, myelomeningocele or omphalocele).

Operations do NOT include: Chest tube placement, cutdown venous access, ECMO, extra digit removal, peripheral arterial line placement, thora/paracentesis and UAL or UVL placement.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, the presence of any minor operation and any major operation were scored separately regardless of type of operation.

PRBC transfusion CC's - Total ccs of blood given in transfusions INITIATED during the SCORING PERIOD, even if some volume was administered AFTER the scoring period. DO not include volume from transfusions initiated BEFORE the scoring period, even if some volume was administered during the scoring period. This can be either packed cells (PC, PRBC) or whole blood. Blood used for exchange transfusion does not count.

Platelets transfusion – Transfusion of platelets GIVEN AT ANY TIME during the SCORING PERIOD.

White blood cells transfusion – White Blood Cell (neutrophil) transfusion INITATED during the SCORING PERIOD.

Partial exchange transfusion – Partial plasma exchange INITATED during the SCORING PERIOD. This is done to treat polycythemai (high hematocrit). It does not matter whether volume is replaced with albumin or normal saline (but not PRBC's or Whole Blood). Fluid given as part of exchange should NOT count as part of volume for the variable VOLUME EXPANSION.

2x volume exchange transfusion – Exchange transfusion INITATED during the SCORING PERIOD. The blood volume used in the exchange transfusion should NOT be counted towards the transfusion or extensive transfusion variable.

Pressors – Use of INTRAVENOUS BLOOD PRESSURE MEDICATIONS (pressors or vasoactive drug infusions) CONCURRENTLY during the SCORING PERIOD. If only a single infusion is administered at once, score as "1". If a second infusion was in use at the same time during the SCORING PERIOD then ">1" should be scored instead. Blood pressure medications include adenosine, doubutamine, dopamine, hydralazine, isoproterenol (isuprel), nitroglycerine (NTG), nitroprusside(nipride), phenylephrine, priscoline, prostaglandins and tolazoline. Epinephrine (epi drip) should be scored here UNLESS given as part of CPR. If given as part of CPR, score as CPR only. Do not score inhaled nitric oxide.

CPR - Cardio Pulmonary Resuscitation (CPR) administered during the SCORING PERIOD. There

must be documentation of cardiac compressions for either bradycardia or electro-mechanical dissociation. The use of bicarbonate and\or epinephrine alone is insufficient.

C) Outcomes

Bacteremia:
Date of positive culture - Record the date of the blood draw for each positive blood or CSF culture.
Organism, culture #__ - Record the 3 to 5 number/letter code for all organism found in positive cultures. Some of the more common ones include: GBS = group B strep, CONS=coag neg staph, AUR =staph aureus, ECOLI = E . Coli, KLEBS=klebsiella and CAND=canddida. If an organism has not yet been coded, call the STUDY COORDINATING CENTRE for a code. Do not record information about resistance to antibiotics. Do not record repeat tests from the same infection. Consider the following to be signs of a new infection: (1) There is a new organism; OR (2) A blood culture DRAWN seven days after the initial positive draw comes up positive. If the same organism is found in BOTH a blood and CSF culture, DO SCORE them separately. Record one blood culture (NOT one organism) per line, so you should contact the STUDY COORDINATING CENTRE for codes for multiple organisms from the same blood culture. You SHOULD record all positive blood cultures, even if they are noted or thought to be contaminants. If patients are transferred in with a positive culture, do NOT record here, but make a note in the comments box.
Source of culture # __ - Source of positive culture, B=blood , C=cerebrospinal fluid.
Total # of blood cultures - Total count of all blood cultures (positive or negative) received by the clinical laboratory during the NICU admission. Two blood cultures taken at the same time from different sites (2 blood draws) count as two blood cultures. Two bottle /anaerobic combination count as 1 culture.
Total # of CSF cults – Total number of CSF cultures (positive or negative) received by the clinical laboratory during this NICU admission. If CSF obtained without culture, do not include.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, an eligible bacteremia had to occur after the administration of RBCs.

Necrotizing Enterocolitis – Necrotizing entercolitis according to Bell's criteria, stage 2 or higher. If there is DEFINITE pneumatosis (air in the bowel wall) or portal/hepatic (air in the liver) diagnosed by x-ray, or if there is a surgical or autopsy diagnosis of NEC, score by highest level of treatment ("surgical Rx">" "medical Rx") received. If surgical autopsy diagnosis conflicts with x-ray diagnosis, the surgical/autopsy diagnosis takes priority. X-rays showing free air WITHOUT pneumatosis do NOT count as NEC diagnosis. Blood stools without pneumatosis may lead to a suspected diagnosis and treatment, but is NOT counted as NEC diagnosis. Please make a note of bowel perforations (that are not NEC) in the comments box. Score "None" if there was no NEC diagnosed according to our definition during the hospital stay. Score "Unknown/NA" if the baby died shortly after birth and no

diagnosis was made.

Intraventricular Hemmorhage: Score the IVH portion of the screen based on all head ultrasounds, CAT scans and MRIs done during the appropriate time periods. If you come across any serious outcomes which are not included on the IVH & ROP screen (e.g. "periventricular calcification", "parenchymal calcification," "cystic parietal lesion"), please call the STUDY COORDINATING CENTRE for advice on possible inclusion in the comments box. The following should NOT be scored anywhere: "possible" or "questionable" diagnoses, subarachnoid hemorrhages, subdural hemorrhages, tentorial bleeds, fluid collections in the brain, arachnoid cysts, caudothalmic groove cysts, choroids plexus cysts, subependymal cysts or other cysts other than those found in the brain parenchyma (the brain itself).

IVH Grade I (SHE) – Grade 1 hemorrhage according to the criteria of Papile: echogenic lesion confined to the germinal matrix area, not extending into the ventricles or adjacent parenchyma. Descriptors include "subependymal hemorrhage" (SEH), and "germinal matrix hemorrhage" (GMH). Grade according to level of certainty. If certain at Grade I, but uncertain of Grade II, mark as such. Score "possible" and "questionable" diagnoses as "None." Score "suggestive of...." and "most likely...." as "probable." Score all IVH bleeds that occur on either side. This should be based on ultrasound in the first two weeks of life. Do not score new IVH occurring after two weeks of life. Score "None" if none of the ultrasounds taken during the first 2 weeks of life showed a grade I IVH. Score "N/A" if there were no ultrasounds taken during the first 2 weeks of life at your hospital.

IVH Grade II – Grade II hemorrhage according to the criteria of Papile: echogenic lesion\density originating in the germinal matrix area AND extending into the ventricles, but not distending the ventricles with blood. Grade according to level of certainty. If certain at Grade I, but uncertain of Grade II, mark as such. Score "possible" and "questionable" diagnoses as "None." Score "suggestive of..." and "most likely.." as "Probable." Score all IVH bleeds that occur on either side. This should be based on ultrasound in the first two weeks of life. Do not score new IVH occurring after two weeks of life. Score "None" if none of the ultrasounds taken during the first 2 weeks of life showed a grade II IVH. Score "N/A" if there were no ultrasounds taken during the first 2 weeks of life at your hospital.

IVH Grade III – Grade III hemorrhage according to the criteria of Papile: echogenic lesion originating in the germinal matrix area AND extending into the ventricles, AND distending the ventricles with blood. Grade according to the level of certainty. If certain of Grade I, but uncertain of Grade II, mark as such. Score "possible" and "questionable" diagnoses as "None." Score "suggestive of...." and "most likely...." as "Probable." Score all IVH bleeds that occur on either side. This should be based on ultrasound in the first two weeks of life. Do not score new IVH occurring after two weeks of life. Score "None" if none of the ultrasounds taken during the first 2 weeks of life showed a grade III IVH. Score "N/A" if there were no ultrasounds taken during the first 2 weeks of life at your hospital.

IVH Grade IV (IPE) - Grade IV hemorrhage according to the criteria of Papile: echogenic lesion in the parenchyma of the brain (white matter or gray matter). This need not be accompanied by intraventricular hemorrhages grade I-III. Alternative nomenclature includes :intraparenchymal hemorrhage" and "intraparenchymal echodensity"(IPE). Grade according to level of certainty. If certain at Grade III but uncertain of Grade IV, mark as such. Score "possible" and "questionable" diangnoses as "None." Score "suggestive of....." and "most likely" as "Probable." Score all IVH bleeds that occur on either side. This should be based on ultrasound in the first two weeks of life. Do not score new IVH occurring after two weeks of life. Score "None" if none of the ultrasounds taken during the first two weeks of life showed a grade IV IVH. Score "N/A" if there were no ultrasounds taken during the first 2 weeks of life at your hospital.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, presence of IVH grade III and IV were collapsed into a single variable.

Retinopathy of prematurity: Stage - Maximum stage of retinopathy of prematurity (ROP) in worst eye as defined by the International Committee on Retinopathy of Prematurity (ICROP). If there is no eye exam, score as not applicable.

Retinopathy of prematurity: Zone – Record location of ROP by Zone. Disease severity is worst in Zone 1 (optic disk to macula),very serious in Zone 2, (macula to periphery) and worrisome in Zone 3 (peripheral vision). If there is no eye exam, score as not applicable.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, the presence of retinopathy of prematurity, regardless of stage, was collapsed into a single variable

Death: For all babies who are admitted to the NICU and die you will need to verify the cause of death by a) asking the attending physician and b) checking the death certificate to see what is listed. You should also be sure to photocopy the death summary (discharge summary) and the autopsy report (if done). The autopsy report may not be completed for several weeks. Keep unaltered copies for your own records and mail copies to the STUDY COORDINATING CENTRE with the name and medical record number deleted. You should be sure to put the baby's study ID# and the Site ID on the STUDY COORDINATING CENTRE copy. You should also talk to your Site Investigators about getting a log of all delivery room deaths(live born babies only) from Pathology. You will need to know date and time of birth and death, and cause of death. You should also find out the birthweight and gestational age of these babies. You may be able to work out a system of checking with Pathology monthly for any delivery room deaths.

D) Interventions/Procedures

Catheter type# : Record the catheter type as: UV = Umbilical Venous PERC = Percutaneous

(spaghetti, perc, pic) BROV = Broviac (surgically placed).
If more than 5 CVLs were placed at your hospital, record the first 5 placed. Score lines regardless of whether central placement is achieved; do NOT score lines that are never successfully placed. Where it is unclear whether the line was successfully placed, score based on whether the line has begun infusing solutions or not. Do NOT record lines that are not present during some part of your hospital admission.

Days on CPAP – Total number of days during which the infant received CPAP. One day is defined as 6 am to 5:59 am the next day. Exclude any days during which IPPV also occurred. Include any days where the infant was only on CPAP part of the day. Score ALL days (or partial days) including CPAP for the duration of a procedure and up to 24 hours after the procedure as a result of the procedure.

For the purposes of the "Effectiveness of Leukoreduction in Neonates" study, days on CPAP was categorized into 0 days, 1-10 days, and greater than 10 days.

ECMO - On extra corporeal membrane oxygenation(ECMO) at any time during the hospital stay. ECMO given as part of an operation SHOULD be scored here, but a note should also be made in the comments box that ECMO was given as part of an operation and not as a procedure unto itself.

Cryo/Laser – Cryotherapy or laser photocoagulation treatment for Retinopathy of Prematurity (ROP) at any time during the hospitalization. This should also be counted as a minor operation under "Total number of operations."

Total number of major operations – Major operations are counted here AND categorized above. If multiple procedures are performed during one anesthesia episode, count as ONE operation (the highest level of any of the procedures), but classify all major operations separately. Major operations include laparotomy, thoracotomy, craniotomy (reservoir or shunt CNS), and ECMO (on this screen but not on NTISS), but NOT Cryo/Laser treatment. If no operations were performed, record 0 (zero).

Total number of minor operations - Minor operations are counted here, and with the exception of Cryo/Laser treatment, are not classified. Minor operations include bronchoscopy, cytoscopy, laryngoscopy, nephrotomy, rectal biopsy, surgically placed catheters, CVL placement (in operating room or without anesthesia), PDA ligation, gastrostomy, tracheostomy, balloon septostomy, cardiac catheterization, herniorrhaphy, examination under anesthesia, cryotherapy or laser therapy for ROP, and skin grafting.

Operation do NOT include chest tube placement, UAL or UVL placement, peripheral arterial line placement, cutdown venous access, extra digit removal and thora/paracentesis.

E) Discharge Variables

Destination on discharge -
Score the disposition on discharge from your NICU
Score "Community hospital Level I" if they baby was transferred to any term (level I/healthy baby) nursery.
Score "Community hospital Level II" if the baby was transferred to a level II community hospital nursery (in which case you should follow up on this baby with the post-transfer screen).
Score "Tertiary hospital transfer" if the baby was transferred to one of the other study sites (in which case you should follow up on this baby with the post-transfer screen) or to another tertiary care centre.
Score "Home" if the baby was discharged home from your NICU.
Score "Other" if the baby was transferred under special circumstances such as to a rehabilitation hospital.
Score "Died" if the baby died during this hospital stay.

Cause of death - Record the principle cause of death as stated by the attending physician and ask the physician to verify the cause of death listed in the clinical notes (and autopsy findings when available). This is typed in as text and may be abbreviated if necessary. Use underlying diagnoses, not terminal events like "cardiac arrest."

Transferred to where - Record the 2 to 8 letter code for the name of the facility when a baby is transferred to another facility on discharge. If a hospital has not yet been coded, call the STUDY COORDINATING CENTRE for a code. Only score this item if you have checked "community hospital transfer", "tertiary hospital" or "other" in the destination column.

F) Transfusion Variables

Leukoreduction: scored according to whether infant received leukoreduced or non-leukoreduced RBC products.

Volume Transfused: total volume (mL) of RBCs transfused during the infants NICU stay

Date Transfused: Date at which RBC volume transfused

Appendix V: Descriptive Statistics

Dichotomous Variables

	Non-Leukoreduced (n=268)			Leukoreduced (n=247)			Missing Data	
		95% CI			95%CI			
	Proportion	Lower	Upper	Proportion	Lower	Upper	NLR *	LR**
Demographics								
Admission Status (% Outborn)	**14.55%**	10.32%	18.78%	**12.65%**	8.48%	16.82%	0	2
Antenatal Corticosteroids (% Yes)	**68.44%**	62.81%	74.07%	**72.77%**	67.06%	78.47%	5	12
Delivery (% Vaginal)	**51.13%**	45.11%	57.15%	**56.61%**	50.35%	62.87%	2	5
Sex (% Males)	**44.03%**	38.08%	49.98%	**49.38%**	43.08%	55.68%	0	4
Day 1 Variables (% Present)								
Any Antibiotic Agent	**99.59%**	98.79%	100.00%	**100.00%**	100.00%	100.00%	16	7
Any Cardiovascular Pressor	**43.03%**	36.89%	49.17%	**38.75%**	32.57%	44.93%	17	7
Any Postnatal Steroid	**7.14%**	3.96%	10.33%	**5.00%**	2.24%	7.76%	16	7
Arterial Line	**77.38%**	72.21%	82.56%	**68.33%**	62.44%	74.23%	16	7
Central Venous	**62.30%**	56.31%	68.30%	**66.67%**	60.69%	72.64%	16	7
Continuous Positive Airway Pressure (CPAP)	**16.67%**	12.06%	21.28%	**51.25%**	44.91%	57.59%	16	7
Cardiopulmonary Resuscitation (CPR)	**2.38%**	0.49%	4.27%	**5.00%**	2.24%	7.76%	16	7
High Frequency Ventilation	**4.76%**	2.13%	7.40%	**9.17%**	5.51%	12.82%	16	7
Intravenous Immunoglobulin (IVIG)	**0.40%**	0.00%	1.17%	**1.67%**	0.00%	3.29%	16	7
Mechanical Ventilation	**89.68%**	85.92%	93.45%	**81.25%**	76.30%	86.20%	16	7
Peripheral IV	**62.70%**	56.72%	68.68%	**60.00%**	53.79%	66.21%	16	7
Platelet Transfusion	**1.19%**	0.00%	2.53%	**1.67%**	0.00%	3.29%	16	7
Supplemental Oxygen	**77.38%**	72.21%	82.56%	**84.58%**	80.01%	89.16%	16	7
Surfactant	**53.57%**	47.40%	59.74%	**67.50%**	61.56%	73.44%	16	7
Ventilation with Muscle Relaxants	**1.19%**	0.00%	2.53%	**1.67%**	0.04%	3.29%	16	7
Volume Exchange	**0.04%**	0.00%	1.17%	**0.00%**	0.00%	0.00%	16	7
WBC Transfusion	**0.00%**	0	0	**0.00%**	0	0	16	7
Catheter Type (% Present)								
Broviac	**4.10%**	1.72%	6.48%	**1.21%**	0.00%	2.58%	0	0
Percutaneous	**32.84%**	27.20%	38.47%	**32.39%**	26.54%	38.24%	0	0
Umbilical	**63.43%**	57.66%	69.21%	**64.78%**	58.81%	70.75%	0	0

* NLR = Non-Leukoreduced, **LR = Leukoreduced

Dichotomous Variables (continued)

	Non-Leukoreduced (n=268)			Leukoreduced (n=247)			Missing Data	
		95% CI			95% CI			
	Proportion	Lower	Upper	Proportion	Lower	Upper	NLR *	LR**
Interventions/Procedures (% Present)								
Any Operation (Major or Minor)	**30.45%**	24.91%	35.99%	**23.65%**	18.28%	29.03%	2	6
Any Operation (Major)	**7.92%**	4.67%	11.18%	**5.42%**	2.55%	8.29%	3	7
Any Operation (Minor)	**27.44%**	22.07%	32.82%	**22.41%**	17.13%	27.68%	2	6
CPAP: 0 Days	**30.86%**	25.19%	36.53%	**29.46%**	23.69%	35.23%	12	6
CPAP: 1-10 Days	**32.03%**	26.30%	37.76%	**30.54%**	24.69%	36.40%	12	8
CPAP: >11 Days	**37.11%**	31.18%	43.04%	**39.75%**	33.53%	45.97%	12	8
Cryo/Laser	**8.96%**	5.53%	12.38%	**5.71%**	2.80%	8.63%	0	2
Outcomes (% Present)								
Any IVH Stage III-IV	**17.37%**	12.75%	22.00%	**13.69%**	9.34%	18.04%	9	6
Any IVH Stage I-IV	**47.88%**	41.78%	53.97%	**45.64%**	39.34%	51.94%	9	6
Any Bacteremia	**41.42%**	35.51%	47.33%	**40.49%**	34.35%	46.62%	0	0
Bacteremia after 1st Transfusion	**29.59%**	24.10%	35.07%	**25.61%**	20.14%	31.08%		
Bronchopulmonary Dysplasia	**52.61%**	46.62%	58.60%	**34.82%**	28.86%	40.77%	0	0
Mortality	**16.79%**	12.31%	21.27%	**17.96%**	13.14%	22.78%	0	2
Necrotizing Enterocolitis	**12.50%**	8.44%	16.56%	**6.67%**	3.50%	9.83%	12	7
Retinopathy of Prematurity	**59.09%**	52.58%	65.60%	**41.74%**	35.35%	48.13%	48	17

* NLR = Non-Leukoreduced, **LR = Leukoreduced

Continuous Variables

	Non-Leukoreduced (n=268)			Leukoreduced (n=247)			Missing Data	
		95%CI			95%CI			
	Mean	LCL	UCL	Mean	LCL	UCL	NLR*	LR**
Birthweight	**814.80**	790.30	839.30	**839.57**	815.73	863.40	0	3
Gestational Age	**26.15**	25.91	26.39	**26.33**	26.02	26.64	1	5
APGAR 1 minute	**4.57**	4.29	4.84	**4.58**	4.29	4.87	2	9
APGAR 5 minute	**7.08**	6.85	7.31	**7.13**	6.91	7.35	5	9
SNAPII on Day 1	**19.95**	18.43	21.47	**19.35**	17.52	21.18	26	22
SNAPII on Day 3	**7.81**	6.73	8.89	**7.69**	6.51	8.88	31	19
SNAPIIPE on Day 1	**36.83**	34.46	39.20	**34.37**	31.73	37.02	30	30
SNAPIIPE on Day 3	**24.13**	22.28	25.99	**22.89**	20.71	25.08	35	28
# of Blood Draws	**6.74**	6.41	7.07	**7.44**	6.92	7.95	16	7
Total CPAP days	**9.20**	7.99	10.40	**10.78**	9.27	12.30	12	8
NICU Length of Stay	**77.14**	72.16	82.12	**70.89**	65.03	76.76	1	4
Total Volume Transfused	**82.14**	71.17	93.10	**113.91**	84.11	143.73	0	0

* NLR = Non-Leukoreduced, **LR = Leukoreduced

Appendix VI: Multiple Regression Models

The odds ratios provided in Appendices VI are the reciprocals of the odds ratios provided in the thesis abstract and Manuscript #3. Traditionally, odds ratios less than 1 suggest benefit in favour of the intervention and therefore the calculated odds ratios provided by the statistical software had to be inversed.

Label used in Modelling	Variable
ADSTAT	Admission Status (% Outborn)
ANT_CORT	Use of Antenatal Corticosteroids
ANYPRESS	Use of Any Cardiac Pressor
APGAR5	APGAR Score @ 5 Minutes
BTHWT	Birth Weight
CPAP1to10	Use of CPAP 1-10 days, Reference: 0 days
CPAP_10	Use of CPAP greater than 10 days, Reference: 0 days
DELIVERY	Type of Delivery (Vaginal)
FLUSEASON	Admitted during Flu Season (December to March)
GESTAGE	Gestational Age
HFVENT	Use of High Frequency Ventilation
LEUKO	Administered Leukoreduced RBCs
MECVENT	Use of Mechanical Ventilation
PERCUTANEOUS	Use of Percutaneous Catheter
SEX	Sex (% Males)
SITE2	Site 2 (Royal University Hospital), Reference: Vancouver
SITE3	Site 3 (Mount Sinai Hospital), Reference: Vancouver
snIIsc1	SNAP II Score on Day 1
STEROID	Use of Steroids on Day 1 of Admission
SUPPO2	Use of Supplemental Oxygen on Day 1 of Admission
SURF	Use of Surfactant on Day 1 of Admission
UMBILICAL	Use of Umbilical Catheter
volumeTRans	Total Volume of RBCs Transfused

Multinomial Logistic Regression Report

Outcome: Eligible Bacteremia

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	ELIGIBLE0BACT	Rows Processed	515
Reference Value	1	Rows Used	457
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	2
Final Log Likelihood	-201.06259	Unique Row Patterns	459
Model R-Squared	0.26068	Sum of Frequencies	457
Actual Convergence	1.82086E-08	Likelihood Iterations	6
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: ELIGIBLE0BACT = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-1.09365	0.33499	0.00356	31.50027
B1: ADSTAT	0.01519	1.01531	0.43346	2.37821
B2: ANT_CORT	-0.00760	0.99242	0.53230	1.85029
B3: ANYPRESS	-0.23230	0.79271	0.44496	1.41223
B4: APGAR5	-0.04060	0.96021	0.82039	1.12385
B5: BTHWT	0.00072	1.00072	0.99909	1.00234
B6: CPAP1to10	-0.58205	0.55875	0.27078	1.15298
B7: CPAP_10	-0.66884	0.51230	0.23957	1.09552
B8: DELIVERY	-0.03161	0.96888	0.55315	1.69705
B9: FLUSEASON	0.36311	1.43780	0.82269	2.51279
B10: GESTAGE	0.12936	1.13810	0.95562	1.35541
B11: HFVENT	1.07797	2.93871	0.94934	9.09691
B12: LEUKO	0.52726	1.69428	0.98812	2.90507
B13: MECVENT	-0.57395	0.56330	0.20350	1.55927
B14: PERCUTANEOUS	-0.57097	0.56498	0.32295	0.98840
B15: SEX	-0.03196	0.96854	0.58010	1.61710
B16: SITE2	-1.89998	0.14957	0.07680	0.29130
B17: SITE3	-1.63680	0.19460	0.04622	0.81927
B18: snIIsc1	-0.01884	0.98134	0.95720	1.00608
B19: STEROID	0.50589	1.65846	0.56633	4.85664
B20: SUPPO2	0.99790	2.71258	0.95958	7.66808
B21: SURF	-0.04251	0.95838	0.52785	1.74008
B22: UMBILICAL	-0.61043	0.54312	0.28908	1.02038
B23: volumeTRans	-0.00507	0.99494	0.99208	0.99781

Estimated Logistic Regression Model(s)
Model For ELIGIBLE0BACT=0
-1.09364856388697 + 1.51940601644058E-02*ADSTAT -7.60467299656491E-03*ANT_CORT - .232296756200603*ANYPRESS -4.06044820562113E-02*APGAR5 + 7.1679307747324E-04*BTHWT - .582045250211688*CPAP1to10 -.66883870631425*CPAP_10 -3.16137401237079E-02*DELIVERY + .363111130794544*FLUSEASON + .129357748708257*GESTAGE + 1.07797063056109*HFVENT + .527255929858158*LEUKO -.573947296350587*MECVENT -.570968283954953*PERCUTANEOUS - 3.19618972966356E-02*SEX -1.89998429815189*SITE2 -1.63680418497711*SITE3 - 1.88389608261819E-02*snIIsc1 + .505887177460326*STEROID + .997901925210903*SUPPO2 - 4.25090750394029E-02*SURF -.610430694342572*UMBILICAL -5.06942494947042E-03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-271.95605	0.00000		
ADSTAT	1	-201.06320	0.26068	0.00000	0.73932
ANT_CORT	1	-201.06288	0.26068	0.00000	0.73932
ANYPRESS	1	-201.37240	0.25954	0.00114	0.74046
APGAR5	1	-201.19135	0.26021	0.00047	0.73979
BTHWT	1	-201.43637	0.25931	0.00137	0.74069
CPAP1to10	1	-202.31872	0.25606	0.00462	0.74394
CPAP_10	1	-202.57959	0.25510	0.00558	0.74490
DELIVERY	1	-201.06870	0.26066	0.00002	0.73934
FLUSEASON	1	-201.88803	0.25764	0.00304	0.74236
GESTAGE	1	-202.18976	0.25654	0.00414	0.74346
HFVENT	1	-202.96398	0.25369	0.00699	0.74631
LEUKO	1	-202.93793	0.25378	0.00690	0.74622
MECVENT	1	-201.69994	0.25834	0.00234	0.74166
PERCUTANEOUS	1	-203.06809	0.25331	0.00737	0.74669
SEX	1	-201.07006	0.26065	0.00003	0.73935
SITE2	1	-218.55558	0.19636	0.06432	0.80364
SITE3	1	-203.59065	0.25138	0.00930	0.74862
snIIsc1	1	-202.15705	0.25666	0.00402	0.74334
STEROID	1	-201.49838	0.25908	0.00160	0.74092
SUPPO2	1	-202.83531	0.25416	0.00652	0.74584
SURF	1	-201.07235	0.26064	0.00004	0.73936
UMBILICAL	1	-202.90932	0.25389	0.00679	0.74611
volumeTRans	1	-208.07257	0.23490	0.02578	0.76510
None(Model)	23	-201.06259	0.26068	0.00000	0.73932
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Mortality

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	died	Rows Processed	515
Reference Value	1	Rows Used	459
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	0
Final Log Likelihood	-116.58347	Unique Row Patterns	459
Model R-Squared	0.44690	Sum of Frequencies	459
Actual Convergence	4.216749E-10	Likelihood Iterations	8
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: died = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-5.06847	0.00629	0.00004	1.07316
B1: ADSTAT	0.20357	1.22577	0.44085	3.40822
B2: ANT_CORT	0.05561	1.05718	0.48964	2.28255
B3: ANYPRESS	0.01278	1.01286	0.44264	2.31765
B4: APGAR5	0.13427	1.14371	0.94186	1.38881
B5: BTHWT	0.00250	1.00250	1.00035	1.00466
B6: CPAP1to10	3.01183	20.32457	7.81052	52.88871
B7: CPAP_10	4.43009	83.93935	18.62365	378.32626
B8: DELIVERY	-0.04484	0.95615	0.45031	2.03023
B9: FLUSEASON	0.17911	1.19616	0.55324	2.58619
B10: GESTAGE	0.04776	1.04892	0.87266	1.26079
B11: HFVENT	-0.48329	0.61675	0.16244	2.34161
B12: LEUKO	-0.19556	0.82238	0.40024	1.68974
B13: MECVENT	-0.12068	0.88632	0.29208	2.68953
B14: PERCUTANEOUS	0.70397	2.02177	0.88130	4.63810
B15: SEX	0.21509	1.23997	0.61526	2.49899
B16: SITE2	-0.31963	0.72642	0.29551	1.78566
B17: SITE3	-0.76666	0.46456	0.07315	2.95042
B18: snIIsc1	0.00509	1.00510	0.97771	1.03327
B19: STEROID	-1.11681	0.32732	0.09536	1.12351
B20: SUPPO2	-0.58423	0.55754	0.13570	2.29064
B21: SURF	0.86331	2.37099	1.02756	5.47077
B22: UMBILICAL	-1.06303	0.34541	0.13852	0.86128
B23: volumeTRans	0.00132	1.00132	0.99804	1.00461

Estimated Logistic Regression Model(s)
Model For died=0
-5.06846674087264 + .203565447804837*ADSTAT + 5.56073015611126E-02*ANT_CORT + 1.27825756090922E-02*ANYPRESS + .134273631518981*APGAR5 + 2.49713975952825E-03*BTHWT + 3.01183070948917*CPAP1to10 + 4.43009450742843*CPAP_10 -4.48410286377202E-02*DELIVERY + .179114968370802*FLUSEASON + 4.77632650302348E-02*GESTAGE - .483291429229394*HFVENT -.195557347498455*LEUKO -.120679159692112*MECVENT + .703972624321781*PERCUTANEOUS + .2150885288893*SEX -.319630589908243*SITE2 - .766658796491693*SITE3 + 5.09043957404397E-03*snIIsc1 -1.11681316353123*STEROID - .584225186923449*SUPPO2 + .863305744569639*SURF -1.06302736618255*UMBILICAL + 1.31962640655556E-03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-210.78259	0.00000		
ADSTAT	1	-116.65966	0.44654	0.00036	0.55346
ANT_CORT	1	-116.59349	0.44685	0.00005	0.55315
ANYPRESS	1	-116.58393	0.44690	0.00000	0.55310
APGAR5	1	-117.49447	0.44258	0.00432	0.55742
BTHWT	1	-119.27596	0.43413	0.01277	0.56587
CPAP1to10	1	-143.51375	0.31914	0.12776	0.68086
CPAP_10	1	-156.60146	0.25705	0.18985	0.74295
DELIVERY	1	-116.59029	0.44687	0.00003	0.55313
FLUSEASON	1	-116.68759	0.44641	0.00049	0.55359
GESTAGE	1	-116.71506	0.44628	0.00062	0.55372
HFVENT	1	-116.83592	0.44570	0.00120	0.55430
LEUKO	1	-116.72532	0.44623	0.00067	0.55377
MECVENT	1	-116.60617	0.44679	0.00011	0.55321
PERCUTANEOUS	1	-117.98768	0.44024	0.00666	0.55976
SEX	1	-116.76486	0.44604	0.00086	0.55396
SITE2	1	-116.82769	0.44574	0.00116	0.55426
SITE3	1	-116.92088	0.44530	0.00160	0.55470
snIIsc1	1	-116.64880	0.44659	0.00031	0.55341
STEROID	1	-118.19253	0.43927	0.00763	0.56073
SUPPO2	1	-116.92571	0.44528	0.00162	0.55472
SURF	1	-118.68444	0.43693	0.00997	0.56307
UMBILICAL	1	-119.31246	0.43395	0.01295	0.56605
volumeTRans	1	-117.26534	0.44367	0.00323	0.55633
None(Model)	23	-116.58347	0.44690	0.00000	0.55310
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Retinopathy of Prematurity

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	ROP	Rows Processed	515
Reference Value	1	Rows Used	408
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	51
Final Log Likelihood	-211.68339	Unique Row Patterns	459
Model R-Squared	0.25137	Sum of Frequencies	408
Actual Convergence	1.894578E-09	Likelihood Iterations	6
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: ROP = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-5.43808	0.00435	0.00005	0.35455
B1: ADSTAT	-0.64215	0.52616	0.22930	1.20734
B2: ANT_CORT	-0.35279	0.70272	0.39133	1.26191
B3: ANYPRESS	0.15922	1.17260	0.65784	2.09015
B4: APGAR5	0.01223	1.01230	0.87329	1.17344
B5: BTHWT	0.00151	1.00151	0.99997	1.00306
B6: CPAP1to10	-2.45830	0.08558	0.03566	0.20537
B7: CPAP_10	-3.25675	0.03851	0.01576	0.09413
B8: DELIVERY	-0.28704	0.75049	0.44206	1.27411
B9: FLUSEASON	-0.26033	0.77080	0.45863	1.29544
B10: GESTAGE	0.30530	1.35703	1.14295	1.61120
B11: HFVENT	0.29108	1.33787	0.49404	3.62299
B12: LEUKO	0.58415	1.79347	1.07448	2.99357
B13: MECVENT	0.75440	2.12634	0.89040	5.07785
B14: PERCUTANEOUS	-0.31361	0.73080	0.42990	1.24230
B15: SEX	-0.00329	0.99671	0.61105	1.62579
B16: SITE2	0.11059	1.11694	0.61491	2.02882
B17: SITE3	-5.16212	0.00573	0.00092	0.03551
B18: snIIsc1	-0.01912	0.98107	0.95863	1.00402
B19: STEROID	-0.12226	0.88492	0.27102	2.88936
B20: SUPPO2	-0.63181	0.53163	0.19797	1.42764
B21: SURF	-0.27993	0.75584	0.41714	1.36956
B22: UMBILICAL	-0.33214	0.71739	0.38695	1.33001
B23: volumeTRans	-0.00436	0.99565	0.99264	0.99868

Estimated Logistic Regression Model(s)
Model For ROP=0
-5.43808053704474 -.642145156893161*ADSTAT -.35279422918113*ANT_CORT +
.159222303385234*ANYPRESS + .012225359238105*APGAR5 + 1.5129608771889E-03*BTHWT -
2.45830158020972*CPAP1to10 -3.25675002158812*CPAP_10 -.287035104473401*DELIVERY -
.260330852277308*FLUSEASON + .305299239665057*GESTAGE + .29107633908563*HFVENT +
.584153494815145*LEUKO + .754403588111084*MECVENT -.31361383796218*PERCUTANEOUS -
3.2908641566055E-03*SEX + .110589440858291*SITE2 -5.16211962081386*SITE3 -
1.91159015902416E-02*snIIsc1 -.122261903958743*STEROID -.631810529502663*SUPPO2 -
.279925789126114*SURF -.332139095574915*UMBILICAL -4.35506003450317E-03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-282.75993	0.00000		
ADSTAT	1	-212.84132	0.24727	0.00410	0.75273
ANT_CORT	1	-212.38619	0.24888	0.00249	0.75112
ANYPRESS	1	-211.82946	0.25085	0.00052	0.74915
APGAR5	1	-211.69656	0.25132	0.00005	0.74868
BTHWT	1	-213.55322	0.24475	0.00661	0.75525
CPAP1to10	1	-230.39868	0.18518	0.06619	0.81482
CPAP_10	1	-245.82472	0.13062	0.12074	0.86938
DELIVERY	1	-212.24998	0.24936	0.00200	0.75064
FLUSEASON	1	-212.16665	0.24966	0.00171	0.75034
GESTAGE	1	-218.53187	0.22715	0.02422	0.77285
HFVENT	1	-211.84710	0.25079	0.00058	0.74921
LEUKO	1	-214.20215	0.24246	0.00891	0.75754
MECVENT	1	-213.14931	0.24618	0.00518	0.75382
PERCUTANEOUS	1	-212.35410	0.24900	0.00237	0.75100
SEX	1	-211.68348	0.25137	0.00000	0.74863
SITE2	1	-211.74930	0.25113	0.00023	0.74887
SITE3	1	-230.59007	0.18450	0.06686	0.81550
snIIsc1	1	-213.00670	0.24669	0.00468	0.75331
STEROID	1	-211.70388	0.25129	0.00007	0.74871
SUPPO2	1	-212.47880	0.24855	0.00281	0.75145
SURF	1	-212.11170	0.24985	0.00151	0.75015
UMBILICAL	1	-212.24283	0.24939	0.00198	0.75061
volumeTRans	1	-216.78457	0.23333	0.01804	0.76667
None(Model)	23	-211.68339	0.25137	0.00000	0.74863
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Necrotizing Enterocolitis

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	NECRO	Rows Processed	515
Reference Value	1	Rows Used	448
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	11
Final Log Likelihood	-111.84510	Unique Row Patterns	459
Model R-Squared	0.21036	Sum of Frequencies	448
Actual Convergence	7.739547E-09	Likelihood Iterations	7
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: NECRO = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-4.78084	0.00839	0.00001	10.79115
B1: ADSTAT	-0.53846	0.58365	0.17759	1.91818
B2: ANT_CORT	-0.81979	0.44052	0.17317	1.12065
B3: ANYPRESS	0.35313	1.42352	0.57500	3.52421
B4: APGAR5	0.03893	1.03970	0.82181	1.31536
B5: BTHWT	-0.00115	0.99885	0.99638	1.00132
B6: CPAP1to10	-0.02297	0.97729	0.33914	2.81625
B7: CPAP_10	-0.02298	0.97728	0.34536	2.76545
B8: DELIVERY	-0.12832	0.87957	0.40515	1.90952
B9: FLUSEASON	0.73104	2.07725	0.88461	4.87783
B10: GESTAGE	0.23424	1.26395	0.95565	1.67170
B11: HFVENT	0.29977	1.34954	0.15225	11.96223
B12: LEUKO	0.93933	2.55827	1.10948	5.89890
B13: MECVENT	-0.49497	0.60959	0.16165	2.29878
B14: PERCUTANEOUS	-0.91053	0.40231	0.17661	0.91646
B15: SEX	0.07405	1.07686	0.51503	2.25156
B16: SITE2	0.62561	1.86939	0.70751	4.93933
B17: SITE3	-0.85244	0.42637	0.05621	3.23396
B18: snIIsc1	0.01052	1.01058	0.97613	1.04625
B19: STEROID	0.47212	1.60339	0.30138	8.53046
B20: SUPPO2	-0.06522	0.93687	0.22146	3.96334
B21: SURF	0.05631	1.05792	0.45453	2.46235
B22: UMBILICAL	1.14076	3.12913	1.27987	7.65037
B23: volumeTRans	-0.00736	0.99267	0.98923	0.99612

Estimated Logistic Regression Model(s)
Model For NECRO=0
-4.78083721518226 -.538456090007427*ADSTAT -.819788921341333*ANT_CORT + .35313109431971*ANYPRESS + 3.89336000508607E-02*APGAR5 -1.15456311361738E-03*BTHWT - 2.29708793567836E-02*CPAP1to10 -2.29779829356353E-02*CPAP_10 - .128319137249389*DELIVERY + .731044818972898*FLUSEASON + .234238290031651*GESTAGE + .299766819829156*HFVENT + .93932994665712*LEUKO -.494969954266974*MECVENT - .91052521236693*PERCUTANEOUS + 7.40480462890805E-02*SEX + .625611519040575*SITE2 - .852443149583428*SITE3 + 1.05240223158164E-02*snIIsc1 + .472121500362947*STEROID - 6.52158380293971E-02*SUPPO2 + 5.63078143648538E-02*SURF + 1.1407564203396*UMBILICAL - 7.36000900781838E-03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-141.64150	0.00000		
ADSTAT	1	-112.22218	0.20770	0.00266	0.79230
ANT_CORT	1	-113.45484	0.19900	0.01136	0.80100
ANYPRESS	1	-112.13944	0.20829	0.00208	0.79171
APGAR5	1	-111.89707	0.21000	0.00037	0.79000
BTHWT	1	-112.26885	0.20737	0.00299	0.79263
CPAP1to10	1	-111.84601	0.21036	0.00001	0.78964
CPAP_10	1	-111.84604	0.21036	0.00001	0.78964
DELIVERY	1	-111.89770	0.20999	0.00037	0.79001
FLUSEASON	1	-113.35986	0.19967	0.01069	0.80033
GESTAGE	1	-113.34112	0.19980	0.01056	0.80020
HFVENT	1	-111.88376	0.21009	0.00027	0.78991
LEUKO	1	-114.43311	0.19209	0.01827	0.80791
MECVENT	1	-112.12565	0.20838	0.00198	0.79162
PERCUTANEOUS	1	-114.23628	0.19348	0.01688	0.80652
SEX	1	-111.86448	0.21023	0.00014	0.78977
SITE2	1	-112.66703	0.20456	0.00580	0.79544
SITE3	1	-112.19027	0.20793	0.00244	0.79207
snIIsc1	1	-112.02504	0.20909	0.00127	0.79091
STEROID	1	-112.00573	0.20923	0.00113	0.79077
SUPPO2	1	-111.84906	0.21034	0.00003	0.78966
SURF	1	-111.85362	0.21030	0.00006	0.78970
UMBILICAL	1	-115.03567	0.18784	0.02253	0.81216
volumeTRans	1	-123.40400	0.12876	0.08161	0.87124
None(Model)	23	-111.84510	0.21036	0.00000	0.78964
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Bronchopulmonary Dysplasia

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	BPD	Rows Processed	515
Reference Value	1	Rows Used	459
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	0
Final Log Likelihood	-209.87917	Unique Row Patterns	459
Model R-Squared	0.33390	Sum of Frequencies	459
Actual Convergence	1.922958E-08	Likelihood Iterations	6
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: BPD = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-9.34123	0.00009	0.00000	0.01238
B1: ADSTAT	-0.37894	0.68458	0.29636	1.58139
B2: ANT_CORT	-0.27026	0.76318	0.42126	1.38262
B3: ANYPRESS	-0.65277	0.52060	0.28992	0.93483
B4: APGAR5	-0.12533	0.88220	0.75495	1.03091
B5: BTHWT	0.00136	1.00136	0.99973	1.00299
B6: CPAP1to10	-3.12433	0.04397	0.01848	0.10460
B7: CPAP_10	-2.61586	0.07311	0.03181	0.16802
B8: DELIVERY	-0.30288	0.73869	0.42940	1.27075
B9: FLUSEASON	0.24126	1.27285	0.74979	2.16078
B10: GESTAGE	0.49503	1.64054	1.34430	2.00208
B11: HFVENT	0.26755	1.30676	0.46530	3.66992
B12: LEUKO	0.87707	2.40384	1.43179	4.03584
B13: MECVENT	-0.31291	0.73132	0.31259	1.71093
B14: PERCUTANEOUS	-0.12804	0.87982	0.50909	1.52053
B15: SEX	0.30975	1.36308	0.82866	2.24215
B16: SITE2	-1.43261	0.23869	0.12717	0.44798
B17: SITE3	-4.40176	0.01226	0.00256	0.05875
B18: snIIsc1	-0.02879	0.97162	0.94841	0.99541
B19: STEROID	-0.96135	0.38237	0.12022	1.21617
B20: SUPPO2	-0.05841	0.94327	0.35714	2.49132
B21: SURF	-0.04389	0.95706	0.53397	1.71538
B22: UMBILICAL	0.69534	2.00439	1.08571	3.70039
B23: volumeTRans	-0.00132	0.99868	0.99671	1.00064

Estimated Logistic Regression Model(s)
Model For BPD=0
-9.34122814783026 -.3789427103965 2*ADSTAT -.270263956275256*ANT_CORT -
.652768670636972*ANYPRESS -.125333208417186*APGAR5 + 1.35520044883321E-03*BTHWT -
3.12433009558003*CPAP1to10 -2.6158579468519*CPAP_10 -.302883329492113*DELIVERY +
.24125604057326*FLUSEASON + .495028177197091*GESTAGE + .267548291720688*HFVENT +
.877068748816807*LEUKO -.312907182130836*MECVENT -.128038295388415*PERCUTANEOUS +
.309746005711914*SEX -1.43260788343631*SITE2 -4.40176116597456*SITE3 -2.87863845557315E-
02*snIIsc1 -.961354873565524*STEROID -5.84057140941357E-02*SUPPO2 -4.38881600837244E-
02*SURF + .695338523221828*UMBILICAL -1.32440284339674E-03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-315.08781	0.00000		
ADSTAT	1	-210.27034	0.33266	0.00124	0.66734
ANT_CORT	1	-210.27919	0.33263	0.00127	0.66737
ANYPRESS	1	-212.27847	0.32629	0.00761	0.67371
APGAR5	1	-211.14556	0.32988	0.00402	0.67012
BTHWT	1	-211.21595	0.32966	0.00424	0.67034
CPAP1to10	1	-241.94337	0.23214	0.10176	0.76786
CPAP_10	1	-233.26920	0.25967	0.07423	0.74033
DELIVERY	1	-210.47932	0.33200	0.00190	0.66800
FLUSEASON	1	-210.28000	0.33263	0.00127	0.66737
GESTAGE	1	-224.11759	0.28871	0.04519	0.71129
HFVENT	1	-210.00842	0.33349	0.00041	0.66651
LEUKO	1	-215.55622	0.31589	0.01802	0.68411
MECVENT	1	-210.14143	0.33307	0.00083	0.66693
PERCUTANEOUS	1	-209.98429	0.33357	0.00033	0.66643
SEX	1	-210.62558	0.33153	0.00237	0.66847
SITE2	1	-220.43702	0.30039	0.03351	0.69961
SITE3	1	-227.50024	0.27798	0.05592	0.72202
snIIsc1	1	-212.64245	0.32513	0.00877	0.67487
STEROID	1	-211.27700	0.32947	0.00444	0.67053
SUPPO2	1	-209.88613	0.33388	0.00002	0.66612
SURF	1	-209.89003	0.33387	0.00003	0.66613
UMBILICAL	1	-212.39835	0.32591	0.00800	0.67409
volumeTRans	1	-211.51447	0.32871	0.00519	0.67129
None(Model)	23	-209.87917	0.33390	0.00000	0.66610
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Any Intraventricular Hemorrhage Grade III or IV

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	ANYIVH3or4	Rows Processed	515
Reference Value	1	Rows Used	456
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	3
Final Log Likelihood	-164.93526	Unique Row Patterns	459
Model R-Squared	0.15638	Sum of Frequencies	456
Actual Convergence	3.232016E-07	Likelihood Iterations	6
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: ANYIVH3or4 = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-6.59757	0.00136	0.00001	0.29928
B1: ADSTAT	-0.55758	0.57259	0.23892	1.37225
B2: ANT_CORT	-0.41347	0.66135	0.33570	1.30289
B3: ANYPRESS	-0.47687	0.62073	0.30331	1.27033
B4: APGAR5	0.03112	1.03161	0.87779	1.21238
B5: BTHWT	-0.00223	0.99777	0.99580	0.99975
B6: CPAP1to10	0.32830	1.38861	0.66387	2.90455
B7: CPAP_10	1.52787	4.60833	2.00505	10.59161
B8: DELIVERY	-0.43571	0.64681	0.34915	1.19821
B9: FLUSEASON	0.28818	1.33400	0.70090	2.53897
B10: GESTAGE	0.32934	1.39005	1.11073	1.73963
B11: HFVENT	0.16598	1.18055	0.38125	3.65556
B12: LEUKO	0.44441	1.55956	0.84974	2.86231
B13: MECVENT	0.39522	1.48471	0.58223	3.78607
B14: PERCUTANEOUS	0.01130	1.01136	0.53229	1.92161
B15: SEX	-0.05611	0.94543	0.52413	1.70539
B16: SITE2	1.22499	3.40412	1.56346	7.41180
B17: SITE3	-0.62150	0.53714	0.09823	2.93725
B18: snIIsc1	-0.01987	0.98032	0.95642	1.00483
B19: STEROID	0.10056	1.10579	0.33491	3.65105
B20: SUPPO2	-0.68998	0.50159	0.13500	1.86367
B21: SURF	0.35310	1.42348	0.72301	2.80256
B22: UMBILICAL	-0.42044	0.65676	0.30704	1.40479
B23: volumeTRans	-0.00024	0.99976	0.99844	1.00109

Estimated Logistic Regression Model(s)
Model For ANYIVH3or4=0
-6.59757101157484 -.557578570321346*ADSTAT -.413471824621217*ANT_CORT -
.476865669516561*ANYPRESS + 3.11184099279701E-02*APGAR5 -2.23218476828894E-03*BTHWT
+ .328304896270784*CPAP1to10 + 1.52786607187459*CPAP_10 -.435706547883509*DELIVERY +
.288183424041492*FLUSEASON + .329341416488868*GESTAGE + .165976975093633*HFVENT +
.444405325669039*LEUKO + .39522250897078*MECVENT + 1.12956073163118E-
02*PERCUTANEOUS -5.61127728731673E-02*SEX + 1.22498781973406*SITE2 -
.621496065785502*SITE3 -1.98727883797536E-02*snIIsc1 + .100557537980728*STEROID -
.689975224641839*SUPPO2 + .353102261925961*SURF -.420440806808599*UMBILICAL -
2.37846684379112E-04*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-195.50883	0.00000		
ADSTAT	1	-165.69835	0.15248	0.00390	0.84752
ANT_CORT	1	-165.66888	0.15263	0.00375	0.84737
ANYPRESS	1	-165.79721	0.15197	0.00441	0.84803
APGAR5	1	-165.00616	0.15602	0.00036	0.84398
BTHWT	1	-167.50787	0.14322	0.01316	0.85678
CPAP1to10	1	-165.31729	0.15443	0.00195	0.84557
CPAP_10	1	-171.96791	0.12041	0.03597	0.87959
DELIVERY	1	-165.90292	0.15143	0.00495	0.84857
FLUSEASON	1	-165.32851	0.15437	0.00201	0.84563
GESTAGE	1	-169.88305	0.13107	0.02531	0.86893
HFVENT	1	-164.97718	0.15617	0.00021	0.84383
LEUKO	1	-165.98102	0.15103	0.00535	0.84897
MECVENT	1	-165.27172	0.15466	0.00172	0.84534
PERCUTANEOUS	1	-164.93586	0.15638	0.00000	0.84362
SEX	1	-164.95263	0.15629	0.00009	0.84371
SITE2	1	-170.04075	0.13027	0.02611	0.86973
SITE3	1	-165.19786	0.15504	0.00134	0.84496
snIIsc1	1	-166.17922	0.15002	0.00636	0.84998
STEROID	1	-164.94901	0.15631	0.00007	0.84369
SUPPO2	1	-165.51208	0.15343	0.00295	0.84657
SURF	1	-165.45398	0.15373	0.00265	0.84627
UMBILICAL	1	-165.53620	0.15331	0.00307	0.84669
volumeTRans	1	-164.99243	0.15609	0.00029	0.84391
None(Model)	23	-164.93526	0.15638	0.00000	0.84362
None(Saturated)	459	0.00000	1.00000		0.00000

Multinomial Logistic Regression Report
Outcome: Any Major NICU Morbidity (Necrotizing Enterocolitis or Bronchopulmonary Dysplasia or Intraventricular Hemorrhage Grade III or IV or Retinoptahy of Prematurity)

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	CompORGAN	Rows Processed	515
Reference Value	1	Rows Used	438
Number of Values	2	Rows for Validation	0
Frequency Variable	None	Rows X's Missing	56
Numeric Ind. Variables	23	Rows Freq Miss. or 0	0
Categorical Ind. Variables	0	Rows Prediction Only	21
Final Log Likelihood	-177.55350	Unique Row Patterns	459
Model R-Squared	0.28388	Sum of Frequencies	438
Actual Convergence	2.010582E-08	Likelihood Iterations	7
Target Convergence	0.000001	Maximum Iterations	20
Model D.F.	24	Max Like Message	Normal
Completion			

Odds Ratios Section (Reference Value: CompORGAN = 1)

Parameter	Regression Coefficient (B or Beta)	Odds Ratio Exp(B)	Lower 95% Confidence Limit	Upper 95% Confidence Limit
B0: Intercept	-8.66512	0.00017	0.00000	0.01966
B1: ADSTAT	-0.96461	0.38113	0.14814	0.98056
B2: ANT_CORT	-0.12193	0.88521	0.46894	1.67100
B3: ANYPRESS	-0.29793	0.74236	0.38116	1.44583
B4: APGAR5	-0.04280	0.95810	0.81076	1.13222
B5: BTHWT	-0.00007	0.99993	0.99825	1.00161
B6: CPAP1to10	-2.03404	0.13081	0.05469	0.31285
B7: CPAP_10	-2.13148	0.11866	0.05262	0.26759
B8: DELIVERY	-0.76019	0.46758	0.25484	0.85790
B9: FLUSEASON	0.15752	1.17061	0.65591	2.08920
B10: GESTAGE	0.44938	1.56733	1.30156	1.88738
B11: HFVENT	-0.20439	0.81515	0.22689	2.92851
B12: LEUKO	1.17122	3.22592	1.79050	5.81209
B13: MECVENT	0.52437	1.68939	0.68911	4.14161
B14: PERCUTANEOUS	-0.44326	0.64194	0.34318	1.20077
B15: SEX	0.14969	1.16148	0.67777	1.99038
B16: SITE2	0.41580	1.51558	0.81394	2.82206
B17: SITE3	-5.47408	0.00419	0.00031	0.05716
B18: snIIsc1	-0.03170	0.96880	0.94435	0.99388
B19: STEROID	-0.50757	0.60196	0.13926	2.60197
B20: SUPPO2	-0.45111	0.63692	0.23197	1.74877
B21: SURF	-0.26908	0.76409	0.39695	1.47077
B22: UMBILICAL	0.04569	1.04675	0.54443	2.01254
B23: volumeTRans	-0.00765	0.99238	0.98782	0.99695

Estimated Logistic Regression Model(s)
Model For CompORGAN=0
-8.66511560861904 -.964611312411443*ADSTAT -.121929624706518*ANT_CORT -
.297926275246319*ANYPRESS -4.28025705777322E-02*APGAR5 -7.10157499341361E-05*BTHWT -
2.03403581983783*CPAP1to10 -2.13147629864095*CPAP_10 -.760188852014171*DELIVERY +
.157520751012435*FLUSEASON + .449375740137706*GESTAGE -.204388526466377*HFVENT +
1.17121850819809*LEUKO + .524366265504523*MECVENT -.443262406646313*PERCUTANEOUS
+ .149692816792416*SEX + .415796511773439*SITE2 -5.47407862084879*SITE3 -
3.16980942683255E-02*snIIsc1 -.507571829707132*STEROID -.451106099486794*SUPPO2 -
.269075558963729*SURF + 4.56907141653872E-02*UMBILICAL -7.65047941979592E-
03*volumeTRans

Note that each model gives XB, where Logit(Y) = XB. To calculate a probability, transform the logit using Prob(Y=0) = 1/(1+Exp(-XB)) or Prob(Y=1) = Exp(-XB)/(1+Exp(-XB)).

Log Likelihood & R-Squared Section

Term(s) Omitted	DF	Log Likelihood	R-Squared Of Remaining Term(s)	Reduction From Model R-Squared	Reduction From Saturated R-Squared
All	1	-247.93705	0.00000		
ADSTAT	1	-179.69831	0.27523	0.00865	0.72477
ANT_CORT	1	-177.62395	0.28359	0.00028	0.71641
ANYPRESS	1	-177.93848	0.28232	0.00155	0.71768
APGAR5	1	-177.67864	0.28337	0.00050	0.71663
BTHWT	1	-177.55694	0.28386	0.00001	0.71614
CPAP1to10	1	-189.30157	0.23649	0.04738	0.76351
CPAP_10	1	-192.41249	0.22395	0.05993	0.77605
DELIVERY	1	-180.66324	0.27133	0.01254	0.72867
FLUSEASON	1	-177.69513	0.28331	0.00057	0.71669
GESTAGE	1	-190.78322	0.23052	0.05336	0.76948
HFVENT	1	-177.60333	0.28368	0.00020	0.71632
LEUKO	1	-185.67321	0.25113	0.03275	0.74887
MECVENT	1	-178.22209	0.28118	0.00270	0.71882
PERCUTANEOUS	1	-178.53373	0.27992	0.00395	0.72008
SEX	1	-177.70191	0.28328	0.00060	0.71672
SITE2	1	-178.41526	0.28040	0.00348	0.71960
SITE3	1	-191.38007	0.22811	0.05577	0.77189
snIIsc1	1	-180.66281	0.27134	0.01254	0.72866
STEROID	1	-177.79870	0.28289	0.00099	0.71711
SUPPO2	1	-177.93081	0.28235	0.00152	0.71765
SURF	1	-177.87751	0.28257	0.00131	0.71743
UMBILICAL	1	-177.56290	0.28384	0.00004	0.71616
volumeTRans	1	-184.86447	0.25439	0.02949	0.74561
None(Model)	23	-177.55350	0.28388	0.00000	0.71612
None(Saturated)	459	0.00000	1.00000		0.00000

Multiple Regression Report
Outcome: NICU Length of Stay

Run Summary Section

Parameter	Value	Parameter	Value
Dependent Variable	NLOS	Rows Processed	515
Number Ind. Variables	23	Rows Filtered Out	0
Weight Variable	None	Rows with X's Missing	56
R2	0.3820	Rows with Weight Missing	0
Adj R2	0.3494	Rows with Y Missing	0
Coefficient of Variation	0.4777	Rows Used in Estimation	459
Mean Square Error	1235.568	Sum of Weights	459.000
Square Root of MSE	35.15064	Completion Status	Normal Completion
Ave Abs Pct Error	136.085		

Regression Equation Section

Independent Variable	Regression Coefficient b(i)	Standard Error Sb(i)	T-Value to test H0:B(i)=0	Prob Level	Reject H0 at 5%?	Power of Test at 5%
Intercept	112.1493	27.3211	4.105	0.0000	Yes	0.9837
ADSTAT	-7.9000	5.6972	-1.387	0.1663	No	0.2826
ANT_CORT	-0.3474	3.9822	-0.087	0.9305	No	0.0509
ANYPRESS	4.8928	4.1230	1.187	0.2360	No	0.2197
APGAR5	2.5427	1.0346	2.458	0.0144	Yes	0.6887
BTHWT	-0.0246	0.0105	-2.345	0.0195	Yes	0.6482
CPAP1to10	39.2852	4.8137	8.161	0.0000	Yes	1.0000
CPAP_10	46.8909	4.7030	9.970	0.0000	Yes	1.0000
DELIVERY	-3.9065	3.7016	-1.055	0.2918	No	0.1835
FLUSEASON	-3.4415	3.6512	-0.943	0.3464	No	0.1558
GESTAGE	-2.5079	1.0030	-2.501	0.0128	Yes	0.7037
HFVENT	7.2709	7.2086	1.009	0.3137	No	0.1717
LEUKO	-7.2201	3.5385	-2.040	0.0419	Yes	0.5303
MECVENT	0.3449	5.6653	0.061	0.9515	No	0.0504
PERCUTANEOUS	14.7094	3.7903	3.881	0.0001	Yes	0.9721
SEX	-4.3925	3.4224	-1.283	0.2000	No	0.2490
SITE2	-2.9450	4.1745	-0.705	0.4809	No	0.1084
SITE3	13.9524	9.8028	1.423	0.1554	No	0.2950
snIIsc1	0.1349	0.1529	0.882	0.3781	No	0.1424
STEROID	-9.9454	7.6692	-1.297	0.1954	No	0.2533
SUPPO2	-1.2445	6.6508	-0.187	0.8517	No	0.0540
SURF	-2.1132	4.0607	-0.520	0.6031	No	0.0814
UMBILICAL	-7.3505	4.1310	-1.779	0.0759	No	0.4269
volumeTRans	0.0659	0.0096	6.888	0.0000	Yes	1.0000

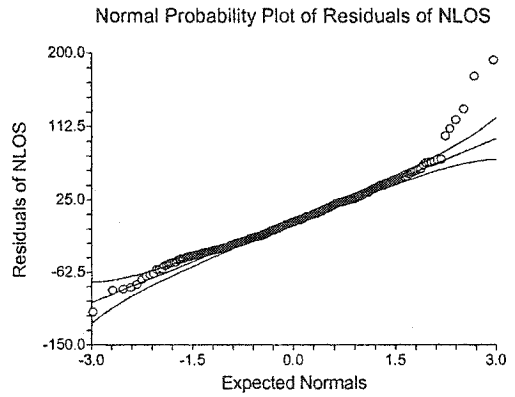
Multiple Regression Report

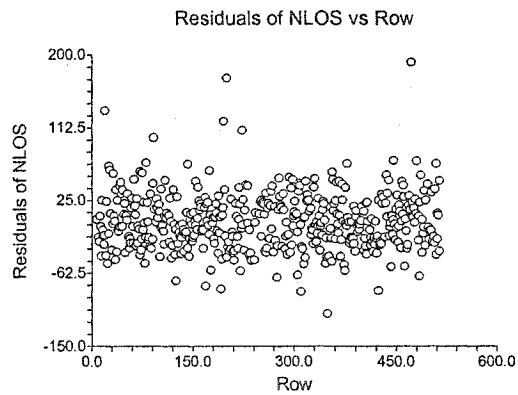
Page/Date/Time	2 10/9/02 2:07:53 PM
Database	C:\1MASTER\FINALMODEL\FINAL.S0
Dependent	NLOS

Analysis of Variance Section

Source	DF	R2	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (5%)
Intercept	1		2485001	2485001			
Model	23	0.3820	332287.9	14447.3	11.693	0.0000	1.0000
Error	435	0.6180	537471.9	1235.568			
Total(Adjusted)	458	1.0000	869759.9	1899.039			

Plots Section





Appendix VII: Imputed versus Non-imputed Regression Models

*(OR< 1 shows a beneficial effect for leukoreduction)

	Crude			Adjusted with Imputations**			Adjusted without Imputations		
	Odds Ratio	95% CI Lower	Upper	**Odds Ratio**	95% CI Lower	Upper	**Odds Ratio**	95% CI Lower	Upper
Primary Outcomes									
Bacteremia	0.82	0.56	1.20	0.59	0.34	1.01	0.65	0.37	1.12
Mortality	1.09	0.68	1.72	1.22	0.59	2.50	1.61	0.72	3.57
Major NICU Morbidities									
Retinopathy of Prematurity	0.50	0.34	0.72	0.56	0.33	0.93	0.69	0.41	1.18
Bronchopulmonary Dysplasia	0.48	0.34	0.68	0.42	0.25	0.70	0.44	0.26	0.76
Necrotizing Enterocolitis	0.50	0.27	0.93	0.39	0.17	0.90	0.55	0.25	1.20
IVH Grade III or IV	0.76	0.46	1.23	0.65	0.35	1.19	0.88	0.47	1.67
Any Major NICU Morbidity***	0.39	0.26	0.61	0.31	0.17	0.56	0.46	0.25	0.83
Lengths of Stay (in Days)	**Days**	Lower	Upper	**Days**	Lower	Upper	**Days**	Lower	Upper
NICU Length of Stay	-6.25	-13.90	1.40	-7.22	-14.16	-0.28	-7.06	-14.45	0.33

*All multivariate models included: Site, Admission Status (outborn/inborn), Gestational Age, Sex, Birthweight, Delivery, Antenatal Steroids, volume transfused, APGAR@ 5 minutes, Snap II on day 1, Any cardiovascular pressor on Day 1, CPAP Categorized (0days, 1-10 days, 11-25 days, >25 days), Fluseason (Dec-March), Mechanical Ventilation on Day 1, High Frequency ventilation on Day 1, Supplemental O2 support on Day 1, Surfactant Use on Day 1, Steroids on Day 1, Umbilical Catheter, Percutaneous Catheter

** imputations for Birthweight (3 patients) APGAR @ 5 minutes(14 patients) and Snap II on Day 1 (48 patients)

*** Either Retinopathy of Prematurity or Bronchopulmonary Dysplesia or Necrotizing Enterocolitis or IVH Grade III or IV

Appendix VIII : Non-transfused Comparison between Periods

	Non-Leukoreduced PERIOD (N=175)	Leukoreduced PERIOD (N=224)	Absolute Difference	Lower	Upper
Demographics					
Sex (Males) (%)	51.1	46.9	4.3	-5.8	14.4
Birthweight (grams)*	1008.3	964.8	-43.5	-87.6	0.6
Gestational Age (weeks)*	28.6	28.5	-0.1	-0.7	0.5
Antenatal Corticosteroids (%)	58.0	55.9	2.1	-7.9	12.1
Delivery (Vaginal) (%)	40.5	42.3	-1.8	-11.8	8.1
APGAR @ 1 minutes*	5.4	5.3	-0.1	-0.6	0.4
APGAR @ 5 minutes*	7.4	7.1	-0.3	-0.8	0.2
Admission Status (outborn) (%)	12.6	9.5	3.1	-3.1	9.4
Illness Severity					
SNAPII on Day 1*	11.6	8.9	-2.7	-5.1	-0.3
SNAPII on Day 3*	1.76	1.86	0.1	-0.9	1.1
SNAPIIPE on Day 1*	20.2	16.9	-3.3	-6.8	0.2
SNAPIIPE on Day 3*	9.85	9.26	-0.6	-3.0	1.8
Day 1 (1st 24 hrs after admission)					
Supplemental O2 (%)	79.0	72.4	6.7	-2.2	15.5
CPAP (%)	17.9	19.6	-1.7	-9.8	6.4
Mechanical Ventilation (%)	69.1	66.8	2.3	-7.4	12.0
Ventilation with relax (%)	3.7	2.5	1.2	-2.4	4.8
high frequency ventilation (%)	6.2	7.5	-1.4	-6.6	3.8
Surfactant (%)	48.1	47.7	0.4	-10.0	10.8
Peripheral IV (%)	59.9	69.3	-9.5	-19.4	0.4
Arterial Line (%)	63.6	47.7	15.8	5.7	26.0
Central Venous (%)	52.5	36.7	15.8	5.6	26.0
Any Antibiotic Agent (%)	100.0	100.0	0.0	0.0	0.0
IVIG (%)	0.0	0.0	0.0	0.0	0.0
# of Blood Draws	6.2	5.7	-0.5	-1.1	0.1
Platelet Transfusions (%)	0	0.1	-0.5	-1.5	0.5
WBC Transfusions (%)	0	0	0.0	0.0	0.0
Volume Exchange (%)	0	0.1	-0.5	-1.1	0.1
Any Cardiovascular Pressor (%)	21.0	17.1	3.9	-4.3	12.1
CPR (%)	8.0	6.0	2.0	-3.3	7.3
Any Postnatal Steroid (%)	1.9	4.5	-2.7	-6.2	0.9

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Authors:	Dean Fergusson, Paul Hebert, Stan Shapiro
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Authors:	Dean Fergusson, Paul Hebert, Shoo Lee, Robin Walker, Keith Barrington, Lawrence Joseph, Morris Blajchman, Stan Shapiro

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Harcourt

30 October 2002

Mr. Dean Fergusson
University of Ottawa Centre for Transfusion Research
Ottawa Health Research Institute, Dept. of Clinical Epidemiology

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Transfusion Medicine Reviews 2002; Vol. 16(4):1-8

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Sincerely,

Tara Routh

Tara

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Research Administrative Assistant
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