Effect of the transfusion of leukoreduced packed red blood cell units on the incidence rate of hospital-acquired infections in the pediatric critical care setting

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ABSTRACT

Hospital-acquired infections (HAI) are common, with an estimated 1.4 million patients worldwide being affected at any given time. HAIs cause a significant burden of disease, especially in critically ill patients. It is estimated that 42% of patients admitted to adult, pediatric, or neonatal intensive care units (ICU) are diagnosed with at least one HAI during their stay. One factor that may increase the risk of HAIs is the administration of red blood cells (RBC). It is hypothesized that the increased risk is a result of immunological changes that follow the transfusion of blood products known as transfusion-related immunomodulation (TRIM). Importantly, between 20 and 79% of ICU patients receive RBC transfusions during their stay.

The exact mechanisms surrounding TRIM remain unclear. Thus, the first study of this MSc thesis was a scoping review of published literature to provide an overview of the existing evidence on TRIM mechanisms. Our results show that TRIM mechanisms can be grouped into four categories: 1) effects related to the presence of allogeneic white blood cells (WBCs) in transfused blood, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic RBCs, and 4) hemolysis of RBCs. One of the mechanisms linked to the presence of allogeneic WBCs is the lack of expression of costimulatory molecules, which leads to T cell anergy and consequent immunosuppression. Another immunosuppressive mechanism related to WBCs is microchimerism due to direct allorecognition. This involves donor dendritic cells sharing an HLA-DR antigen with the recipient. Finally, the apoptosis of WBCs and hemolysis of RBCs contained in transfused blood lead to the release of bioactive substances, such as histamine and arginase, among others, that lead to immunosuppression. Notably, some of these mechanisms may be related to the length of storage time before the RBC units are transfused.

The most pronounced TRIM mechanisms involve the presence of allogeneic WBCs in transfused RBC units. Consequently, pre-storage leukoreduction of RBC units, which involves filtering them to remove WBCs, was instituted in many different countries to prevent TRIM. However, pre-storage leukoreduction does not remove all WBCs from RBC units, and the minimum number of allogeneic WBCs that must be present in RBC units to potentially trigger TRIM is currently unknown. Thus, an important research question that remains is if the transfusion of leukoreduced RBC units could also be associated with the development of TRIM and the consequent increase in risk for HAIs.

The second and third studies of this MSc thesis involves the secondary analysis of the "Transfusion Requirements in Pediatric Intensive Care Units" (TRIPICU) study, a randomized controlled trial of 637 critically ill children, with the aim of evaluating the association between transfusing leukoreduced RBCs and the incidence rate of HAIs. Multiple studies have evaluated the association of transfusing leukoreduced RBCs on the incidence of HAIs compared to the transfusion of non-leukoreduced RBCs. However, the individual effect of leukoreduced RBCs has not been well studied yet. Therefore, study 2 primarily aimed to evaluate the use of a restrictive packed leukoreduced RBC transfusion strategy, compared to a liberal leukoreduced RBC transfusion strategy, on the incidence rate of HAI in critically ill children. In doing so, we aimed to analyze if a restrictive transfusion strategy could mitigate the potentially harmful effect of transfusion of leukoreduced RBCs on the incidence rate of HAI. Furthermore, we aimed to evaluate the association between 1) leukoreduced RBC transfusion and HAI incidence rate, 2) the number of leukoreduced RBC transfusions and HAI incidence rate, and 3) the volume of leukoreduced RBC transfusions and HAI incidence rate (secondary aims). The incidence rate ratio (IRR) for the association of a restrictive transfusion strategy was found to be 0.91 (95% confidence interval [CI] 0.70, 1.19). The results of our quasi-Poisson multivariable regression models showed that the association of transfusion of leukoreduced RBCs (IRR 1.15; 95% CI 0.69, 1.91) and volume of leukoreduced RBC transfusions (IRR 1.73; 95% CI 0.94, 3.18) on HAI incidence rate were inconclusive. However, we observed a statistically significant association between the number of leukoreduced RBC transfusions and HAI incidence rate when critically ill children receive ≥3 blood transfusions (IRR 2.32; 95% CI 1.14, 4.71).

In study 3, we aimed to evaluate the association between the length of storage of transfused leukoreduced RBC units and the HAI incidence rate. The results of our quasi-Poisson multivariable model showed a statistically significant association between the transfusion of RBC units stored for ≥35 days and an increase in HAI incidence rate (IRR 3.66; 95% CI 1.22, 10.98).

In conclusion, this MSc thesis provides an overview and evaluation of TRIM mechanisms associated with the transfusion of RBC units. In addition, it shows that multiple leukoreduced RBC transfusions and the transfusion of leukoreduced RBC units stored for ≥35 days are associated with a statistically significant increase in HAI incidence rate. The results of this MSc thesis help to inform transfusion practices and future research in the pediatric critical

care setting as it demonstrates that reducing the number of transfusions of leukoreduced RBCs performed in patients and limiting the transfusion of very old leukoreduced blood are associated with a reduction in the incidence of HAI in this patient population.

RÉSUMÉ

Les infections nosocomiales (IAS) sont l'un des événements indésirables les plus fréquents dans le système de santé. On estime que plus de 1,4 million de patients dans le monde sont touchés par des IAS à un moment donné. Les IAS entraînent importante morbidité chez les patients gravement malades. On estime que 42% des patients admis aux unités de soins intensifs (USI) adultes, pédiatriques ou néonatals sont diagnostiqués avec au moins une IAS pendant leur séjour. L'administration de globules rouges (RBC) est l'un des facteurs qui peut augmenter le risque des IAS. Une hypothèse c'est que l'augmentation du risque des IAS associé aux transfusions sanguines serait le résultat des changements immunologiques qui suivent la transfusion de produits sanguins connus comme immunomodulation transfusionnelle (TRIM). Entre 20 et 79% des patients aux USI reçoivent des transfusions de globules rouges pendant leur séjour.

Les mécanismes entourant TRIM ne sont toujours pas clairs. Ainsi, la première étude de ce mémoire de maîtrise a été une étude de portée de la littérature publiée avec le but de présenter les données probantes sur les mécanismes de la TRIM. Nos résultats montrent que les mécanismes de la TRIM peuvent être regroupés en quatre catégories: 1) les effets liés à la présence de globules blancs allogéniques (GB) dans le sang transfusé, 2) l'apoptose des globules blancs allogéniques, 3) les effets liés aux globules rouges allogéniques, et 4) l'hémolyse des globules rouges. Un des mécanismes liés à la présence de globules blancs allogéniques est le manque d'expression des molécules costimulatrices, qui conduit à une anergie des lymphocytes T et à immunosuppression. Un autre mécanisme immunosuppresseur lié aux globules blancs est le microchimérisme dû à une allorecognition directe. Cela implique que les cellules dendritiques présentes dans le sang du donneur partageant un antigène HLA-DR avec le receveur. Enfin, l'apoptose des globules blancs et l'hémolyse des globules rouges contenus dans le sang transfusé libèrent des substances bioactives, telles que l'histamine et l'arginase, entre autres, qui conduisent à une immunosuppression. Notamment, certains de ces mécanismes sont liés à la durée d'entreposage des unités de globules rouges avant la transfusion.

Les mécanismes de la TRIM les plus démontrés impliquent la présence de globules blancs allogéniques dans les unités de globules rouges transfusés. Par conséquent, le processus de réduction des globules blancs présents dans les unités de globules rouges par filtrage a été

institué à nombreux pays avec le but de faire la prévention de la TRIM. Cependant, la réduction leucocytaire n'enlève pas tous les globules blancs présents dans les unités de globules rouges. En plus, le nombre minimum de globules blancs allogéniques qui doivent être présents dans les unités de globules rouges pour potentiellement causer la TRIM est inconnu. Ainsi, une importante question de recherche est si la transfusion d'unités de globules rouges déleucocytés pourrait également être associée au développement de la TRIM et à l'augmentation conséquente du risque d'IAS.

Les deuxième et troisième études de ce mémoire de maîtrise impliquent l'analyse secondaire de l'étude «Transfusion Requirements in Pediatric Intensive Care Units» (TRIPICU), un essai contrôlé randomisé portant sur 637 enfants gravement malades. Plusieurs études ont évalué l'effet des transfusions de globules rouges déleucocytés sur l'incidence des IAS par rapport aux transfusions des unités non- déleucocytées globules rouges. Cependant, l'effet individuel des transfusions de globules rouges déleucocytés n'a pas encore été bien étudié. Par conséquent, le but principal de l'étude 2 était d'évaluer l'existence d'une association entre une stratégie restrictive de transfusion de globules rouges déleucocytés, par rapport à une stratégie libérale de transfusion de globules rouges déleucocytés, et le taux d'incidence des IAS chez les enfants gravement malades. Avec cet étude, nous voulions analyser si une stratégie de transfusion restrictive pouvait atténuer l'effet délétère potentiel des transfusions de globules rouges déleucocytés sur le taux d'incidence des IAS. De plus, nous voulions évaluer des associations entre 1) la transfusion de globules rouges déleucocytés et le taux d'incidence des IAS, 2) le nombre de transfusions d'érythrocytes déleucocytés et le taux d'incidence des IAS, et 3) le volume de transfusion de globules rouges déleucocytés et le taux d'incidence des IAS (objectifs secondaires).

Le ratio du taux d'incidence (RTI) pour l'effet d'une stratégie transfusionnelle restrictive était de 0,91 (intervalle de confiance à 95% [IC] 0,70, 1,19). Les résultats de nos modèles de régression quasi-Poisson multivariée ont montré que l'effet de la transfusion de globules rouges déleucocytés (RTI 1,15; IC à 95% 0,69, 1,91) et du volume de transfusion de globules rouges déleucocytés (RTI 1,73; IC à 95% 0,94, 3,18) sur le taux d'incidence des IAS n'étaient pas concluants. Cependant, nous avons observé une association statistiquement significative entre le nombre de transfusions de globules rouges déleucocytés et le taux d'incidence des IAS lorsque

des enfants gravement malades reçoivent ≥3 transfusions sanguines (RTI 2,32; IC à 95% 1,14, 4,71).

Dans l'étude 3, nous avons évalué l'effet de la durée d'entreposage des unités transfusées de globules rouges déleucocytés sur le taux d'incidence des IAS. Les résultats de notre modèle quasi-Poisson multivarié ont montré une association statistiquement significative entre la transfusion d'unités de globules rouges déleucocytés entrposées pendant ≥35 jours et une augmentation du taux d'incidence des IAS (RTI 3,66; IC à 95% 1,22, 10,98).

En conclusion, ce mémoire de maîtrise donne un aperçu des mécanismes de la TRIM associés à la transfusion d'unités de globules rouges. De plus, il montre que la transfusion multiple de globules rouges déleucocytés et la transfusion d'unités de globules rouges déleucocytés entrposées pendant ≥ 35 jours sont associées à une augmentation statistiquement significative du taux d'incidence des IAS. Les résultats de ce mémoire de maîtrise contribuent à éclairer les pratiques transfusionnelles et les recherches futures dans le contexte des soins intensifs pédiatriques, car ils démontrent que la réduction du nombre de transfusions effectuées et la limitation de la transfusion de sang très ancien peuvent réduire l'incidence de l'IAS chez cette population de patients.

PREFACE

This thesis focuses on blood transfusions and their association with the risk of hospital-acquired infections (HAIs). An introduction to blood transfusions and their potential impact on HAIs is presented in Chapter 1. In addition, the rationale, hypotheses, and primary and secondary objectives for the three manuscripts included in this thesis are outlined. Subsequently, a detailed summary of the literature on the epidemiology of blood transfusions and HAIs is presented, as well as the HAI risk factors and the corresponding costs, in Chapter 2. The results of the first manuscript addressing immunological mechanisms associated with blood transfusions are reported in Chapter 3. The second manuscript is then presented in Chapter 4, evaluating the association between transfusing leukoreduced packed RBCs and the overall HAI incidence rate in critically ill children. The third manuscript is presented in Chapter 5, evaluating the association between length of storage time of leukoreduced RBC transfusions and the overall HAI incidence rate in critically ill children. Finally, a summary and concluding remarks are given in Chapter 6. I wrote all chapters of this thesis, which were then revised and edited by Dr. Patricia S. Fontela and Dr. Dean A. Fergusson.

This thesis has been prepared according to the guidelines for a manuscript-based thesis, and includes the following three manuscripts:

> Leah K. Flatman, Kim C. Noël, Genevieve Gore, Catherine Goudie, Philippe Bégin, Jacques Lacroix, Jesse Papenburg, Dean A. Fergusson, Patricia S. Fontela. Transfusion-related immunomodulation mechanisms: a scoping review. Submitted to *Transfusion Medicine Reviews.*
>
> Leah K. Flatman, Dean A. Fergusson, Jacques Lacroix, Thierry Ducruet, Jesse Papenburg, Patricia S. Fontela. Association between leukoreduced red blood cell transfusions and hospital-acquired infections in critically ill children: a secondary data analysis of the TRIPICU study. To be submitted to *Transfusion.*

Leah K. Flatman, Dean A. Fergusson, Jacques Lacroix, Thierry Ducruet, Jesse Papenburg, Patricia S. Fontela. Association between length of storage of transfused red blood cell units and hospital-acquired infections in critically ill children: a secondary data analysis of the TRIPICU study. To be submitted to *Transfusion Medicine*.

Details of co-authors' contributions to each manuscript are outlined on pages **xiv-xv.**

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CONTRIBUTION OF AUTHORS

Dr. Patricia S. Fontela and Dr. Dean A. Fergusson developed the original research questions for this thesis, in collaboration with Dr. Jacques Lacroix.

I conducted all data management, statistical analyses, and interpretation of results. In addition, I drafted all three manuscripts included in this thesis, which were reviewed and edited by Dr. Fontela and Dr. Fergusson, as well as other co-authors.

The contribution of co-authors to the manuscripts included in this thesis are as follows:

Transfusion-related immunomodulation mechanisms: a scoping review
Conception: Patricia S. Fontela; *Protocol development:* Leah K. Flatman, PS Fontela; *Search strategy:* LK Flatman, PS Fontela, Genevieve Gore; *Studies selection:* LK Flatman, Kim Chloé Noël; *Data collection:* LK Flatman, KC Noël; *Data analysis:* LK Flatman, PS Fontela; *Manuscript writing:* LK Flatman; *Critical review of results and manuscript:* PS Fontela, KC Noël, Dean A. Fergusson, Jacques Lacroix, Catherine Goudie, Jesse Papenburg, Philippe Bégin; *Expertise on hematology:* C Goudie; *Expertise in immunology:* P Bégin.

Association between red blood cell transfusions and hospital-acquired infections in critically ill children: a secondary data analysis of the TRIPICU study
Conception: PS Fontela, DA Fergusson, J Lacroix;
Original study: J Lacroix, Paul C. Hébert, James S. Hutchison, Heather A. Hume, Marisa Tucci, Thierry Ducruet, France Gauvin, Jean-Paul Collet, Baruch J. Toledano, Pierre Robillard, Ari Joffe, Dominique Biarent, Kathleen Meert, and Mark J. Peters, for the TRIPICU Investigators, the Canadian Critical Care Trials Group, and the Pediatric Acute Lung Injury and Sepsis Investigators Network
Protocol development: LK Flatman, PS Fontela, DA Fergusson, J Lacroix, T Ducruet, J Papenburg; *Data analysis:* LK Flatman; *Interpretation of results:* LK Flatman, PS Fontela, DA Fergusson, J Lacroix, T Ducruet, J Papenburg; *Manuscript writing:* LK Flatman; *Critical review of results and manuscript:* PS Fontela, DA Fergusson, J Lacroix, T Ducruet, J Papenburg.

Association between length of storage of transfused red blood cell units and hospital-acquired infections in critically ill children: a secondary data analysis of the TRIPICU study

Conception: PS Fontela, DA Fergusson, J Lacroix;

Original study: J Lacroix, PC Hébert, JS Hutchison, HA Hume, M Tucci, T Ducruet, F Gauvin, JP Collet, BJ Toledano, P Robillard, A Joffe, D Biarent, K Meert, and MJ Peters, for the TRIPICU Investigators, the Canadian Critical Care Trials Group, and the Pediatric Acute Lung Injury and Sepsis Investigators Network

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LIST OF ABBREVIATIONS

APC – antigen presenting cell

CAUTI – catheter-related urinary tract infection

CDC – Centers for Disease Control

CI – confidence interval

CLABSI – central line-associated bloodstream infection

CNISP – Canadian Nosocomial Infection Surveillance Program

DALY – disability-adjusted life year

DAMPs – damage-associated molecular patterns

ECDC – European Centre for Disease Prevention and Control

FasL – Fas-ligand

HAI – hospital-acquired infection

HBV – hepatitis B virus

HCV – hepatitis C virus

HIV – human immunodeficiency virus

HLA – human leukocyte antigen

ICU – intensive care unit

IFN-γ – interferon gamma

IL – interleukin

INSPQ – Institut national de santé publique du Québec

IQR – interquartile range

IR – incidence rate

IRR – incidence rate ratio

MHC – major histocompatibility complex

MODS – multiple organ dysfunction syndrome

MSc – Master of Science

NICU – neonatal intensive care unit

NHSN – National Healthcare Safety Network

NK – natural killer

OR – odds ratio

PELOD – pediatric logistic organ dysfunction

PGE2 – prostaglandin E2

PICU – pediatric intensive care unit

PRISM – pediatric risk of mortality

PRISMA-ScR – Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews

RR – risk ratio/relative risk

RBC – red blood cell

RCT – randomized controlled trial

sHLA-I – soluble HLA-I

sIL-2-R – soluble interleukin 2 receptor

SD – standard deviation

SIRS – systemic inflammatory response syndrome

SPIN – Surveillance provinciale des infections nosocomiales

SSI – surgical site infection

TACO – transfusion-associated circulatory overload

TGF-β – transforming growth factor-beta

TNFα – tumour necrosis factor alpha

TRALI – transfusion-related acute lung injury

TRIM – transfusion-related immunomodulation

TRIPICU – Transfusion Requirements in Pediatric Intensive Care Units

VAP – ventilator-associated pneumonia

WBC – white blood cell

WHO – World Health Organization

YLD – years of life lived with disabilities

YLL – years of life lost due to pre-mature mortality

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CHAPTER 1 – INTRODUCTION

Hospital-acquired infections (HAI) are a widespread adverse event, with more than 1.4 million patients affected by HAIs worldwide at any one time.[1] The prevalence of patients in acute care hospitals who acquire at least one HAI during their stay ranges between 2.9% to 10.0% depending on the country.[2,3] When explicitly focusing on intensive care units (ICUs), 43% of HAIs occurred in adult, pediatric, or neonatal ICUs.[2]

Numerous infection control measures have been implemented by healthcare institutions to control this problem.[4-7] However, this is not sufficient to eliminate HAIs, as only 10% to 70% of HAIs have been shown to be prevented through the implementation of such measures.[8] Thus, it is hypothesized that other important factors contribute to patients acquiring infections during their hospital stay. To further reduce the incidence rate of HAIs, it is crucial to better understand the biological mechanisms that contribute to increase the risk of patients having such infections. ***One important hypothesis is that red blood cell (RBC) transfusions lead to immunomodulation, increasing the risk for HAIs.***

The first objective of this Master of Science (MSc) thesis was to conduct a scoping review to synthesize the existing literature on transfusion-related immunomodulation mechanisms (TRIM). TRIM refers to immunological changes that follow blood products' transfusion.[9] Studies have proposed multiple TRIM mechanisms that can be associated with the increase of HAIs.[10-12] Early studies suggested measures to prevent TRIM, including autologous RBC transfusion (i.e., transfusion of blood from the same individual), or the removal of white blood cells (WBCs) from RBC units by a process called leukoreduction.[13] However, leukoreduction does not entirely remove WBCs present in RBC units.[14] Therefore, it is possible that the remaining WBCs present could lead to a downregulation of the immune system of the patient receiving the transfusion. ***We hypothesized that there is an association between the transfusion of leukoreduced RBCs and an increase in the overall HAI incidence rate.***

For the second and third objectives of this MSc thesis, we performed a secondary analysis of the "Transfusion Requirements in Pediatric Intensive Care Units" (TRIPICU) randomized controlled trial by Lacroix et al.[15] Our second objective was to determine the association between transfusing leukoreduced RBCs and HAI incidence rate. Specifically, we aimed to determine the association of a restrictive-transfusion strategy compared to a liberal-

transfusion strategy on HAI incidence rate. In addition, we also evaluated the association between receiving a leukoreduced RBC transfusion and HAI incidence rate, as well as the existence of associations between 1) the number of leukoreduced RBC transfusions and HAI incidence rate, and 2) the volume of leukoreduced RBC transfused and HAI incidence rate. Finally, the third objective of this MSc was to evaluate the association between length of storage of transfused leukoreduced RBC units and the HAI incidence rate.

CHAPTER 2 – LITERATURE REVIEW

2.1 Hospital-Acquired Infections (HAIs)

2.1.1 Types of HAIs

HAIs, also known as nosocomial infections, are among the most frequent adverse events in healthcare worldwide. They are defined as infections that occur on or after the third day of hospital admission and that were not present on hospital admission.[16] There are many different types of HAIs, and the most frequent type of HAI changes depending on the population and region. According to the World Health Organization (WHO), the most commonly reported HAIs were urinary tract infection and surgical site infection (SSI) in high-income and low-income countries, respectively.[17] SSI was found to be nine times higher in limited-resource countries than in high-income countries.[17]

The European Centre for Disease Prevention and Control (ECDC) reported in 2016 that there are six main types of HAIs in Europe: hospital-acquired pneumonia, urinary tract infection, SSI, *Clostridioides difficile* infection, neonatal sepsis, and primary bloodstream infection.[3] A few of the most frequently isolated microorganisms causing these infections in European acute care hospitals are *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* spp., and *Enterococcus* spp.[3] Focusing on ICUs exclusively, a survey across European acute care hospitals found that the most common types of HAIs in critical care units were respiratory and bloodstream.[18]

According to the Centers for Disease Control (CDC), the six main types of HAIs in the United States are central line-associated bloodstream infections (CLABSI), catheter-associated urinary tract infections (CAUTI), SSI, ventilator-associated events, including ventilator-associated pneumonia (VAP), *Clostridioides difficile* infection, and methicillin-resistant *Staphylococcus aureus* bacteremia.[19] The CDC's 2015 HAI Hospital Prevalence Survey reported that pneumonia, *Clostridioides difficile* infections, and SSI were the most common HAIs in United States acute care hospitals.[20] The CDC/National Healthcare Safety Network (NHSN) 2018 HAI progress report stated that there were 19,188 events of CLABSI, and of which, 7,194 events (37.5%) occurred in ICUs.[21] Similarly, they reported that there were 22,015 events of CAUTI, with 9,957 (45.2%) occurring in ICUs.[21] Finally, they reported that 96.8% of ventilator-associated events occurred in ICUs.[21]

The NHSN reported that between 2011 – 2014, there were 408,151 pathogens from 365,490 HAIs.[22] Of those reported pathogens, fifteen pathogen groups accounted for 87%, with the most common being *Escherichia coli* (15%), *Staphylococcus aureus* (12%), *Klebsiella* spp. (8%), and coagulase-negative staphylococci (8%).[22] In a subgroup analysis by NHSN on pediatric HAIs in 2011 – 2014, it was reported that there were 22,323 pathogens associated with 20,390 HAIs.[23] The most common pathogens accounting for 60% of HAIs reported were *Staphylococcus aureus* (17%), coagulase-negative staphylococci (17%), *Escherichia coli* (11%), *Klebsiella pneumoniae* and/or *oxytoca* (9%), and *Enterococcus faecalis* (8%).[23]

The Canadian Nosocomial Infection Surveillance Program (CNISP) stated that the most common type of HAI reported over the last three surveys (2002, 2009, 2017) were urinary tract infections (31.9%), pneumonia (23.4%), SSI (20.2%), bloodstream infection (15.2%), and *Clostridioides difficile* infection (9.3%).[24] In 2017, 35.6% of all HAIs were due to device-associated infections (VAP, CAUTI, SSI, and CLABSI), with CAUTI accounting for 37.1% of those device-associated infections.[24] The three most frequently reported HAIs in Canadian pediatric patients of all ages were bloodstream infections (30.7%), pneumonia (16.1%), and viral gastroenteritis (13.7%).[25] When separated into age groups, SSI was highest in adolescents (12 years to <18 years) with a prevalence of 2.9%, while in children, infants, and neonates, the highest was bloodstream infections ranging from 3.0-3.5%.[25]

Focusing on the Province of Quebec, Canada, the Institut national de santé publique du Québec (INSPQ; Quebec Institute of Public Health) and Surveillance provinciale des infections nosocomiales (SPIN; Provincial Nosocomial Infection Surveillance) committee reported that the overall incidence rate of bloodstream infections in hospitalized patients was 5.43 per 10,000 patient days (95% confidence interval [CI] 5.22, 5.64).[26] Compared to other units (incidence rate [IR] 4.81 per 10,000 patient days; 95% CI 4.61, 5.02), ICUs had a higher incidence rate of 14.05 per 10,000 patient days (95% CI 12.82, 15.41).[26] Specifically focusing on pediatric intensive care units (PICUs), the incidence rate of bloodstream infections was found to be 20.2 per 10,000 patient days (95% CI 13.85, 29.45).[26] HAIs are a global issue, and no matter the demographics, they cause significant clinical burden, as well as on the healthcare system and the society.

2.1.2 Prevalence of HAIs around the world

According to the WHO, more than 1.4 million patients worldwide are affected by HAIs at any one time.[1] In Europe, the ECDC estimated that, in 2018, the prevalence of patients in acute care hospitals who acquired at least one HAI during their stay was 6.5% (95% CI 5.4, 7.8%).[3] The prevalence of HAIs increased to 19.2% in ICUs.[3] Based on the prevalence in acute care hospitals in Europe, it is estimated that 3.7 patients per 100 admissions will have a HAI during their stay resulting in a total of 4.5 million HAIs per year, ranging from 2.6 to 7.6 million.[3]

In the United States, the prevalence of HAIs in acute care hospitals was 4.0% (95% CI 3.7, 4.4%) in 2011.[2] This translated into approximately 1 out of every 25 patients admitted to acute care hospitals in the United States acquiring at least one HAI during their stay.[2] Importantly, out of the 721,800 HAIs diagnosed in acute care hospitals in 2011, 300,000 of them (42%) occurred in adult, pediatric, or neonatal ICUs.[2] The prevalence of HAIs has decreased in the United States over time; in 2015, the CDC's HAI Hospital Prevalence Survey found that an estimated 687,200 HAIs occurred in United States' acute care hospitals, corresponding to 3.2% (95% CI 2.9, 3.5%) of hospitalized patients having one or more HAIs.[20]

In 2017, the CNISP reported that there was a decline in the prevalence of HAI in Canadian acute care hospitals compared to other survey years.[24] The prevalence of patients having at least one HAI was found to be 7.9% (95% CI 6.8, 9.0%).[24] However, in ICUs, the prevalence increased to 12.6% (95% CI 10.1, 15.7%).[24] Regarding children, two point prevalence surveys of HAIs focusing on pediatric patients were performed in Canada in 2002 and 2009.[25,27] In 2002, the overall prevalence of pediatric patients with HAIs was 8%, while in PICUs, the prevalence was 19%.[27] In a subgroup analysis based on age, it was found that neonates were 1.5 times more likely to acquire an HAI than the other age groups.[27] The prevalence of HAIs in Canadian pediatric patients has remained steady as it was reported to be 8.7% in 2009.[25]

As previously shown, the risk of acquiring HAIs is higher among critically ill patients, independent of the age group. ICUs patients are at a higher risk of developing a HAI due to being exposed to invasive procedures and equipment needed for their care.[28] Also, the patients in critical care units are more susceptible to HAIs than other patients in the hospital because of underlying conditions and concurrent disease processes leading to decreased immune defences.[28,29]

Critically ill patients have innate and adaptive immunological derangements that put them at a higher risk for HAI.[30] It has been shown that the magnitude of the physiologic insult dictates the patient's response to pathogens.[31] Additionally, the patient's specific immune response depends on their genetic characteristics and comorbidities, as well as on the pathogen's load and virulence.[31,32] Severe infections present in critically ill patients, such as sepsis, can result in pro-inflammation, which leads to excessive inflammation and decreases the patient's ability to respond to a new infection stimulus, or to the triggering of anti-inflammatory mechanisms, leading to immunosuppression. In both cases, patients become more susceptible to secondary infections.[32] In critically ill patients, neutrophil function is also reduced compared to healthy controls ($p<0.05$).[33] Furthermore, changes in neutrophil surface receptor expression were shown to be correlated with disease severity ($r^2=0.60$, $p<0.0001$).[33] Thus, this immunological derangement may lead to higher incidences of HAIs in critically ill patients.[34]

The WHO estimated that the ICU HAI incidence rate is 2 to 3 times higher in low- and middle-income countries (42.7 HAI/1000 patient-days; 95% CI 34.8, 50.5) compared to high-income countries (17.0 HAI/1000 patient-days; 95% CI 14.2, 19.8).[35] As a result of the increasing prevalence of HAIs worldwide, HAIs also have an important impact on the healthcare system.

2.1.3 Burden of HAIs across the world

It is estimated that 2,609,911 new HAIs occur every year in the European Union.[36] A metric used to quantify the burden of disease is disability-adjusted life year (DALY), which is the sum of years of life lived with disabilities (YLDs) and years of life lost due to premature mortality (YLLs).[36] Therefore, the number of new HAIs occurring every year in Europe is equivalent to 501 DALYs per 100,000 people in the general population.[36] The same population prevalence-based modelling study found that 91,130 deaths each year in the European Union were due to the HAIs, with 56% of those deaths attributable to pneumonia and bloodstream infections.[36] The worldwide crude excess mortality rate in adult patients is estimated to be 18.5%, 23.6%, and 29.3% for urinary catheter-related, catheter-related bloodstream infections, and VAP, respectively.[35] The WHO reported that HAIs cause 25 million extra-days of hospital stay, 37,000 attributable deaths, and cost approximately €13 – 24 billion annually in Europe (approximately CAN$20 – 37 billion).[1,35]

For patients who present a HAI during their hospital admission, there is an increased financial cost associated with it and a longer length of stay. A study performed by the CDC estimated that the direct medical costs of HAIs across hospitals in the United States ranged from US$28.4 to $33.8 billion per year.[37] When stratified by HAI type, the meta-analysis of Zimlichman et al. showed that CLABSI was the most costly HAI in the United States, at US$45,814 per patient, followed by VAP and SSIs costing US$40,144 and US$20,785 per patient, respectively.[38] The WHO reported that the increased length of stay related to an HAI in emerging economies ranged between 5 and 29.5 days.[35] In Brazil, Cavalcante et al. found that pediatric patients had an increased length of stay for those with HAIs than those without (14.1 days vs. 5.1 days; $p<0.001$).[39] DiGiovine et al. showed that the hospital length of stay for patients in the United States with a hospital-acquired bloodstream infection compared to those patients who were uninfected was 24.2 days and 20.3 days, respectively.[40] In contrast, the regular length of stay for ICU patients without HAIs was 5.7 days and increased to 13.2 days for ICU patients with a HAI.[40] Pittet et al. found similar results regarding the increased length of stay for American patients with bloodstream infections compared to those uninfected.[41]

The Canadian Patient Safety Institute estimated that each case of HAI costs between CAN$2,265 and CAN$22,400 in Canada because of treatments, prolonged lengths of stay, and long-term disabilities.[42] When analyzing CLABSI in Canadian ICU patients, the median excess length of hospital stay is 13.5 days and ICU stay is 2 days.[43] The average attributable cost due to ICU-acquired CLABSI was CAN$25,155.[43]

Specifically investigating critically ill pediatric patients, it was estimated that patients who acquired an infection during their stay in a Spanish PICU had an increased cost of €30,791.40 (approximately CAN$47,000) compared to patients who did not present HAIs.[44] HAIs utilize hospital resources and increase patient morbidity and mortality. Therefore, finding ways to prevent these infections can not only save hospital resources, but also reduce the burden on the healthcare system and on the individual patient.

2.1.4 Risk factors for HAIs

Numerous risk factors can increase a patient's risk of acquiring a HAI during their hospital stay. A study by Sydnor and Perl categorized risk factors for HAIs into three groups: 1) those associated with the host, 2) those associated with treatment strategies, and 3) those

associated with healthcare worker behaviours.[45] Another study by Vincent demonstrated that predisposing HAI factors could be grouped into four categories: 1) related to underlying health status, 2) related to the acute disease process, 3) related to invasive procedures, and 4) related to treatment.[29] The majority of HAIs are associated with the presence of invasive devices, e.g., central lines, urinary catheters, and mechanical ventilators, which disrupt normal host protection mechanisms, including the skin barrier or mucosal membranes.[46,47] Furthermore, some acute disease processes, such as sepsis, cause derangements of the immune system making patients more prone to HAIs.[32] Immunocompromised patients also present higher HAI risk due to increased rates of invasive procedures and contact with the healthcare system.[48] Other HAI risk factors include antimicrobial exposure, age, and comorbidities.[49] Deptula et al. found that the prevalence of HAIs in acute care hospitals in Poland was the greatest in children less than one year of age (odds ratio [OR] 2.997, 95% CI 2.148, 4.181), followed by children aged one to four years and adults aged >65 years.[50]

The risk factors for HAIs differ depending on patients' characteristics and their location in the hospital. Strassle et al. reported patients admitted to hospitals with inhalational injury were at higher risk for HAIs (hazard ratio [HR] 1.61; 95% CI 1.17, 2.22; $p < 0.0001$).[51] Additionally, they found that compared to those with < 5% of total body surface area (TBSA) burned, those with large burns also had an increased risk for HAIs (5-10% TBSA, HR 2.92, 95% CI 1.63, 5.23; 10-20% TBSA, HR 6.38, 95% CI 3.64, 11.17; > 20% TBSA, HR 10.33, 95% CI 5.74, 18.60).[51] Deptula et al. showed that the highest prevalence of HAIs was in patients in both adult (39.8%) and pediatric ICUs (30.8%).[50] Among pediatric patients, the risk factors that were shown to be associated with a higher incidence of HAIs were respiratory disease on admission (incidence rate ratio [IRR] 4.0; 95% CI 2.83, 5.72), presence of another disease associated with admission diagnosis (IRR 3.5; 95% CI 2.41, 5.02), nonsurgical clinical disease (IRR 5.9; 95% CI 3.92, 8.85), and PICU admission (IRR 3.5; 9.5% 1.91, 6.28).[39] In neonatal ICUs (NICUs), it was found that the main risk factors for overall HAIs are the use of mechanical ventilation ($p = 0.005$), longer duration of mechanical ventilation ($p < 0.001$) and of central venous catheter placement ($p < 0.001$), a longer length of NICU stay ($p = 0.004$), and a low maximum fraction of inspired oxygen ($p < 0.001$).[48]

A systematic review by the WHO showed that risk factors vary according to the country's facility and socioeconomic status.[35] In high-income countries, the most common risk factors for

HAIs include age, admission to ICUs, presence of an invasive medical device, undergoing surgery, or trauma.[35] These risk factors were also observed in middle- and low-income countries; however, the lack of basic hygiene and limited resource resulting from poverty was an additional risk in those countries.[35] Concerning age and economic status, newborns in emerging economies have a 3 to 20 times higher risk of developing an HAI than in high-income countries.[52] Further comparing emerging economies with high-income countries, out of every 100 hospitalized patients, 10 in emerging economies and 7 in high-income countries will acquire at least one HAI.[52]

2.1.5 When infection control measures are not enough: the need to understand biological mechanisms potentially associated with HAIs

As HAIs continue to be an important burden on healthcare systems worldwide, efforts to prevent them are priorities for multiple organizations, including the Government of Canada's Infection Prevention and Control, The Canadian Nosocomial Infection Control Program (CNISP), U.S. Department of Health and Human Services, and the WHO.[53-55] To date, various infection control measures have been shown to effectively reduce HAI incidence rates after their implementation, including self-study modules on prevention for respiratory therapists and ICU nurses, continuous quality improvement by regularly updating written protocols based on surveillance of HAI rates, implementation of unit-based safety programs along with daily goal sheets, in-services at staff meetings, and formal didactic lectures.[4-7]

However, the use of infection control measures is insufficient to eradicate HAIs. Studies have demonstrated that between 30% and 90% of HAIs still happen despite the implementation of infection control measures.[8] Other risk factors play a key role in whether a patient is more susceptible to acquiring a HAI. Some of these factors include pathogen virulence and host response, which is influenced by the patient's clinical condition and age.[56,57] ICU patient case-mix includes patients receiving invasive and sometimes prolonged care, something crucial for their survival, but that also augments the risk for HAI even if preventive measures are correctly followed. Regarding age, young children and elders are both at higher risk for HAI due to their immune system's immaturity and immunosenescence, respectively. Thus, to further prevent HAIs, a greater understanding and knowledge of the biological mechanisms which result in

patients having a higher risk of bacterial colonization leading to HAI is vital. A mechanism that could increase the risk for HAIs is blood transfusions.

2.2 Blood Transfusions

2.2.1 Blood transfusions through the years

Blood transfusions are medical procedures where blood donated by volunteers is administered to these in need. Transfusions are commonly used to replace the blood that has been lost during surgery or severe injury or disease.[58] Blood transfusions were first described in 1666 when Dr. Richard Lower of Oxford, England, conducted experiments and transferred blood between dogs.[59,60] In 1667, Dr. Jean-Baptiste Denis of Paris, France, transfused blood from a sheep into a 15-year-old boy who had lost a vast amount of blood due to bloodletting that had been performed to reduce his fever.[59,61] It was not until 1818 when the first person was transfused with human blood in London, England, using Dr. James Blundell's device known as a Gravitator.[59,62] This was the beginning of modern transfusion medicine.

2.2.2 Blood safety strategy: how donated blood is processed

After blood is donated, it goes through various stages before it can be transfused to patients. In 1951, the first cell separator was invented by Edwin Cohen; it separated whole blood into different cellular components.[59] Blood is composed of four parts: plasma, RBCs, WBCs, and platelets.[58] The three main blood parts that are transfused are RBCs, plasma, and platelets. Once the plasma is removed from whole blood, packed RBCs are left.[63] Transfusion of RBCs is mainly performed to increase oxygen to tissues and treat hemorrhagic events. In contrast, plasma transfusions are given to reverse anticoagulant effects, and platelet transfusions are common to prevent and/or also treat hemorrhage in patients with bleeding disorders.[63]

Depending on the country, there are variations in the use of whole blood for transfusions compared to other blood products. According to the WHO's Global Database on Blood Safety, whole blood transfusions are seldom performed in high-income countries. However, in lower-middle- and low-income countries, the percentage of whole blood transfused is estimated to be 24% and 85%, respectively.[64] In comparison, RBCs are transfused at a rate of 32.0 units per 1000 population in higher-income countries, while the rate of RBC transfusion is 12.5 units, 5.4

units, and 3.4 units per 1000 people in upper middle-, lower-middle-, and low-income countries, respectively.[64]

Before blood is transfused, it goes through multiple tests and processes, which includes testing for different infectious diseases. According to the WHO's 2016 Global status report on blood safety and availability, 176 out of 180 responding countries reported that they have a policy for screening all blood products for human immunodeficiency virus (HIV) and a policy for hepatitis B virus (HBV), while 174 countries have a policy for hepatitis C virus (HCV).[64]

Compared to high-income countries, emerging economies have a higher proportion of HIV, HBV, and HCV positive blood donations.[64] In Canada, the Canadian Blood Services reported that all blood donations that are found to be positive for infections are destroyed and are not transfused to patients.[65] As a result, the primary source of risk for infections occurs when a blood donor acquired an infection and donated blood before the time period when it was possible to detect the infection using laboratory tests.[65] It is estimated that the residual risk of infection in Canada is 1 in 12.9 million donations for HIV, 1 in 27.1 million donations for HCV, and 1 in 1.38 million donations for HBV.[65]

2.2.3 Blood transfusions: a common intervention in healthcare

The WHO's 2016 Global status report on blood safety and availability reported an estimated 112.5 million blood donations collected in 180 countries worldwide. According to the Canadian Blood Services' Surveillance Report, 801,281 blood donations were collected in Canada in 2019.[65] In the United States, the National Blood Collection and Utilization Survey reported that 12.2 million units were collected in 2017 and of those, 10.7 million blood units were transfused.[66]

The reasons for patients requiring blood transfusions vary around the world. In high-income countries, blood transfusions are mainly performed as supportive measures in cardiovascular and transplant surgery, for patients who present with significant trauma, and as therapy for those with cancer.[64] In comparison, in low- and middle-income countries, blood transfusions are commonly performed to treat severe childhood anemia and pregnancy-related complications such as postpartum hemorrhage.[64] Furthermore, demographics of those receiving transfusions of blood and blood products differ based on socioeconomic status. In low- and middle-income countries, the WHO reported that 67% of all blood transfusions are given to

children under five years of age. The second highest group is females between the ages of 15 and 45 years.[64] In high-income countries, 79% of blood transfusions are directed towards patients over 60 years old.[64]

Across the world, approximately 85 million RBC units are transfused per year.[67] This is equivalent to 2.7 units of blood transfused every second. As previously mentioned, RBC transfusions are given for various medical conditions, including very sick patients who require blood products to increase oxygen delivery to different body organs.[58] Thus, RBC transfusions are a standard supportive measure for critically ill patients. Up to 53% of adult ICU patients receive at least one RBC transfusion during their stay.[68-73] In pediatrics, Bateman et al. reported that 49% of children admitted to 30 North American PICUs received one or more RBC transfusions while they were in the PICU.[74] Importantly, among pediatric post-cardiac surgery patients, the proportion of RBC transfusion increased to 79% for patients whose surgeries required cardiopulmonary bypass and 55% for patients for whom cardiopulmonary bypass was not necessary.[75-77] Despite the clinical benefits of RBC transfusions and a very low rate of transmissible blood-borne infections due to strict screening strategies and policies implemented by worldwide blood service agencies mentioned above, studies have shown increased infection rates and complications after RBC transfusions.

2.3 Complications Associated with RBC Transfusions

2.3.1 The three T's of RBC transfusion complications: TACO, TRALI, and TRIM

RBC transfusions are associated with many different complications, such as transfusion-associated circulatory overload (TACO), transfusion-related acute lung injury (TRALI), and TRIM.[78] TACO occurs when blood is transfused at a pace that is faster than the transfusion recipient's circulatory system can handle.[63] This results in pulmonary edema leading to increased length of hospitalization or even death.[79] TACO is an acute complication, that occurs mainly in infants less than three years old or patients older than 60.[63,79] Roubinian et al. reported that the incidence of TACO in four participating tertiary care hospitals across the United States in 2016 was 1 case per 100 patients transfused.[80]

Another acute transfusion reaction is TRALI. TRALI is also clinically presented as acute pulmonary edema, but differs from TACO as it is not a result of circulatory overload.[81] In TRALI, bioactive substances present in the transfused blood activate patient's neutrophils.[82] The

activated neutrophils can lead to pulmonary leukostasis, capillary leak, endothelial damage, and pulmonary edema.[82] Multiple factors can increase inflammation, ultimately leading to significant lung injury.[82] Both TACO and TRALI present signs and symptoms within 6 hours after blood transfusion.[81]

TRIM refers to the changes in the immune system that follow the transfusion of blood products, including pro-inflammatory and immunosuppressive effects.[11] Multiple studies have proposed several TRIM mechanisms that could be associated with increased HAI incidence.[10-12] Initial studies showed that TRIM was associated with the presence of allogeneic WBCs in blood transfusions[11,12,83], the release of bioactive soluble factors that accumulate during RBC storage,[11,12] and the presence of human leukocyte antigen (HLA) peptides on donor and recipient cells.[10,12]

Thus, it was initially hypothesized that TRIM could be avoided through various measures, including 1) the transfusion of autologous blood (i.e., transfusion of blood collected from the same individual), 2) transfusing fresh blood instead of stored, or 3) removal of WBCs from RBC units through a process called leukoreduction.[13] As the leading TRIM theory was immunosuppression due to the presence of WBCs in RBC transfusions, many countries instituted the use of pre-storage leukoreduction. In Canada, universal pre-storage leukoreduction of RBC units was implemented in across Canada during the summer of 1999.[84]

During the leukoreduction process, whole blood is passed through a specialized filter that removes WBCs and is processed into various components, as previously mentioned.[85] This process removes the vast majority of the donor's WBCs from blood units, but not all of them.[85] In each leukoreduced unit, up to $5X10^6$ WBCs can be present.[14] Consequently, it is possible that the remaining immunologically active WBCs present in RBC units could lead to a TRIM-related downregulation of the transfusion recipient's immune system. A study by Frabetti et al. showed that the number of remaining WBCs in blood units was higher during the first 7 days post-donation and decreased by two-thirds between 7 and 14 days of storage.[86] Importantly, the number of WBCs required to be present in blood units to cause TRIM is still unknown.

Therefore, the thesis' first objective was to perform a scoping review of the current literature to map out the existing evidence on TRIM mechanisms (study 1).

2.3.2 Blood transfusions and the risk for HAIs

Studies show an association between RBC transfusions and increased HAI incidence rate.[10-12,87-94] A meta-analysis by Hill et al. reported an OR of 3.45 (95% CI 1.43, 15.15) for postoperative HAIs occurring after allogeneic blood transfusions.[94] This association was even stronger in the subgroup of trauma patients when comparing those who were transfused with those who were not (OR 5.26; 95% CI 5.03, 5.43). Similarly, a prospective cohort study by Claridge et al. showed that the HAI incidence proportion in transfused trauma patients was higher compared to the non-transfused trauma (33% and 7.6%, respectively; $p<0.0001$).[92] In a study evaluating perioperative blood transfusions after bariatric surgery, Higgins et al. observed that patients who received ≥1 unit of whole blood or RBCs had a 5.7-fold increased risk of developing a SSI (OR 5.7; 95% CI 4.5, 7.4).[93] In addition, several studies showed that RBC transfusions are independently associated with a higher incidence of bacterial infections in a dose-dependent manner, i.e., the higher the number of transfusions, the higher the risk for HAIs.[95-97] Chelemer et al. found that rate of HAI increased with the number of transfusions in adult coronary artery bypass surgery patients (no transfusion 4.8%, 1-2 units 15.2%, 3-5 units 22.1%, ≥ 6 units 29.0%; $p < 0.001$).[95] Similarly, Yu et al. discovered that each additional unit of leukoreduced RBC transfusion in cardiac surgery patients increased the incidence of pneumonia (1 unit 0.0% vs. 2 units 0.7% vs. 3 units 2.3%; $p < 0.013$) and sepsis (1 unit 0.0% vs. 2 units 0.3% vs. 3 units 2.3%; $p < 0.006$).[97] Finally, Everhart et al. showed increasing HAIs with increasing number of leukoreduced RBCs in patients undergoing shoulder arthroplasty (IRR 1.68 per unit of RBC; 95% CI 1.21, 2.35; $p = 0.002$).[96] One of the main challenges with all of the aforementioned studies is the control of potential confounders. Due to the studies' design, there may have been unmeasured confounders presented that were not accounted for in the analyses, which may alter the results of the studies.

Patients that receive repeated blood transfusions are generally sicker. Rajasekaran et al. showed that critically ill children who received leukoreduced RBC transfusions had significantly higher pediatric risk of mortality (PRISM) and Pediatric Logistic Organ Dysfunction (PELOD) scores, thus indicating a higher severity of illness.[98] As mentioned earlier, critically ill patients also have immunological derangements that put them at a higher risk for HAI.[30-34] However, a possible explanation for an increase in HAI is that blood transfusions are immunosuppressive. This may be explained by potential TRIM mechanisms modulating the immune system.

Two meta-analyses have evaluated the effect of leukoreduced RBC transfusions, compared to the transfusion of non-leukoreduced RBCs, on the incidence of HAIs.[99,100] Vamvakas reported an association between postoperative infection and post-storage leukoreduction (OR 2.25; 95% CI 1.12, 4.25).[101] However, this benefit may be due to selection bias as one of the studies transfused only 44% of randomized patients.[88] Another study included in this meta-analysis mentioned that RBC storage time varied between the two trial arms, but no statistical adjustment was performed for such difference.[91] A meta-analysis by Fergusson et al. showed that bedside leukoreduction had a protective effect against HAIs (risk ratio [RR] 0.38; 95% CI 0.26, 0.54).[99] Similarly to the meta-analysis performed by Vamvakas et al., one of the included RCTs was the aforementioned study that included a significant proportion of patients who were not transfused.[88] Furthermore, another study did not adjust for important confounders, including the use of immunosuppressive drugs and comorbidities.[102] Importantly, both meta-analyses faced the challenge of including studies that used different HAI definitions.

One current hypothesis is that the transfusion of leukoreduced RBCs would still lead to a downregulation of the transfusion recipient's immune system due to the presence of few but immunologically active WBCs, along with the potential immunosuppressive effects of the RBCs themselves. It is important to note that the aforementioned meta-analyses do not erase a possible association between the transfusion of leukoreduced RBC units and HAIs. As previously discussed, TRIM can potentially still happen after the use of leukoreduced blood.

Thus, the second objective was to evaluate the association between transfusing leukoreduced packed RBCs in stable, critically ill children and HAI. Specifically, we aimed to evaluate the association between 1) a leukoreduced RBC restrictive-transfusion strategy and HAI incidence rate, 2) transfusing leukoreduced RBC and HAI incidence rate, 3) the number of leukoreduced RBC transfusions and HAI incidence rate, and 4) the volume of leukoreduced RBC transfusions and HAI incidence rate. This was accomplished through a secondary analysis of the TRIPICU RCT.[15]

Another important hypothesis is that the length of storage of transfused leukoreduced RBCs could be associated with an increase in HAIs. The transfusion of RBC units stored for a prolonged time is potentially associated with the release of RBC bioactive substances that could lead to immunosuppression. On the other hand, transfusing fresh blood could lead to an increased risk for HAIs due to the presence of immunologically active donor WBCs that can

modulate our capacity to fight infections. Numerous studies have researched the association between the storage age of RBCs and HAIs in critically ill patients. The primary hypothesis was that fresh blood was superior. However, no individual RCT was able to prove that a specific length of storage for RBC units was superior regarding an increased risk of HAIs.[46,49,103-107]

Finally, this thesis' third objective was to evaluate the association between transfusing "fresh" and "stored" leukoreduced RBC units and HAI incidence rate in stable, critically ill pediatric patients. To do so, we performed a secondary analysis of a subset of 257 patients who received a single RBC transfusion during their participation in the TRIPICU RCT.[15]

CHAPTER 3 – IMMUNOLOGICAL MECHANISMS ASSOCIATED WITH BLOOD TRANSFUSIONS

3.1 Preamble

Numerous studies propose various TRIM mechanisms that may explain the association between blood transfusions and the increased risk for HAIs. However, despite the multiple mechanisms reported, evidence that confirms the clinical impact of these mechanisms is still controversial.

Therefore, the first manuscript of this thesis aimed to conduct a scoping review of the literature evaluating TRIM mechanisms to synthesize the existing evidence and provide a narrative on the various immunological mechanisms associated with blood transfusions.

3.2 Manuscript 1

Transfusion-Related Immunomodulation Mechanisms: A Scoping Review

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Authors have no conflicts of interest to report.

ABSTRACT

Introduction: Red blood cell (RBC) transfusions are a common life-saving treatment. However, studies suggest they can raise the risk of hospital-acquired infections (HAI). This scoping review aims to map out described RBC transfusion-related immunomodulation (TRIM) mechanisms that might increase HAI incidence.

Methods: We performed a systematic search of Ovid/MEDLINE, Ovid/EMBASE, and Cochrane/CENTRAL from their inception to June 13, 2019, for original clinical or laboratory-based studies that described RBC TRIM. Using PubMed's Similar articles feature on February 21, 2020, we identified and screened additional eligible articles related to the primary search. Two trained researchers screened the citations and independently collected data.

Results: Of the 8,179 articles screened, 82 studies met eligibility and were included (54 were laboratory-based, and 28 were clinical studies). Cytokine concentrations varied after blood transfusions due to the effect of transfusions on T cells. We grouped TRIM mechanisms into four categories: 1) effects related to the presence of allogeneic white blood cells (WBCs) in transfused RBC units, 2) release of bioactive substances due to WBC apoptosis, 3) effects of allogeneic RBCs on T helper and T regulatory cells, and 4) release of bioactive substances by hemolytic allogeneic RBCs. Various TRIM mechanisms were identified related to the length of storage time of RBC units that occur primarily in either fresh or stored blood. The transfusion of stored RBCs was associated with 1) the absence of the costimulatory signal required for T cell activation, with consequent T cell anergy, and 2) the release of bioactive soluble factors, including histamine, eosinophil cationic protein, and soluble Fas ligand. The transfusion of fresh RBCs was associated with microchimerism.

Conclusions: Several different TRIM mechanisms may result in an increased risk of developing HAIs. Importantly, some of these mechanisms appear to be linked to the length of storage of transfused RBC units. Understanding these mechanisms will inform future studies and the development of blood bank strategies to mitigate HAI risks in transfused patients to improve their clinical outcomes.

INTRODUCTION

Each year around 117 million blood donations are collected worldwide, and 85 million red blood cell (RBC) units are transfused.[1, 2] In the United States alone, approximately 11 million transfusions were given in 2017, equivalent to one unit of blood transfused every 4 seconds.[3] In 2019, the Canadian Blood Services reported 801,281 donations in Canada.[4]

Blood cells have many different functions, including transporting oxygen and nutrients, immunological functions, and coagulation to prevent excess bleeding.[5] In critically ill patients, the main reasons for transfusing RBCs include low hemoglobin levels and the need to increase oxygen delivery to better support dysfunctional organs.[6-8] Despite the positive aspects associated with RBC transfusions, there are also risks linked to them, such as transfusion-transmitted infectious diseases like hepatitis and transfusion-related adverse events like transfusion-associated circulatory overload (TACO).[9, 10] In addition, some studies suggest an increased risk for hospital-acquired infections (HAI) with RBC transfusion.[11-18]

Blood service agencies have implemented rigorous screening strategies worldwide to avoid the direct transmission of infectious diseases by contaminated RBC units.[19, 20] However, despite transmissible blood-borne infection rates being rare, studies still show a trend of increased rates of HAI after patients receive RBC transfusions. The mechanisms behind the increased observed risk are relatively unknown. One hypothesis is that RBC transfusions can lead to infections through transfusion-related immunomodulation (TRIM).[17, 21]

TRIM refers to the changes in the immune system that follow the transfusion of blood products, which includes pro-inflammatory and immunosuppressive effects.[22] Most known TRIM mechanisms are related to the presence of white blood cells (WBCs) in transfused RBC units.[21] This scoping review aimed to map out TRIM mechanisms that might increase the risk of HAI. A better knowledge of such mechanisms may help develop strategies to modulate the potential HAI risk associated with RBC transfusions.

MATERIALS AND METHODS

We developed the study protocol using the Joanna Briggs Institute approach to conducting scoping reviews.[23, 24] We performed the study in accordance with the Preferred Reporting

Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guidelines.[23, 25]

Information Sources and Search Strategy

We developed an electronic search strategy (Appendix A) in collaboration with a liaison Health Sciences librarian (GG). We searched Ovid/MEDLINE, Ovid/EMBASE, and Cochrane/CENTRAL databases for eligible studies published from their inception to June 13, 2019. Based on a selection of included studies from the primary search, we used PubMed's Similar articles feature on February 21, 2020, to find additional eligible articles related to the original citations' titles, abstracts, and MeSH terms. We screened the first 300 highest ranked records. As a second search strategy, we read TRIM review articles captured by our primary searches to identify seminal papers that described biological mechanisms that were later identified as TRIM mechanisms.

Study Selection

Two independent and trained reviewers (LKF and KCN) screened titles and abstracts (first screen) and full-text reports (second screen). Discrepancies were resolved through consensus or consultation with an arbitrator (PSF). Articles that passed the two stages of screening were charted for relevant data. We included published abstracts or full reports of original studies that were either clinical or laboratory-based and described potential biological mechanisms that could lead to an increased risk of HAI after RBC transfusion. Inclusion was limited to articles published in English. For clinical studies, we included papers that compared two groups of patients and described potential TRIM mechanisms. Comparisons included patients who received RBC transfusions vs. non-transfused patients or patient groups that received RBCs with differing storage lengths (fresh vs. stored blood). We described potential TRIM mechanisms associated with the age of blood, as studies suggest that varying lengths of RBC storage lead to HAIs.[26-32] For laboratory-based studies, we included studies that utilized animal models or blood cell models to test the hypothesis of TRIM mechanisms.

Data Collection Process

Two reviewers (LKF and KCN) extracted data from each included study. Disagreements among reviewers were resolved by consensus. Data extracted included first author, publication year and country, study design, type of model (clinical, animal, or cell), intervention group (fresh/stored, transfused/not transfused, leukoreduced/non-leukoreduced, other), outcomes, results, and if the author's proposed or studied a mechanism.

Analysis

Included studies were grouped based on their characteristics, including study design, country of origin, and publication year. Next, studies were analyzed together in broad categories based on the proposed mechanisms: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs and charted in respective tables. Finally, the proposed mechanisms were mapped in a figure along with information gathered on the expression of cytokines.

RESULTS

Study Selection

Our systematic search yielded 7,879 potentially relevant publications (Figure 1). After removing 1,678 duplicates, we excluded a further 5,951 publications during the title and abstract screening and examined the full text of the remaining 250 articles. A total of 47 articles met our inclusion criteria. Using the Similar Articles feature in PubMed, within the highest ranked 300 records (out of a total of 1,881), we identified 5 additional publications that met our inclusion criteria. A further 30 articles were identified by screening personal libraries and the reference list of relevant review articles. Overall, a total of 82 articles were included in the scoping review (Appendix Table B.1).[33-114]

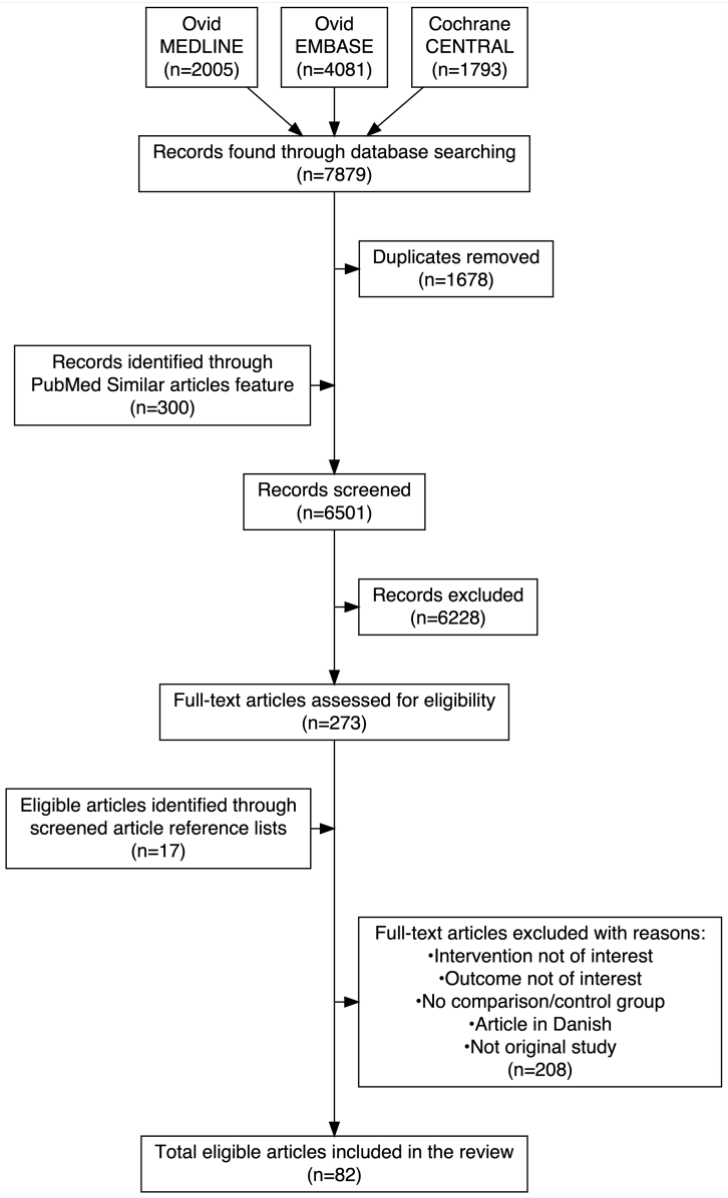

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of systematic literature search for studies related to TRIM.

Study Characteristics

Forty-one studies (50%) were conducted in North and South America (Table 1). Two-thirds of the studies (54 of 82) were laboratory-based, with cell models (38; 70%) being the most common model for hypothesis testing. Among clinical studies, cohort studies (22; 79%) were the

most common. The number of papers included from periods 1980-1989, 1990-1999, 2000-2009, and 2010-2019 were 5, 25, 20, and 32, respectively.

Table 1 Characteristics of articles on TRIM mechanisms after RBC transfusions

Characteristics	Number (%) of 82 articles
Type of article	
Laboratory-based	54 (66%)
Cell model	38
Murine model	14
Canine model	1
Porcine model	1
Clinical study	28 (34%)
Cohort study	22
Randomized controlled trial	6
Country of origin [a]	
North and South America	41 (50%)
Eastern Mediterranean	1 (1%)
Europe	33 (40%)
South-East Asia	1 (1%)
Western Pacific	6 (7%)
Year of publication	
1980 – 1989	5 (6%)
1990 – 1999	25 (30%)
2000 – 2009	20 (24%)
2010 – 2019	32 (39%)

[a] based on World Health Organization country groupings[115]
Percentages may not sum to 100 because of rounding.

Change in cytokines concentrations associated with blood transfusion

Thirty-three articles described the effect of blood transfusions on cytokine concentrations. Fifteen articles compared the concentration of cytokines between transfused and non-transfused patients (Table 2);[44, 50, 53, 56, 57, 66, 67, 71, 79, 92, 102, 107, 108, 110, 113] no overall trend in the difference of pro- and anti-inflammatory cytokine levels were observed, whichever the model (cell, animal, or human). The blood concentration of interleukin-6 (IL-6), which is a systemic pro-inflammatory cytokine, was higher in transfused patients; however, in

laboratory-based models, there was no difference in IL-6 levels between transfused and non-transfused animals, while the level of IL-6 was greater in cell models not exposed to blood.

Table 2 Comparison of cytokine concentrations between transfused and non-transfused groups

Model	Cytokine Concentration in Transfusion Recipients					
	Transfused > Non-Transfused		**No difference between groups**		**Non-Transfused > Transfused**	
	Pro-inflammatory	**Anti-inflammatory**	**Pro-inflammatory**	**Anti-inflammatory**	**Pro-inflammatory**	**Anti-inflammatory**
Cell			IL-2 [102] IFN-γ [102]	IL-10 [102]	IL-6 [102]	
Animal			IL-1 [44] IL-6 [44] IL-21 [44]	IL-10 [44]		
Human	sIL-2-R [113] IL-6 [53, 56, 67, 110, 113] IL-8 [53] IFN-γ [107]	IL-4* [57] IL-10 [53, 57, 110] TGF-β [57]	IL-1β [50, 71] IL-2 [50, 71] IL-6 [50, 71] IL-8 [50, 71] IL-12 [71] IL-17A [50] TNFα [50, 66, 71, 79] IFN-γ [50, 71, 108]	IL-4* [50, 71, 108] IL-5* [50, 71] IL-10 [50, 71, 79] TGF-β [50, 66]	IL-8 [92] IL-9 [107] TNFα [53] MIP-1α [107]	

Abbreviations: IFN-γ, interferon-gamma; IL, interleukin; MIP-α, macrophage inflammatory protein 1 alpha; sIL-2-R, soluble interleukin 2 receptor; TGF-β, transforming growth factor-beta; TNF-α, tumour necrosis factor-alpha. * Type 2 cytokines which modulate allergic/parasitic immune response and decrease type 1 response against bacteria.

The remaining 18 articles focused on the difference in cytokine levels between recipients of fresh blood compared to stored blood (Table 3).[35-39, 47, 48, 51, 60, 73, 81, 85-87, 91, 94, 103, 111] No major trend in the difference of cytokine levels was observed, independently of the model (cell, animal, or human) or use of pre-storage leukoreduction. However, when specifically looking at cell models, there is an increase in pro-inflammatory cytokines in both fresh and stored blood.

Table 3 Comparison of cytokine concentrations in transfusion recipients between fresh and stored blood, separated by pro-inflammatory and anti-inflammatory, model, and blood processing.

Model	Blood Processing	Cytokine Concentration in Transfusion Recipients					
		Fresh > Stored		No difference between groups		Stored > Fresh	
		Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory	Pro-inflammatory	Anti-inflammatory
Cell	Leukoreduced (pre-storage)	IL-1β [37] IL-2 [37] IL-6 [51] IL-8 [51] IL-17 [81] IFN-γ [37] TNF-α [37, 51, 81] MIP-1α [51]	IL-4* [37] IL-10 [37, 81]	IL-8 [35, 36, 48] IL-12 [51] MCP-1 [51] MIP-1β [51] TNF-α [39] IFN-α [51] IFN-γ [35, 81]	IL-10 [36] IL-13 [35] TGF-β [37]	IL-17 [35] MCP-1 [39]	
	Not pre-stored leukoreduced		IL-22 [35]	IL-8 [35, 36, 48] TNF-α [39, 111] IFN-γ [35] IL-6 [47, 94] IL-8 [47] MCP-1 [47]	IL-10 [36] IL-13 [35] TGF-β [94]	IL-1β [36] IL-6 [36] IL-8 [111] TNF-α [36] MCP-1[39]	TGF-β [35]
Animal	Leukoreduced (pre-storage)						
	Not pre-stored leukoreduced			IL-6 [38, 47] IL-8 [47] IFN-γ [38] TNF-α [38] MCP-1 [38]	IL-10 [38]	IL-6 [86, 103] MIP-1α [38] MIP-2 [38] MCP-1 [47]	IL-10 [85]
Human	Leukoreduced (pre-storage)			IL-1β [91, 103] IL-2 [86, 87, 91, 103] IL-6 [87, 91] IL-7 [91, 103] IL-8 [73, 91, 103] IL-12 [91, 103] IL-17 [91, 103] IL-21 [91, 103] IL-23 [91, 103] IFN-γ [86, 91] TNF-α [73, 86, 87, 91] MCP-1 [87] MIP-1α [91, 103] MIP-1β [91, 103]	IL-4* [86] IL-10 [86, 103]		
	Not pre-stored leukoreduced				TGF-β [60]		

Abbreviations: IFN-α, interferon-alpha; IFN-γ, interferon-gamma; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MIP-1α, macrophage inflammatory protein 1 alpha; MIP-1β, macrophage inflammatory protein 1 beta; MIP-2, macrophage inflammatory protein 2; TGF-β, transforming growth factor-beta; TNFα, tumor necrosis factor-alpha. * Type 2 cytokines which modulate allergic/parasitic immune response and decrease type 1 response against bacteria.

TRIM Mechanisms

The TRIM mechanisms proposed by the included articles were grouped into four major categories: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs (Figure 2).

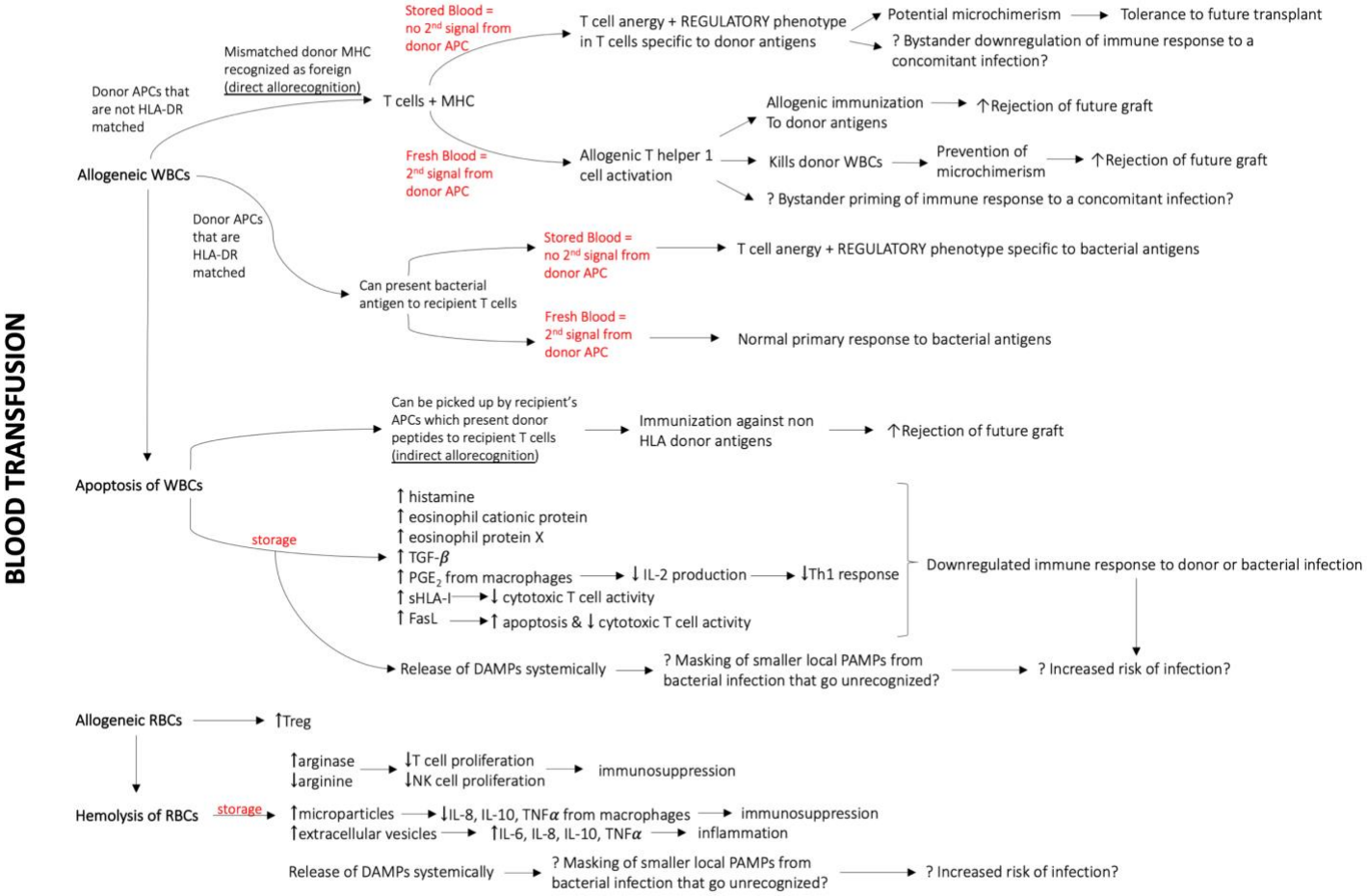

Figure 2 Described TRIM mechanisms separated by WBCs and RBCs

Abbreviations: APC, antigen-presenting cell; DAMPs, damage-associated molecular patterns; FasL, Fas ligand; HLA-DR, human leukocyte antigen – DR isotype; IFN-γ, interferon-gamma; IL, interleukin; MHC, major histocompatibility complex; NK, natural killer; PAMPS, pathogen-associated molecular patterns; PGE_2, prostaglandin E2; sHLA-1, soluble human leukocyte antigen 1; RBCs, red blood cells; TGF-β, transforming growth factor-beta; Th1, T helper 1; TNFα, tumour necrosis factor-alpha; Treg, T regulatory cells; TRIM, transfusion-related immunomodulation; WBCs, white blood cells.

TRIM Mechanisms Associated with the Presence of Allogeneic WBCs

1) Absence of a secondary costimulatory signal triggered by the donor's WBCs

Nine studies described a mechanism where the presence of donor WBCs leads to the absence of the secondary costimulatory signal necessary to release IL-2 and fully activate T cells (Table 4; see Appendix C for a description of the T cell activation process).[52, 61, 64, 69, 70, 74, 80, 83, 84] Jenkins and Schwartz were the first to show that the absence of this secondary costimulatory signal may cause T cells to enter an unresponsive state, including a decrease in IL-2 production.[52, 69, 83] They also discovered that the IL-2 receptor is not inhibited during the unresponsive state, as T cells were able to respond to exogenous IL-2.[69] Therefore, the T cell's state of unresponsiveness results in a decrease of IL-2 production but does not affect the IL-2 receptor function. Further analyzing the mechanism behind T cell unresponsiveness, Mueller et al. showed that the binding of an antigen to the T cell receptor increases intracellular calcium and protein kinase C activation. However, without the costimulatory signal, these two secondary messengers are insufficient to induce T cell proliferation, causing an unresponsiveness state.[84]

Mincheff et al. described a mechanism where the presence of donor WBCs leads to the absence of the secondary costimulatory signal.[83] In this study, donor antigen-presenting cells (APCs) lost the ability to induce either alloreactive T cell response or T cell response to a new exogenous antigen by day 13 of storage.[83] However, T cell proliferation could be rescued by the exogenous addition of IL-2, which indicated T cell anergy. The authors hypothesized that such inability to activate T cells could be explained by the loss of the costimulatory signal on donor APCs from stored blood units.[83] This would lead to tolerance against the antigen presented.[83] Therefore, when patients are transfused with stored blood, and the secondary signal is absent, T cells would not proliferate or release IL-2. This jeopardizes patients' ability to trigger an inflammatory reaction, making them more susceptible to bacterial infections.

2) Microchimerism and HLA antigens

Eight studies described a mechanism involving the sharing, or sometimes mismatch, of HLA antigens between the blood donor and transfusion recipient with the potential to lead to microchimerism (Table 4; see Appendix C for a definition of microchimerism).[55, 75, 76, 78, 82, 97, 99, 109] Studies have demonstrated that microchimerism is 1) caused by donor WBCs in the recipient and 2) not dependent on the number of WBCs present.[55, 78] Lagaaij et al.

observed an increase in ex vivo measurements of cell-mediated cytotoxicity against donor lymphocytes when the donor WBCs and the recipient did not share an HLA-DR antigen ("mismatch"), which peaked 2 weeks after transfusion.[76]

Presentation of donor antigens can occur via two different pathways: 1) direct allorecognition and 2) indirect allorecognition.[116] Direct allorecognition occurs when donor APCs present antigens directly to the recipient's T helper cells.[116] Indirect allorecognition happens when the recipient's APCs process and present an antigen from the recipient's cells to the T helper cells.[116]

Lagaaij et al. observed that the chance of sensitization against donor cells decreased when blood donors and transfusion recipients shared HLA-DR antigens, hypothesizing that direct allorecognition of a matched HLA-DR leads to the downregulation of the transfusion recipient's T cells.[75] Contrary to what Lagaaij et al. reported, Middleton et al. found no difference in sensitization between those patients awaiting a renal transplant and who received one HLA-DR antigen-matched blood transfusion compared to those who received random blood.[82] However, they observed a reduction in the incidence of renal transplant rejection episodes for those patients receiving matched blood.[82] Additionally, van Twuyver et al. found that when the blood donor and recipient share one HLA haplotype, there is an absence of T cell response against donor alloantigens.[109]

Regarding the indirect allorecognition pathway, Roelen et al. demonstrated that in vitro-generated regulatory T cells can recognize synthetic peptides that are presented in self-HLA class II molecules by autologous T cells or dendritic cells.[104] The regulatory T cells downregulate the response of these autologous T cells by killing them.[99]

TRIM mechanisms involving direct allorecognition may only be relevant for the transfusion of fresh blood since donor dendritic cells are only functional for 7 days after blood is donated.[117] Reed et al. further showed that the freshness of stored blood influenced the survival and function of WBCs, as microchimerism could not be detected in patients who received blood stored for more than 12 days.[97] The clinical consequences of microchimerism for transfused patients is unknown, but it could include graft-vs.-host or auto-immune effects. Importantly, when the donor and recipient are mismatched for HLA-DR antigens, alloreactive T lymphocyte activity increases, which may have immunomodulatory effects.

3) Release of bioactive substances during WBC apoptosis

Sixteen studies described mechanisms involving the release of bioactive substances from transfused WBCs when they undergo apoptosis (Table 4).[33, 43, 46, 57-60, 63, 65, 68, 85, 88-90, 95, 100] The release of eosinophil cationic protein and eosinophil protein X was observed to inhibit lymphocyte proliferation through the formation of enhanced suppressor activity leading to immunosuppression.[89, 95]

Furthermore, Bury et al. showed that released histamine inhibits T cells proliferation and human neutrophil chemotaxis via H2 receptors.[46] Studies showed that pre-storage leukoreduction reduces the amount of histamine present in stored RBC units.[88, 90]

Three studies reported that RBC transfusions increase prostaglandin E2 (PGE2) levels.[43, 57, 100] They showed that PGE2 inhibits IL-2 and interferon-gamma (IFN-γ) production from T helper 1 cells but does not inhibit T helper 2 cells from producing IL-4 and IL-5, thus causing further suppression of Th1 response.[43, 57, 100] It has also been shown that PGE2 is generated during the storage of blood and that there is a positive correlation between the number of WBCs and PGE2 levels.[68, 100]

Soluble Fas ligand (FasL) is a transmembrane protein that induces apoptosis when cells bind to its cell-surface receptor Fas.[33, 63, 65] Soluble HLA class I (sHLA-I) has been shown to induce FasL expression, specifically in cytotoxic T cells.[33, 58, 59, 63, 65] The upregulation in the expression of Fas and FasL on T cells is due to the increase in sHLA-I.[65] Studies demonstrated that the concentrations of soluble FasL and sHLA-I molecules were higher in RBC units containing residual WBCs, which is expected with fresher RBC units.[59, 65] It was further shown that pre-storage leukoreduction prevented the accumulation of these bioactive substances from increasing in a storage-dependent manner.[65] This decrease in bioactive substances would prevent changes in the cytotoxic T cells membrane during storage.[58-60, 65] Several studies showed that the development of immune tolerance depends on the apoptosis of cytotoxic T cells.[33, 58, 59, 63, 65] Ghio et al. found that sHLA-I, sFasL, and transforming growth factor-beta (TGF-β) are involved in the downregulation of NK cell-mediated cytolysis.[60] As a result, in the downregulation of cytotoxic T cell activity due to the release of these bioactive substances, patients would be limited in their ability to develop an immune response against bacterial infections.

Table 4 Studies that validated TRIM mechanisms associated with WBCs

Mechanism	Studies that validated or mentioned mechanism
Secondary signal: expression of costimulatory molecules on donor's WBCs	
Transfusion of blood independent of the length of storage	DeSilva et al. (1991) [52] Gimmi et al. (1991) [61] Harlan et al. (1994) [64] Jenkins and Schwartz (1987) [69] Jenkins et al. (1991) [70] Koulova et al. (1991) [74] Linsley et al. (1991) [80] Mueller et al. (1989) [84]
Transfusion of stored blood	Mincheff et al. (1993) [83]
Microchimerism and HLA Antigens	
Transfusion of blood independent of the length of storage	Flesland et al. (2004) [55] Lagaaij et al. (1989) [75] Lagaaij et al. (1991) [76] Lapierre et al. (2007) [78] Middleton et al. (1994) [82] Roelen et al. (2002) [99] van Twuyver et al. (1991) [109]
Transfusion of fresh blood	Reed et al. (2007) [97]
Release of Bioactive Substances	
Transfusion of blood independent of the length of storage	Arase et al. (1995) [33] Betz and Fox (1991) [43] Bury et al. (1992) [46] Gafter et al. (1996) [57] Ghio et al. (2001) [59] Griffith et al. (1996) [63] Hashimoto et al. (2004) [65] Peterson et al. (1986) [95] Zavazava and Kronke (1996) [112]
Transfusion of stored blood	Ghio et al. (1999) [58] Ghio et al. (2001) [59] Ghio et al. (2011) [60] Jacobi et al. (2000) [68] Mukherjee et al. (2014) [85] Nielsen et al. (1996) [88] Nielsen et al. (1996) [89] Nielsen et al. (1997) [90] Ross et al. (1990) [100]

TRIM Mechanisms Associated with Allogeneic Red Blood Cells:

1) Effects of RBC transfusions on T regulatory cells

Three studies described a mechanism where the generation of T regulatory cells was induced by RBC transfusion (Table 5).[37, 54, 114] Baumgartner et al. showed that RBC supernatant induces T regulatory cell phenotype and that this effect was unaltered by pre-storage leukoreduction or prolonged storage.[37] Alternatively, Efron et al. found that T regulatory cells' generation depends on the age of transfused allogeneic RBCs.[54] T regulatory cells induced by RBC supernatant were found to suppress the proliferation of effector T cells, including cytotoxic cells, leading to immunosuppression.[37] Zou et al. discovered that patients who received a transfusion had a lower number of NK cells than those who did not receive a transfusion and hypothesized this could be due to regulatory T cells' effects.[114] In the event of a bacterial infection, the T helper 1 response could be downregulated by T regulatory cells' inhibitor activity. Therefore, other immune cells, including NK cells, would not be activated, and cytokine production would be affected, increasing the patient's susceptibility to bacterial infections.

2) Release of bioactive substances during RBC hemolysis

Sixteen studies described a mechanism involving the release of bioactive substances from hemolytic RBCs (Table 5).[34, 40-42, 45, 49, 62, 72, 77, 93, 96, 98, 101, 104-106] The release of the enzyme arginase I from RBCs depletes L-arginine, leading to T cells having reduced expression of the T cell receptor zeta chain (CD3 zeta). This results in decreased T cell proliferation.[40-42, 72, 96, 98] Additional studies showed that arginase I also impairs NK cells' function and proliferation and IFN-γ secretion.[77, 93] The secondary decrease in T cell and NK cell proliferation could impair the immune response in the event of a bacterial infection.

In addition, several studies showed an increase in bioactive RBC-derived microparticle and microvesicle formation as storage time increases.[45, 49, 62, 101, 104-106] Their membranes consist primarily of lipids, which increase after 2 to 3 weeks of storage.[118] Such microparticles have been shown to inhibit IL-8, IL-10, and tumour necrosis factor-alpha (TNFα) being released from macrophages.[101] Importantly, it was observed that there was a higher concentration of RBC extracellular vesicles during the storage of RBC units.[104] Such vesicles modify T cell responses and have dose-dependent pro-inflammatory capabilities by increasing IL-6, IL-8, IL-10, and TNFα.[49, 104-106] Barkkour et al. found that damage-associated

molecular patterns (DAMPs) were also associated with stored blood.[34] They showed that the concentration of mitochondrial DNA accumulated early during RBC storage, within 7 to 14 days.[34] DAMPs are associated with inflammation as they are known immune mediators.[119] Based on these studies, RBC-derived microparticles, extracellular vesicles, and DAMPs can result in immunosuppression or increased inflammation, thus altering the response to bacterial pathogens depending on the bioactive substance released.

Table 5 Studies that validated TRIM mechanisms associated with RBCs

Mechanism	Studies that validated or mentioned mechanism
Effects due to T Regulator Cells	
Transfusion of blood independent of the length of storage	Baumgartner et al. (2009) [37] Efron et al. (2010) [54] Zou et al. (2016) [114]
Release of Bioactive Substances	
Transfusion of blood independent of the length of storage	Bernard et al. (2007) [41] Bernard et al. (2010) [42] Kim et al. (2002) [72] Lamas et al. (2012) [77] Oberlies et al. (2009) [93] Rodriguez et al. (2002) [98]
Transfusion of stored blood	Bakkour et al. (2016) [34] Bernard et al. (2008) [40] Bosman et al. (2008) [45] Danesh et al. (2014) [49] Greenwalt et al. (1980) [62] Prins et al. (2001) [96] Sadallah et al. (2008) [101] Straat et al. (2014) [105] Straat et al. (2015) [104] Straat et al. (2015) [106]

DISCUSSION

This scoping review identified and described several RBC TRIM mechanisms. We classified TRIM mechanisms into four groups: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs. In addition, some of these mechanisms

seem to be related to the length of storage of RBC units, with the transfusion of both fresh and stored blood being able to lead to immunosuppression.

Traditionally, TRIM has been studied in the context of immunization against organ donor antigens and how it affects future graft transplantation. TRIM has not been studied explicitly in the context of the immediate immune response to inflammation, although it has been hypothesized to contribute in a number of papers.[17, 21]

The most well-known TRIM mechanisms are associated with the presence of WBCs in the transfused blood and their apoptosis. It has been hypothesized that these TRIM mechanisms could be partly prevented by pre-storage leukoreduction and transfusing fresh instead of stored blood.[120] However, leukoreduction does not entirely remove all WBCs. In each leukoreduced RBC unit, up to $5X10^6$ WBCs can still be present.[121] These remaining WBCs are immunologically active in leukoreduced RBC units during the first 10 days of storage.[122, 123] Furthermore, the exact number of WBCs needed to trigger a TRIM mechanism is unknown. Thus, the small number of WBCs remaining in leukoreduced blood could still be enough to trigger TRIM.

RBC-related TRIM mechanisms could also be significant, but they were less frequently studied. Such mechanisms may potentially explain why studies have demonstrated an association between the transfusion of leukoreduced RBCs and HAIs.[11-18] A double-blinded, randomized controlled trial (RCT) by Titlestad et al. comparing infection rates in leukocyte-depleted RBC transfusions and non-leukocyte-depleted RBCs found that there was no significant difference between the two transfusion groups (leukoreduced vs. non-leukoreduced) in terms of rates of infection.[14] The infection rates were 38% in the leukoreduced RBC group and 45% in the non-leukoreduced RBC group ($P = 0.5250$).[14] However, this study may have been underpowered as only 45% of those randomized to receive a transfusion were actually transfused leading to a sample size of only 112 participants. Despite the evidence behind RBC mechanisms presented in this review, we still need to understand better how the presence of donor RBCs can lead to the development of T regulatory cells and why this does not happen to all patients who receive a transfusion. Additionally, we need to clarify the clinical relevance of these RBC-induced T regulatory T cells.

Some of the mechanisms described in this scoping review, specifically the apoptosis of WBCs, the expression of costimulatory signals, and the hemolysis of RBCs, may be linked to

blood transfusions' product storage time. As storage time increases, the concentration of bioactive substances related to these mechanisms also increases. This was the rationale for hypothesizing that the transfusion of fresh blood would lead to better clinical outcomes. Numerous RCTs have evaluated the effect of length of storage of RBCs on HAIs in adult, pediatric and neonatal critical patients.[26-32] However, no individual RCT proved that fresh blood was superior to stored blood with respect to patient outcomes.

Furthermore, some of the aforementioned RCTs showed a trend towards higher HAI incidence when patients received fresh blood. Fergusson et al. showed that the relative risk (RR) of HAIs in neonatal intensive care unit (ICU) patients receiving RBC transfusions was 2% higher; however, this was not statistically significant (RR 1.02, 95% confidence interval [CI] 0.88, 1.19).[27] Similarly, Spinella et al. identified a HAI relative risk of 1.1 (95% CI 0.6, 1.8) for pediatric ICU patients receiving fresh RBC transfusions.[31] Lacroix et al. reported a higher incidence rate of nosocomial infections in critically ill adults who received fresh vs. older RBC units (34.1% vs. 31.3%; absolute risk reduction in the standard delivery group = 2.8, 95%CI -0.9, 6.5).[28] Finally, Schrieber et al. demonstrated that 30% of adult trauma patients transfused with fresh leukoreduced packed RBCs had an infection.[29] In comparison, only 26% of patients receiving old blood (P = 0.77), but this result did not reach statistical significance.[29]

Such results may be partially explained by the fact that microchimerism, due to direct allorecognition, is linked to the presence of donor's dendritic cells in fresh blood. A study by Markowicz and Engleman showed that dendritic cells are only viable for up to one week after blood is donated, which may explain why Reed et al. found that microchimerism was associated with fresh blood.[97, 117] Thus, based on the data presented in our scoping review, it is plausible that the transfusion of both fresh blood and stored blood may lead to immunosuppression by different mechanisms.

Knowing the effects of length of storage time on T cells, and subsequently, cytokines is important for understanding how blood transfusions can lead to immunosuppression or inflammation, ultimately leading to an increased risk for developing a HAI. Understanding these mechanisms will inform future studies in the transfusion community resulting in the development of blood bank strategies to mitigate HAI risks in transfused patients to improve their clinical outcomes. Additionally, as most of these studies were performed in laboratory-based models instead of in vivo, even if we demonstrate relative differences in cytokine and

bioactive substances concentrations in units of blood stored for different lengths of time, it would be more relevant to study the absolute concentrations of these substances. This would allow us to determine whether the change in concentrations is significant, considering the volume transfused and the distribution volume in the patient.

Regarding post-transfusion cytokine expression, we found no real pattern when comparing transfused to non-transfused groups, neither when comparing fresh to stored blood transfusions. This may be since RBC transfusions can trigger both pro-inflammatory and anti-inflammatory reactions. In addition, the lack of a pattern might be because we have included clinical studies and laboratory experiments of varied quality in this review, which poses a challenge for the comparison of study results. Finally, most clinical studies included sick patients in their study population, most of whom have diseases caused by different conditions and are in varying stages of their disease evolution. Since these patients were previously ill before receiving a blood transfusion, their comorbidities may have primed their immune system; therefore, when they received a blood transfusion, this acted as a second insult activating an inflammatory response. Depending on the patient, this insult could exacerbate a pro-inflammatory reaction or trigger an anti-inflammatory response.[124]

Our study has limitations. Although we have performed a comprehensive search, we may have missed studies published in other languages and possibly in grey literature as we focused on published English literature. There is also a potential for publication bias; despite having searched for articles that did not validate the TRIM mechanisms presented in this scoping review, we could not find any. Furthermore, scoping reviews do not evaluate the quality of evidence. Therefore, the conclusions of this review are based on the studies' existence instead of their quality. Finally, most of TRIM literature studies T cell related mechanisms because the term is also used to describe the perspective of RBC effect on future graft rejection in transplant patients. This may explain the lack of studies focusing on the effect of RBC transfusion on innate immunity.

Nevertheless, our scoping review has several strengths. We have used a robust methodology to perform this study. We have also reviewed the literature using a comprehensive approach that allowed for the inclusion of evidence from the bench and clinical studies. In this review, the clinical studies included were all, except for one study, performed on sick

hospitalized patients instead of healthy volunteers. Thus, the mechanisms reported herein are probably applicable only in hospitalized patients who are at higher risk of acquiring HAIs.

CONCLUSION

Despite the significant reduction in the incidence of transmissible blood-borne infections transmitted by the transfusion of blood products, studies still show an association between RBC transfusions and increased risk of HAI potentially due to TRIM. Our scoping review described four main categories of TRIM mechanisms that are due to the presence of both WBCs and RBCs in blood transfusions and the breaking down of these cells over time. Such mechanisms can partly explain the variability in cytokine levels after RBC transfusions, independently of the RBC unit storage time. Understanding the immunological mechanisms that occur after blood transfusions is crucial to inform the development of strategies that can mitigate HAI risks associated with blood transfusions to improve hospitalized patients' outcomes worldwide.

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Appendix A Search Strategies

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily <1946 to Present> Search Strategy:

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1 Erythrocyte Transfusion/ or *blood transfusion/ae, im (8437)

2 (((Transfus* or stored or unit?) adj3 (red blood cell* or red cell* or erythrocyte* or RBC*)) or allogen?ic transfusion* or allogen?ic blood transfusion*).mp. (19928)

3 1 or 2 (19935)

4 exp Immunomodulation/ (304404)

5 (immunomodulat* or immunosuppress* or (immun* adj3 (mediat* or modulat* or suppress*))).mp. or immun* response*.ti. (386570)

6 exp chimerism/ or exp cytokines/bl, an, im, me or eosinophil granule proteins/ or eosinophils/me or exp HLA antigens/an, im, me or exp interferons/ or exp interleukins/an, bl, me or exp leukocytes/an, im, me or peroxidase/an or plasminogen activator inhibitor/an or exp prostaglandins/ or exp transforming growth factor beta/me or exp transforming growth factors/ or exp tumor necrosis factors/ or (((4-1BB or cd27 or cd30 or cd40 or OX40 or RANK) adj ligand*) or antigen presenting or b-cell activating factor* or basophil* or chimerism* or dendritic cell* or ectodysplasin* or eosinophil* or fas ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen activator inhibitor* or prostaglandin* or shla* or t helper* or th1 or th2 or th 1 or th 2 or tnf* or transforming growth factor* or tumo?r necrosis factor* or wbc or white blood cell?).ti,kf. (1262225)

7 4 or 5 or 6 (1689636)

8 3 and 7 (2195)

9 ((transfusion related or transfusion associated) adj2 (immunomodulat* or immun* modulat* or immunosuppress* or immun* suppress*)).mp. (142)

10 8 or 9 (2258)

11 10 and english.lg. (2005)

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Database: Embase Classic+Embase <1947 to 2019 June 12> Search Strategy:

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1 Erythrocyte Transfusion/ (25001)

2 (((Transfus* or stored or unit?) adj3 (red blood cell* or red cell* or erythrocyte* or RBC*)) or allogen?ic transfusion* or allogen?ic blood transfusion*).mp. (43255)

3 1 or 2 (43255)

4 exp Immunomodulation/ (75393)

5 (immunomodulat* or immunosuppress* or (immun* adj3 (mediat* or modulat* or suppress*))).mp. or immun* response*.ti. (585135)

6 exp *antigen presenting cell/ or exp *cytokine/ec or cytotoxic t lymphocyte/ or exp dendritic cell/ or *eosinophil cationic protein/ or eosinophil cationic protein/ec or *eosinophil granule protein/ or eosinophil/ or exp *HLA antigen/ec or HLA system/ or exp *interferon/ or exp *interleukin/ec or interleukin 2/ or interleukin 2 receptor/ or interleukin 4/ or interleukin 10/ or interleukin 12/ or exp *leukocyte antigen/ or exp *leukocyte/ or *microchimerism/ or *myeloperoxidase/ec or *plasminogen activator inhibitor/ or exp *prostaglandin/ or t lymphocyte/ or TH1 cell/ or TH2 cell/ or exp *transforming growth factor beta/ or exp transforming growth factor beta/ec or exp *transforming growth factor/ec or exp *tumor necrosis factor/ (1430788)

7 (((4-1BB or cd27 or cd30 or cd40 or OX40 or RANK) adj ligand*) or antigen presenting or b-cell activating factor* or basophil* or chimerism* or dendritic cell* or ectodysplasin* or eosinophil* or fas ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen activator inhibitor* or prostaglandin* or shla* or t helper* or th1 or th2 or th 1 or th 2 or tnf* or transforming growth factor* or tumo?r necrosis factor* or wbc or white blood cell?).ti,kw. (933532)

8 4 or 5 or 6 or 7 (2131538)

9 3 and 8 (4214)

10 red blood cell transfusion related immunomodulation/ or ((transfusion related or transfusion associated) adj2 (immunomodulat* or immun* modulat* or immunosuppress* or immun* suppress*)).mp. (242)

11 9 or 10 (4316)

12 11 and english.lg. (4081)

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Search Name: CENTRAL

Date Run: 13/06/2019 17:05:02

Comment:

ID Search Hits

#1 (((Transfus* or stored or unit?) near/3 (red next blood next cell* or red next cell* or erythrocyte* or RBC*)) or allogen?ic next transfusion* or allogen?ic next blood next transfusion*) 8083

#2 (immunomodulat* or immunosuppress* or (immun* near/3 (mediat* or modulat* or suppress*))) or immun* next response*:ti 24272

#3 ((("4 1BB" or cd27 or cd30 or cd40 or OX40 or RANK) near/1 ligand*) or antigen next presenting or b next cell next activating next factor* or basophil* or chimerism* or dendritic next cell* or ectodysplasin* or eosinophil* or fas next ligand or granular or nongranular* or hla* or interferon* or interleukin* or killer next cell* or leukocyte* or lymphocyte* or lymphotoxin* or macrophag* or microchimerism* or monocyte* or myeloperoxidase or neutrophil* or peroxidase or plasminogen next activator next inhibitor* or prostaglandin* or shla* or t next helper* or th1 or th2 or th next 1 or th next 2 or tnf* or transforming next growth next factor* or tumo?r next necrosis next factor* or wbc or white next blood next cell?):ti,kw 63275

#4 #2 OR #3 80973

#5 #1 AND #4 1871

#6 ((transfusion next related or transfusion next associated) near/2 (immunomodulat* or immun* next modulat* or immunosuppress* or immun* next suppress*)) 31

#7 #5 OR #6 in Trials 1793

Appendix B Included Studies

Table B.1 Individual study characteristics of included studies

Author	Country	Year of Publication	Study Design	Study Model
Arase et al.	Japan	1995	Animal research study	Mouse
Bal et al.	Turkey	2018	Test-tube lab research	Cell
Baumgartner et al.	USA	2009	Test-tube lab research	Cell
Baumgartner et al.	USA	2009	Test-tube lab research	Cell
Bakkour et al.	USA	2016	Test-tube lab research	Cell
Belizaire et al.	USA	2012	Animal research study	Mouse
Benson et al.	USA	2012	Test-tube lab research	Cell
Bernard et al.	USA	2007	Test-tube lab research	Cell
Bernard et al.	USA	2008	Test-tube lab research	Cell
Bernard et al.	USA	2010	Test-tube lab research	Cell
Betz and Fox	USA	1991	Animal research study	Mouse
Biagini et al.	Brazil	2017	Animal research study	Pig
Bosman et al.	Netherlands	2008	Test-tube lab research	Cell
Bury et al.	Belgium	1992	Prospective cohort study	Human
Callan et al.	USA	2013	Animal research study	Dog
Chin-Yee et al.	Canada	1997	Test-tube lab research	Cell
Danesh et al.	USA	2014	Test-tube lab research	Cell
De Andrade Pereira et al.	Brazil	2012	Prospective cohort study	Human
Dean et al.	Australia	2011	Test-tube lab research	Cell
DeSilva et al.	USA	1991	Animal research study	Mouse
Drosos et al.	Greece	2012	Prospective cohort study	Human
Efron et al.	USA	2010	Animal research study	Mouse
Flesland et al.	Norway	2004	Prospective cohort study	Human
Fransen et al.	Netherlands	1999	Prospective cohort study	Human
Gafter et al.	Israel	1996	Prospective cohort study	Human
Ghio et al.	Italy	1999	Test-tube lab research	Cell

Ghio et al.	Italy	2001	Test-tube lab research	Cell
Ghio et al.	Italy	2011	Prospective cohort study	Human
Gimmi et al.	USA	1991	Test-tube lab research	Cell
Greenwalt et al.	USA	1980	Test-tube lab research	Cell
Griffith et al.	USA	1996	Animal research study	Mouse
Harlan et al.	USA	1994	Animal research study	Mouse
Hashimoto et al.	Brazil	2004	Animal research study	Mouse
Hassani et al.	Iran	2017	Prospective cohort study	Human
Ishijima and Suzuki	Japan	1998	Prospective cohort study	Human
Jacobi et al.	Germany	2000	Test-tube lab research	Cell
Jenkins and Schwartz	USA	1987	Animal research study	Mouse
Jenkins et al.	USA	1991	Test-tube lab research	Cell
Jiwaji et al.	United Kingdom	2014	Randomized controlled trial	Human
Kim et al.	USA	2002	Test-tube lab research	Cell
Kor et al.	USA	2011	Randomized controlled trial	Human
Koulova et al.	USA	1991	Test-tube lab research	Cell
Lagaaij et al.	Netherlands	1989	Prospective cohort study	Human
Lagaaij et al.	Netherlands	1991	Prospective cohort study	Human
Lamas et al.	France	2012	Test-tube lab research	Cell
Lapierre et al.	France	2007	Randomized controlled trial	Human
Leal-Noval et al.	Spain	2010	Prospective cohort study	Human
Linsley et al.	USA	1991	Test-tube lab research	Cell
Long et al.	USA	2014	Test-tube lab research	Cell
Middleton et al.	Ireland	1994	Prospective cohort study	Human
Mincheff et al.	USA	1993	Test-tube lab research	Cell
Mueller et al.	USA	1989	Animal research study	Mouse
Mukherjee et al.	USA	2014	Animal research study	Mouse
Muszynski et al.	USA	2015	Prospective cohort study	Human
Neuman et al.	USA	2015	Randomized controlled trial	Human
Nielsen et al.	Denmark	1996	Test-tube lab research	Cell
Nielsen et al.	Denmark	1996	Test-tube lab research	Cell

Nielsen et al.	Denmark	1997	Test-tube lab research	Cell
Norris et al.	USA	2019	Prospective cohort study	Human
Nunn et al.	United Kingdom	2013	Randomized controlled trial	Human
Oberlies et al.	United Kingdom	2009	Test-tube lab research	Cell
Osei-Hwedieh et al.	USA	2015	Animal research study	Mouse
Peterson et al.	Sweden	1986	Test-tube lab research	Cell
Prins et al.	Netherlands	2001	Test-tube lab research	Cell
Reed et al.	USA	2007	Prospective cohort study	Human
Rodriguez et al.	USA	2002	Test-tube lab research	Cell
Roelen et al.	Netherlands	2002	Test-tube lab research	Cell
Ross et al.	United Kingdom	1990	Animal research study	Rat
Sadallah et al.	Switzerland	2008	Test-tube lab research	Cell
Shao et al.	USA	1998	Animal research study	Mouse
Spinella et al.	USA	2019	Prospective cohort study	Human
Straat et al.	Netherlands	2014	Test-tube lab research	Cell
Straat et al.	Netherlands	2015	Test-tube lab research	Cell
Straat et al.	Netherlands	2015	Test-tube lab research	Cell
Suksompong et al.	Thailand	2019	Prospective cohort study	Human
Sun et al.	Taiwan	2001	Prospective cohort study	Human
van Twuyver et al.	Netherlands	1991	Prospective cohort study	Human
Ydy et al.	Brazil	2007	Prospective cohort study	Human
Zallen et al.	USA	2000	Test-tube lab research	Cell
Zavazava and Kronke	Germany	1996	Test-tube lab research	Cell
Zhao et al.	China	2018	Randomized controlled trial	Human
Zou et al.	China	2016	Prospective cohort study	Human

Appendix C Immunologic concepts/definitions used in this scoping review

1. T cells activation process

The first step involved in T cell activation is the interaction between T cell receptors and major histocompatibility complexes (MHC) located on the antigen-presenting cell (APC).[1-5] This step occurs when MHC binds an antigen peptide, and together, this MHC-peptide complex is recognized by the T cell receptor.[1-5] The occupancy of the T cell receptor by an MHC-peptide complex results in the expression of interleukin (IL)-2 receptors.[1-5]

A secondary costimulatory signal is simultaneously required to activate T cells fully, leading IL-2 production, which in turn acts in an autocrine fashion on the cell's own IL-2 receptors to induce proliferation.[6] High expression of IL-2 is therefore indicative of T cell proliferation, and the expression of the IL-2 receptor on T cell is used as a marker of T cell activation. In the absence of a costimulatory signal, T cell receptor activation will result in T cell anergy or the induction of a T regulatory phenotype characterized by persistently high expression of the IL-2 receptor and anti-inflammatory properties.

2. Microchimerism

Microchimerism is the persistence of donor cells in recipients years after blood transfusions that result in a state of reciprocal tolerance.[7] For this to happen, the recipient must mount a sufficiently low immune response to accommodate these cells allowing for their survival.[7]

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CHAPTER 4 – ASSOCIATION BETWEEN LEUKOREDUCED RED BLOOD CELL TRANSFUSIONS AND HOSPITAL-ACQUIRED INFECTIONS

4.1 Preamble

The results from our scoping review demonstrated that TRIM might be explained by mechanisms involving the presence of donor's WBCs in transfused RBC units and mechanisms related to the transfused RBCs. As both WBCs and RBCs can trigger TRIM, we focused our next studies on evaluating if RBCs are associated with HAIs. Leukoreduced RBCs have been shown to reduce HAIs when compared to non-leukoreduced units.[99,108] However, after leukoreduction, some WBCs remain in RBC units. Since we do not know the necessary volume of WBCs present in transfused RBC units to trigger TRIM, we hypothesize that the transfusion of leukoreduced RBC units could still be associated with an increase in the HAI incidence rate in stable critically ill pediatric patients.

Thus, the second study of this thesis aims to evaluate the existence of an association between the transfusion of leukoreduced RBC units and an increase in the HAI incidence rate in the aforementioned patient population. To do so, we have performed a *post hoc* secondary analysis of the TRIPICU database. The TRIPICU study was a large, pediatric, noninferiority RCT performed in 19 PICUs across Belgium, Canada, the United Kingdom, and the United States from 2001 to 2005. In TRIPICU, pre-storage leukoreduced packed RBCs were used for all transfused patients. In addition, the TRIPICU trial used a very robust and high-quality methodology.

The TRIPICU study's primary aim was to determine whether a restrictive transfusion strategy (intervention) was as safe as a liberal transfusion strategy. However, the investigators had HAIs as a secondary outcome and consequently recorded granular data on HAI events and risk factors, as well as on blood transfusions. Due to these reasons, the use of the TRIPICU database allowed us to use high-quality data to answer our proposed research questions.

4.2 Manuscript 2

Association Between Leukoreduced Red Blood Cell Transfusions and Hospital-Acquired Infections in Critically Ill Children: A Secondary Data Analysis of the TRIPICU Study

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Short title:

Association between leukoreduced red blood cell transfusions and hospital-acquired infections

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Conflicts Of Interest

The authors have no conflicts of interest.

ABSTRACT

Background: Hospital-acquired infections (HAI) are an important problem in critically ill children. Evidence shows that not all HAIs are caused by a lack of compliance with infection control protocols. One hypothesis is that the transfusion of leukoreduced red blood cell units (RBC) is associated with an increase in the HAI incidence rate (IR).

Objectives: 1) To determine an association between leukoreduced RBC restrictive transfusion strategy and HAI IR in critically ill children, 2) to evaluate the existence of an association between leukoreduced RBC transfusions and HAI IR, and 3) to determine the presence of an association between the number or volume of leukoreduced RBC transfusions and HAI IR.

Methods: This is a *post-hoc* secondary analysis of the "Transfusion Requirement in Pediatric Intensive Care Units" (TRIPICU) randomized controlled trial (637 patients). We used descriptive statistics and quasi-Poisson multivariable regression models to estimate the HAI incidence rate ratio (IRR) and their corresponding 95% confidence intervals (CI).

Results: The IRR for the association of a restrictive transfusion strategy was 0.91 (95% CI 0.70, 1.19). The results of our quasi-Poisson multivariable regression models showed that the association of transfusing leukoreduced RBCs (IRR 1.15; 95% CI 0.69, 1.91) and volume of leukoreduced RBC transfusions (IRR 1.73; 95% CI 0.94, 3.18) on HAI IR were not statistically significant. HAI IR increased with the number of leukoreduced RBC transfusions showing a significant association between critically ill children who received ≥3 leukoreduced RBC transfusions and HAI IR (IRR 2.32; 95% CI 1.14, 4.71).

Conclusion: Exposing critically ill children to ≥3 leukoreduced RBC transfusions was associated with a higher HAI IR, suggesting that the association between leukoreduced RBC transfusions and HAI IR follows a dose-response pattern. Clinically significant associations were demonstrated regarding any given transfusion of leukoreduced RBCs and the volume of leukoreduced RBC transfusions on HAI IR.

INTRODUCTION

Hospital-acquired infections (HAI) are one of the most frequent adverse events in healthcare.[1] The World Health Organization estimates that more than 1.4 million patients worldwide are affected by HAIs at any one time.[2] In two Canadian point prevalence surveys, the overall prevalence of hospitalized pediatric patients with HAIs was 8%, while the prevalence in pediatric intensive care units (PICUs) ranged from 17.7% – 19.0%.[3,4]

HAIs are associated with prolonged hospital stays, higher mortality, long-term disability, antimicrobial resistance, and increased economic burden at the healthcare, societal, and individual levels.[2] Studies show that the implementation and compliance with infection control measures are not sufficient to eradicate HAIs.[5-8] To help prevent HAIs, a greater understanding and knowledge of the biological mechanisms that result in patients having a higher risk of bacterial colonization leading to HAIs is vital. One important hypothesis is that red blood cell (RBC) transfusions lead to immunosuppression, increasing the risk for HAIs.

It is estimated that 4.8% of pediatric patients will receive a blood transfusion during their hospital admission, with 60.2% being RBC transfusions.[9] Despite the clinical benefits associated with RBC transfusions, it is hypothesized that they can lead to an increase in the risk of HAIs through transfusion-related immunomodulation (TRIM). The most known TRIM mechanisms suggest that the presence of white blood cells (WBC) in transfused RBC units is associated with immunsupression.[10-13]

Proposed measures to prevent TRIM included the use of leukoreduction, which is a process that removes WBCs from blood products.[14] Leukoreduction can either occur pre-storage, post-storage, or bedside. Pre-storage leukoreduction has been shown to reduce the number of cytokines released from the RBCs during storage, which may partially mitigate some of TRIM's potentially harmful effects.[15] However, leukoreduction does not fully remove WBCs from RBC units. It is possible that the remaining immunologically active WBCs present in leukoreduced RBC units could lead to a downregulation of the immune system of patients receiving the transfusion. Numerous randomized controlled trials (RCTs) have evaluated the association between transfusing leukoreduced RBCs and HAIs, but their results were controversial.[16-20] Additionally, these studies compared the transfusion of leukoreduced RBCs to non-leukoreduced RBCs and based on these studies, leukoreduced blood seems to be associated with fewer HAIs.

However, we still do not know if the transfusion of leukoreduced blood itself is associated with HAIs.

Therefore, to evaluate the existence of an association between the transfusion of leukoreduced RBCs and HAIs, we performed a *post hoc* secondary analysis of the Transfusion Requirements in Pediatric Intensive Care Units (TRIPICU) RCT.[21] In the TRIPICU RCT, all transfused patients received pre-storage leukoreduced packed RBCs, which allowed us to evaluate the individual associations between transfusing leukoreduced RBCs and the incidence rate of HAIs.

We hypothesize that the transfusion of leukoreduced RBCs may be associated with an increase in the HAI incidence rate (IR) in stable critically ill pediatric patients and that the use of a restrictive transfusion strategy would mitigate this effect. In addition, we hypothesize that the association between transfusion of leukoreduced RBCs and HAI IR would follow a dose-response pattern. Thus, our study's primary aim was to determine if a leukoreduced RBC restrictive transfusion strategy compared with a leukoreduced RBC liberal transfusion strategy is associated with a reduction in the HAI incidence rate (IR). Furthermore, we aimed to determine the association between any given transfusion of leukoreduced RBCs and HAI IR and the existence of a dose-response association between the number and/or volume of leukoreduced RBC transfusions and HAI IR compared to no RBC transfusion.

METHODS

Study Design

The TRIPICU study was a parallel, noninferiority RCT performed in 19 PICUs across Canada, Belgium, the United Kingdom, and the United States from 2001 to 2005.[21] For this secondary analysis, data were analyzed either as a superiority RCT (primary aim) or as an observational study (secondary aims).

Participants

Critically ill children between 3 days and 14 years old were included in the study if they were hemodynamically stable (defined as mean arterial pressure not < 2 standard deviations (SD) below the normal mean for age and absence of increase of cardiovascular treatments at least 2

hours before enrolment) and they had at least one hemoglobin concentration of ≤ 9.5 g/dL within the first 7 days of PICU admission. Children were excluded if they were expected to stay < 24 hours in the PICU, patient's study participation was not approved by treating physician, had acute blood loss, weighed < 3.0 kg, had cardiovascular problems, were never discharged from neonatal ICU, had hemolytic anemia, or were enrolled in another study.

The study population for this secondary analysis varied according to the study aim. For the primary aim, we analyzed data from all 637 patients in the TRIPICU database. For secondary aims 1 (association between any given leukoreduced RBC transfusion and HAI IR) and 2 (association between the number of RBC transfusions and HAI IR), we included a subset of 498 patients who received no previous blood transfusions during the hospital stay that led to study participation and had no protocol suspensions. For secondary aim 3 (association between the volume of RBC transfusion and HAI IR), we analyzed data from a subset of 496 patients for whom the number of received RBC transfusions and volume of blood transfused had been recorded and that had no previous blood transfusions or protocol suspensions. We excluded patients who had had previous blood transfusions during the hospital stay to ensure that our results would not be biased by the presence of residual effects from pre-trial transfusions. We also excluded patients that had a protocol suspension to avoid selection bias, given that suspensions primarily occurred due to emergencies (e.g., acute respiratory distress syndrome, worsened shock, or increased bleeding) whose management could have increased the patient's HAI risk.

Interventions

In our *post hoc* secondary analysis, our primary aim's intervention is the original intervention of the TRIPICU RCT: the use of a restrictive leukoreduced RBC transfusion strategy compared to the use of a liberal leukoreduced RBC transfusion strategy.[21] In the restrictive transfusion strategy group, the hemoglobin threshold for transfusion was set at 7g/dL with a target range after transfusion of 8.5 to 9.5g/dL. The threshold for the patients randomized to the liberal transfusion strategy group was 9.5g/dL with a target range after transfusion of 11 to 12g/dL.

The interventions of our secondary aims included 1) at least one leukoreduced RBC transfusion, 2) the number of leukoreduced RBC transfusions, and 3) volume (in cc/kg) of

leukoreduced RBC transfusions. The control group for all secondary aims included patients who received no RBC transfusion.

Outcomes

The primary outcome for all aims of this secondary analysis was HAI IR. A comprehensive list of HAIs recorded for the TRIPICU study can be found in Table 1.[22] In TRIPICU, HAIs were defined according to the Centers for Disease Control and Prevention (CDC, 1988) definitions, as they were considered the gold standard at the time.[22] However, to increase the specificity of central line-associated bloodstream infection (CLABSI) and catheter-related urinary tract infection (CAUTI), their definitions were modified to only include cases that presented positive bacterial cultures.[23] Importantly, these definitions are very similar to the CLABSI and CAUTI definitions currently used by CDC.[24] HAIs during the TRIPICU study were diagnosed by medical teams and/or infection control teams. Medical teams were not blinded for the intervention. HAIs were validated by TRIPICU site investigators and/or infection control teams. All HAIs recorded in the TRIPICU study were diagnosed after randomization up to the first of the following events: death, PICU discharge, or 28 days post-randomization.

We accounted for all HAI episodes that happened after randomization as the numerator to calculate the HAI IR, even if more than one episode happened per patient. Our denominator was the number of patient-days in the PICU after randomization, defined as the number of days between randomization date and PICU discharge date, date of death during PICU stay, or 28 days after randomization, whichever came first.

Data Collection

We recorded data on demographics (age, weight, sex), PICU length of stay before randomization, TRIPICU study group, comorbidities, use of immunomodulatory drugs, presence of inflammatory diseases and hematological problems, surgery, and severity of illness measured by the Pediatric Risk of Mortality (PRISM) score on the day of randomization.[25] Detailed definitions of the aforementioned variables can be found in Appendix 1.

Statistical Analysis

We estimated that using the TRIPICU study population (637 patients) and a superiority analytical approach, we would be able to detect a HAI IR reduction of 22% between groups, using an alpha of 0.05 and 80% power.[26] We used descriptive statistics to summarize the characteristics of the study groups. Data were analyzed using means (SD) or medians (interquartile ranges [IQR]) for continuous variables and proportions for categorical variables. Due to the data distribution's lack of normality, we used the Wilcoxon rank-sum test or the Kruskal-Wallis test to compare continuous variables. Categorical variables were analyzed using the chi-square test or Fisher's exact test.

We analyzed the association between the restrictive transfusion strategy and HAI IR (primary aim) by calculating the incidence rate ratio (IRR) and its 95% confidence interval (CI). For the analysis of our secondary outcomes, we used multivariable quasi-Poisson regression models as study data did not follow a Poisson distribution. An offset of log(ICU patient-days) was used to account for the patients' varying follow-up days. Analyses were conducted using R version 4.0.0.[27]

RESULTS

Primary Aim: Association Between Leukoreduced RBC Restrictive Transfusion Strategy and HAI IR

All 637 patients randomized in the TRIPICU study were included in this primary aim. Table 2 presents the baseline demographic and clinical characteristic variables for participants. The HAI IR in the leukoreduced RBC restrictive transfusion strategy group was 40.39 per 1000 patient-days (95% CI 32.83, 49.18) and 44.36 per 1000 patient-days (95% CI 36.66, 53.20) for the leukoreduced RBC liberal transfusion strategy group, corresponding to an IRR of 0.91 (95% CI 0.70, 1.19) (Table 3, Figure 1).

Secondary Aim 1: Association Between Transfusion of Leukoreduced RBC and HAI IR

Table 4 shows the baseline characteristics of the 498 patients that were included in this secondary aim. There were significant differences between no RBC transfusion and

leukoreduced RBC transfusion when comparing hematological problems, multiple trauma, cardiac surgery, and PRISM score at randomization. The IRs of the groups are shown in Figure 1. The adjusted effect of leukoreduced RBC transfusion on HAI IR (Table 3) was calculated using a quasi-Poisson multivariable model, including the aforementioned variables and the original TRIPICU study group assignment, and it was not statistically significant (adjusted IRR 1.15; 95% CI 0.69, 1.91).

Secondary Aim 2: Association Between Number of Leukoreduced RBC Transfusions and HAI IR

Table 5 shows the baseline demographic and clinical characteristic variables for the 498 TRIPICU participants included in this secondary aim. There were significant differences between the study groups (no transfusion, 1 transfusion, 2 transfusions, and ≥3 transfusions) for patient age, inflammatory diseases, hematological problems, multiple trauma, cardiac surgery, abdominal surgery, other surgery, PRISM score at randomization, and immunomodulatory drugs. After adjusting for these variables and the study group in the original TRIPICU RCT in our multivariable quasi-Poisson model (Table 3), we observed a statistically significant association between exposure to ≥3 leukoreduced RBC transfusions and HAI IR (adjusted IRR 2.32; 95% CI 1.14, 4.71). The IRs of the groups are shown in Figure 1.

Secondary Aim 3: Association Between Volume of Leukoreduced RBC Transfused and HAI IR

Table 6 presents the baseline characteristics of the 496 participants included in this secondary aim. There were significant differences between the four groups (no transfusion, ≤10cc/kg transfusion, 10-20cc/kg transfusion, and > 20cc/kg transfusion) regarding hematological problems, multiple trauma, cardiac surgery, and PRISM score at randomization. Group HAI IRs are shown in Figure 1. After adjusting for hematological problems, multiple trauma, cardiac surgery, PRISM score at randomization, and study group assignments in the original TRIPICU RCT using a multivariable quasi-Poisson regression model, there was no statistically significant association between volume of leukoreduced RBC transfused and HAI IR (Table 3).

DISCUSSION

Our *post-hoc* secondary analysis of the TRIPICU study showed that using a leukoreduced RBC restrictive transfusion strategy did not demonstrate a statistically significant association with HAI IR when compared to a leukoreduced RBC liberal transfusion strategy. Similarly, there was no association between leukoreduced RBC transfusions and an increase in the overall HAI IR. However, when we specifically studied the association between the number of leukoreduced RBC transfusions and the HAI IR, we observed that exposure to ≥3 transfusions was associated with an increase in HAI IR. When analyzing data on the volume of leukoreduced RBC received, our results suggested a trend towards an association between RBC transfusion volume and HAI IR, but this was not statistically significant.

Several studies have evaluated the effect of transfusing leukoreduced RBCs, compared to the transfusion of non-leukoreduced blood, on HAIs.[16-20,28,29] Despite evidence demonstrating a benefit of transfusing leukoreduced RBC units compared to non-leukoreduced blood, the question about the former's effect compared to no transfusion remains. We showed that the HAI IR in patients who received at least one leukoreduced RBC transfusion is higher compared to the HAI IR in non-transfused patients. However, the result of our quasi-Poisson multivariable regression model regarding the association of transfusing leukoreduced RBCs was inconclusive. Contrarily, a study by Horvath et al. showed that adult patients undergoing cardiac surgery who received leukoreduced RBC transfusions had a higher HAI risk than those who were not transfused (pneumonia: 3.59% [transfused] vs. 1.23% [not transfused]; bloodstream: 1.93% [transfused] vs. 0.26% [not transfused]).[30] Similarly, a study by Salvin et al. including 802 PICU cardiac surgery patients found that the HAI incidence was higher in the groups who received high transfusion (>15 ml/kg) and low transfusion (≤15 mL/kg) volume of leukoreduced RBCs compared to no transfusion (14% vs. 13% vs. 4%; $p < 0.001$).[31]

We also evaluated the association between a restrictive transfusion strategy and HAI IR. However, differently from the original TRIPICU study, we used HAI IR, not the proportion of study participants with HAI, as the secondary analysis outcome. This allowed us to account for patients with varying risk for developing HAI due to different PICU lengths of stay. Similar to our results, Lacroix et al. also found no statistically significant difference in the HAI risk between the two transfusion groups (absolute risk reduction 4.6; 95% CI -1.9, 11.1; $p = 0.16$) in

the original TRIPICU study.[21] Based on our sample size calculation for this *post-hoc* analysis, we can say that the TRIPICU study was underpowered to detect a 10% reduction in HAI IR associated with the use of a restrictive leukoreduced RBC transfusion strategy.[22]

Nevertheless, our results showed that the HAI IR in stable, critically ill children who received ≥3 leukoreduced RBC transfusions was 2.32 times the HAI IR in children who had not been transfused. In addition, the comparison between patients who received 1 and 2 transfusions and non-transfused children showed a statistically nonsignificant, but clinically relevant, trend towards an increase in HAI IR with the number of received transfusions. We speculate that a dose-response associated with an increasing number of leukoreduced RBC transfusions may exist and that our study was underpowered to detect it. The aforementioned study by Horvath et al. demonstrated a dose-related association between the number of leukoreduced RBC units transfused and the HAI risk in adult patients.[30] The latter increased by an average of 29% with each RBC unit transfused ($p < 0.001$).[30] Similarly, Yu et al. discovered that each additional unit of leukoreduced RBC transfusion in cardiac surgery patients increased the incidence of pneumonia (1 unit 0.0% vs. 2 units 0.7% vs. 3 units 2.3%; $p < 0.013$) and infectious septicemia (1 unit 0.0% vs. 2 units 0.3% vs. 3 units 2.3%; $p < 0.006$).[32] Finally, Everhart et al. showed increasing HAIs with an increasing number of leukoreduced PRBCs in patients undergoing shoulder arthroplasty (IRR 1.68 per unit of PRBC; 95% CI 1.21, 2.35; $p = 0.002$).[33]

Our study also suggested a dose-response related to blood transfusion volume, but this result was not statistically significant. Woods et al. reported similar but statistically significant findings.[34] In this study, the authors reported an association between higher perioperative blood transfusion volume and postoperative infection in adult lumbar spine surgery patients (OR 2.87; 95% CI 1.63 to 5.06).[34]

The dose-responses mentioned above may be related to different patient factors. Patients that receive repeated blood transfusions are generally sicker. Rajasekaran et al. showed that critically ill children who received leukoreduced RBC transfusions had significantly higher PRISM and Pediatric Logistic Organ Dysfunction (PELOD) scores, thus indicating a higher severity of illness.[35] Critically ill patients also have innate and adaptive immunological derangements that put them at a higher risk for HAI.[36] It has been shown that the magnitude of the physiologic insult dictates the patient's response to pathogens.[37] Additionally, the patient's specific response depends on their genetic characteristics and comorbidities and the pathogen's

load and virulence.[37,38] Neutrophil function is also reduced in critically ill patients compared to healthy controls ($p < 0.05$).[39] Furthermore, critically ill patients with sepsis may present an exacerbation of both pro-inflammatory and anti-inflammatory responses, leading to excessive inflammation or immunosuppression.[38] In both cases, the patient's susceptibility to secondary infections is increased. Thus, all these immunological derangements may lead to higher incidences of HAIs in critically ill patients.[40]

RBC transfusions may also lead to TRIM. TRIM includes both pro-inflammatory and immunosuppressive effects due to residual WBCs in the transfused RBC units and the release of biological substances by both WBCs and RBCs.[12,41] Studies show that the presence of donor WBCs leads to the absence of the secondary costimulatory signal necessary to release IL-2 and fully activate T cells, leading to a state of T cell unresponsiveness.[42-50] Thus, repeated transfusions in critically ill children could act as secondary insults to an already dysregulated immune system, increasing even further the HAI risk in this patient population.[51,52]

One interesting study finding was that the HAI IR of patients who received no transfusion was slightly higher than the HAI IR of patients who received one transfusion or a transfusion of ≤10cc/kg. We speculate that patients who received one transfusion may not have received enough blood to trigger a negative effect. It is also possible that these patients presented only mild metabolic derangements that were improved by the RBC transfusion, allowing them to better cope with future infectious insults.

Our study has limitations. The date when the HAI was diagnosed was not recorded in the TRIPICU study. However, all events happened after patient randomization. Furthermore, the original study did not collect device days, except for mechanical ventilation, which prevented us from calculating the IR for different HAIs. Fortunately, this does not impact the calculation of the overall HAI IR as its denominator is PICU-patient days. The follow-up period for HAIs ended on the day of PICU discharge, which made it impossible to capture HAIs infections occurring within 24 hours after PICU discharge. According to the CDC's infection control definitions, such HAIs would still be attributable to the PICU.[24] An additional limitation would be the risk of protopathic bias if HAIs happened after randomization but before blood transfusion. However, this risk is low as the time from randomization to transfusion was 0.1 days in the liberal transfusion strategy group and 1.7 days in the original restrictive transfusion

strategy group. Finally, there is always the risk for unmeasured confounders as the database used, despite being extremely comprehensive, did not include data on all the risk factors for HAI.

Nevertheless, our study has strengths. The TRIPICU study had HAIs as a secondary outcome and consequently recorded high-quality granular data on HAI risk factors and blood transfusions. In addition, the HAI definitions used in the TRIPICU study followed the CDC 1988 definitions, and some were modified to include the presence of positive bacterial cultures.[22,23] The use of laboratory-confirmed HAI definitions aligns well with the current HAI definitions proposed by the CDC.[24]

In conclusion, our study showed that exposure to ≥3 leukoreduced RBC transfusions is associated with increased HAI IR in stable, critically ill children. We were unable to detect an association between using a restrictive transfusion strategy or transfusing leukoreduced RBC and HAI IR. However, we observed a dose-response regarding the number of leukoreduced RBC transfusions and their volume, suggesting that children who receive multiple transfusions and/or higher volume may be at a higher risk for HAI. Our results highlight important research questions that must be studied to inform the transfusion practices in the pediatric critical care setting, with the hope of reducing HAI IR in this setting.

Table 1 HAIs recorded in the TRIPICU study

HAI GROUP	HAI TYPE
Bloodstream infection	Central line-associated bloodstream infections (CLABSI), bloodstream infection, sepsis
Respiratory system	Pneumonia, tracheitis, upper respiratory tract infection
Urinary tract system	Catheter-associated urinary tract infection (CAUTI), urinary tract infection
Surgery-related	Surgical site infection (SSI)
Central nervous system (CNS)	Meningitis, ventriculoperitoneal shunt infection
Cardiovascular system (CVS)	Mediastinitis
Eye	Conjunctivitis
Ear, nose, and throat (ENT)	Otitis media

Table 2 Clinical characteristics of the patients - restrictive-strategy vs. liberal-strategy

Variable (total N= 637)	Restrictive-Strategy Group (N = 320)	Liberal-Strategy Group * (N = 317)	P Value
Demographics			
Age – months			
Mean ± SD	35.8 ± 46.2	39.6 ± 51.9	0.33
Median [IQR]	14.0 [3.0 – 48.2]	12.0 [4.0 – 61.0]	0.64
Weight – kilograms			
Mean ± SD	14.1 ± 14.8	15.2 ± 15.3	0.38
Median [IQR]	10.0 [5.0 – 16.0]	9.0 [6.0 – 18.0]	0.53
Male sex – no. (%)	190 (59.4)	191 (60.3)	0.88
Previous blood transfusion in PICU – no. (%)	45 (14.1)	59 (18.6)	0.15
Length of stay in PICU before randomization – days			
Mean ± SD	2.3 ± 1.7	2.3 ± 1.8	0.72
Median [IQR]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	0.97
Time between randomization and transfusion - days	1.7	0.1	<0.001
Immunomodulatory drugs – no. (%)	76 (24.3)	78 (25.3)	0.87
Comorbidities – no. (%)			
Inflammatory diseases	202 (63.7)	202 (63.1)	0.94
Hematological problems	54 (16.9)	53 (16.7)	1.00
Multiple trauma	19 (5.9)	21 (6.6)	0.85
Surgery – no. (%)			
Cardiac	63 (19.7)	62 (19.6)	1.00
Abdominal	15 (4.7)	16 (5.0)	0.98
Transplantation	3 (0.9)	5 (1.6)	0.71
Other surgery	51 (16.1)	54 (16.9)	0.87
Severity of illness (PRISM score) on day of randomization			
Mean ± SD	4.8 ± 4.4	4.8 ± 4.3	0.99
Median [IQR]	4.0 [2.0 – 7.0]	4.0 [1.0 – 7.0]	0.93

Percentages may not sum to 100 because of rounding.
Abbreviations: SD, standard deviation; IQR, interquartile region; PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality
* Reference group.

Table 3 Adjusted and Non-Adjusted Incidence Rate Ratios for each study aim

	Number of Patients	Number of Infections	Patient days (per 1000 person-days)	IR (95 % CI)	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)*
Primary Aim: Association Between Leukoreduced RBC Restrictive Transfusion Strategy and HAI IR						
Liberal (reference)	317	116	2615	44.36 (36.66, 53.20)	1.00 (reference)	Not applicable
Restrictive	320	99	2451	40.39 (32.83, 49.18)	0.91 (0.70, 1.19)	
Secondary Aim 1: Association Between Transfusion of Leukoreduced RBC and HAI IR						
No Transfusion (reference)	157	30	1033	29.04 (19.59, 41.46)	1.00 (reference)	1.00 (reference)
Transfused	341	95	2687	35.36 (28.60, 43.22)	1.21 (0.79, 1.88)	1.15 (0.69, 1.91)
Secondary Aim 2: Association Between the Number of Leukoreduced RBC Transfusions and HAI IR						
No transfusion (reference)	157	30	1033	29.04 (19.59, 41.46)	1.00 (reference)	1.00 (reference)
1 Transfusion	259	44	1699	25.90 (18.82, 34.77)	0.89 (0.54, 1.48)	1.02 (0.57, 1.80)
2 Transfusions	62	29	627	46.25 (30.98, 66.43)	1.59 (0.91, 2.78)	1.80 (0.95, 3.39)
3+ Transfusions	20	22	361	60.94 (38.19, 92.27)	2.10 (1.15, 3.82)	2.32 (1.14, 4.71)
Secondary Aim 3: Association Between Volume of Leukoreduced RBC Transfusions and HAI IR						
No transfusion (reference)	157	30	1033	29.04 (19.59, 41.46)	1.00 (reference)	1.00 (reference)
≤10cc/kg	88	12	568	21.13 (10.92, 36.90)	0.73 (0.35, 1.51)	0.74 (0.36, 1.52)
10-20cc/kg	182	41	1199	34.20 (24.54, 46.39)	1.18 (0.70, 1.97)	1.32 (0.73, 2.42)
>20cc/kg	69	41	891	46.02 (33.02, 62.43)	1.58 (0.95, 2.65)	1.73 (0.94, 3.18)

Abbreviations: IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval; HAI, hospital-acquired infections; RBC, red blood cells

*Quasi-Poisson model for secondary aim 1 adjusted for hematological problems, multiple trauma, cardiac surgery, PRISM score at randomization, and TRIPICU RCT study group. Quasi-Poisson model for secondary aim 2 adjusted for age of patient, presence of comorbidities (inflammatory diseases, hematological problems, and/or multiple trauma), cardiac surgery, abdominal surgery, other surgery, PRISM score at randomization, immunomodulatory drugs, and TRIPICU RCT study group. Quasi-Poisson model for secondary aim 3 adjusted for hematological problems, multiple trauma, cardiac surgery, PRISM score at randomization, and TRIPICU RCT study group.

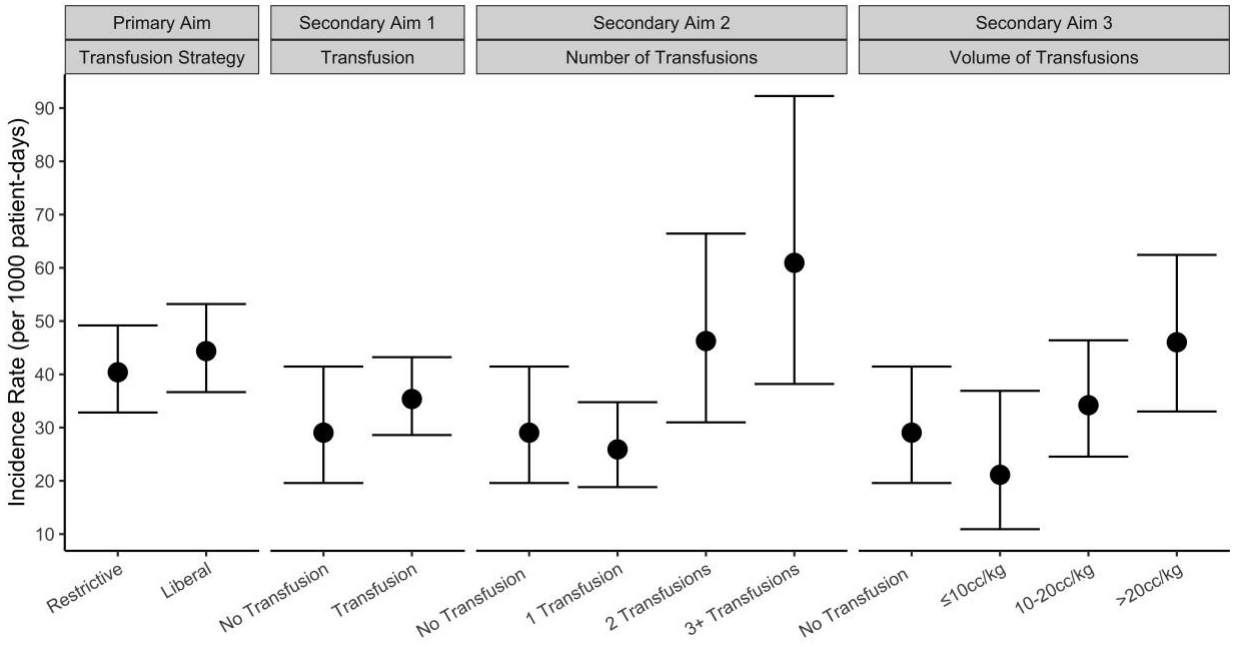

Figure 1 Incidence rates with corresponding 95% confidence intervals for the different interventions

Table 4 Clinical characteristics of the patients – Secondary aim 1

Variable (total N= 498)	No Transfusion of RBC Group * (N = 157)	Transfusion of RBC Group (N = 341)	P Value
Demographics			
Age – months			
Mean ± SD	35.7 ± 45.3	34.1 ± 47.7	0.72
Median [IQR]	15.0 [4.0 – 48.0]	11.0 [3.0 – 44.0]	0.30
Weight – kilograms			
Mean ± SD	13.7 ± 12.2	13.6 ± 14.1	0.98
Median [IQR]	10.0 [5.0 – 15.0]	9.0 [5.0 – 15.0]	0.41
Male sex – no. (%)	92 (58.6)	219 (64.2)	0.27
Length of stay in PICU before randomization – days			
Mean ± SD	2.2 ± 1.7	2.2 ± 1.7	0.72
Median [IQR]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	0.69
Restrictive strategy group – no. (%) †	150 (95.5)	98 (28.7)	<0.01
Immunomodulatory drugs – no. (%)	29 (18.5)	75 (22.0)	0.44
Comorbidities – no. (%)			
Inflammatory diseases	89 (56.7)	209 (61.3)	0.38
Hematological problems	11 (7.0)	49 (14.4)	0.03
Multiple trauma	1 (0.6)	21 (6.2)	<0.01
Surgery – no. (%)			
Cardiac	36 (22.9)	61 (17.9)	0.23
Abdominal	4 (2.5)	21 (6.2)	0.12
Transplantation	3 (1.9)	3 (0.9)	0.39
Other surgery	20 (12.7)	57 (16.7)	0.31
Severity of illness (PRISM score) on day of randomization			
Mean ± SD	3.9 ± 3.8	4.8 ± 4.5	0.03
Median [IQR]	3.0 [1.0 – 6.0]	4.0 [1.0 – 7.0]	0.06

Percentages may not sum to 100 because of rounding.

Abbreviations: RBC, red blood cells; SD, standard deviation; IQR, interquartile region; PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality

* Reference group.

† In the restrictive-strategy group of the TRIPICU study, the hemoglobin threshold for transfusion was set at 7 g/dL, with a target range after transfusion of 8.5 to 9.5 g/dL.

Table 5 Clinical characteristics of the patients – Secondary aim 2

Variable (total N= 498)	No Transfusion Group * (N = 157)	1 Transfusion Group (N = 259)	2 Transfusions Group (N = 62)	3+ Transfusions Group (N =20)	P Value
Demographics					
Age – months					
Mean ± SD	35.7 ± 45.3	30.8 ± 43.2	45.4 ± 60.9	42.2 ± 52.4	0.13
Median [IQR]	15.0 [4.0 – 48.0]	10.0 [3.0 – 37.5]	13.5 [4.0 – 82.0]	15.0 [3.8 – 85.5]	0.32
Weight – kilograms					
Mean ± SD	13.7 ± 12.2	12.5 ± 11.9	17.3 ± 18.7	17.4 ± 20.5	0.05
Median [IQR]	10.0 [5.0 – 15.0]	9.0 [5.0 – 15.0]	9.5 [5.2 – 19.2]	10.0 [5.8 – 20.0]	0.43
Male sex – no. (%)	92 (58.6)	169 (65.3)	38 (61.3)	12 (60.0)	0.58
Length of stay in PICU before randomization – days					
Mean ± SD	2.2 ± 1.7	2.1 ± 1.6	2.3 ± 1.8	2.5 ± 1.9	0.62
Median [IQR]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	2.0 [1.0 – 4.0]	0.81
Restrictive strategy group – no. (%) †	150 (95.5)	81 (31.3)	12 (19.4)	5 (25.0)	<0.01
Immunomodulatory drugs – no. (%)	29 (18.5)	54 (20.8)	14 (22.6)	7 (35.0)	0.38
Comorbidities – no. (%)					
Inflammatory diseases	89 (56.7)	155 (59.8)	38 (61.3)	16 (80.0)	0.25
Hematological problems	11 (7.0)	31 (12.0)	11 (17.7)	7 (35.0)	<0.01
Multiple trauma	1 (0.6)	16 (6.2)	5 (8.1)	0 (0.0)	0.01
Surgery – no. (%)					
Cardiac	36 (22.9)	47 (18.1)	11 (17.7)	3 (15.0)	0.64
Abdominal	4 (2.5)	14 (5.4)	3 (4.8)	4 (20.0)	0.02
Transplantation	3 (1.9)	2 (0.8)	1 (1.6)	0 (0.0)	0.54
Other surgery	20 (12.7)	42 (16.2)	9 (14.5)	6 (30.0)	0.23
Severity of illness (PRISM score) on day of randomization					
Mean ± SD	3.9 ± 3.8	4.6 ± 4.4	5.7 ± 5.0	5.7 ± 4.2	0.04
Median [IQR]	3.0 [1.0 – 6.0]	4.0 [1.0 – 7.0]	4.0 [2.0 – 9.0]	5.5 [2.8 – 8.2]	0.08

Percentages may not sum to 100 because of rounding.

Abbreviations: SD, standard deviation; IQR, interquartile region; PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality

* Reference group.

† In the restrictive-strategy group of the TRIPICU study, the hemoglobin threshold for transfusion was set at 7 g/dL, with a target range after transfusion of 8.5 to 9.5 g/dL.

Table 6 Clinical characteristics of the patients – Secondary aim 3

Variable (total N= 496)	No Transfusion Group * (N = 157)	≤10cc/kg Transfusion Group (N = 88)	10-20cc/kg Transfusion Group (N = 182)	>20+cc/kg Transfusion Group (N = 69)	P Value
Demographics					
Age – months					
Mean ± SD	35.7 ± 45.3	41.1 ± 55.8	34.9 ± 46.2	24.1 ± 38.5	0.15
Median [IQR]	15.0 [4.0 – 48.0]	11.0 [3.0 – 61.2]	11.0 [3.0 – 46.5]	9.0 [3.0 – 23.0]	0.40
Weight – kilograms					
Mean ± SD	13.7 ± 12.2	16.0 ± 17.6	13.8 ± 13.5	10.5 ± 9.4	0.09
Median [IQR]	10.0 [5.0 – 15.0]	9.0 [5.0 – 18.5]	9.0 [5.0 – 16.0]	8.0 [5.0 – 12.0]	0.33
Male sex – no. (%)	92 (58.6)	57 (64.8)	115 (63.2)	47 (68.1)	0.54
Length of stay in PICU – days					
Mean ± SD	2.2 ± 1.7	2.2 ± 1.5	2.1 ± 1.7	2.2 ± 1.7	0.97
Median [IQR]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	1.0 [1.0 – 3.0]	0.89
Restrictive strategy group – no. (%) †	150 (95.5)	45 (51.1)	41 (22.5)	11 (15.9)	<0.01
Immunomodulatory drugs – no. (%)	29 (18.5)	24 (27.3)	34 (18.7)	16 (23.2)	0.32
Comorbidities – no. (%)					
Inflammatory diseases	89 (56.7)	55 (62.5)	109 (59.9)	44 (63.8)	0.72
Hematological problems	11 (7.0)	13 (14.8)	23 (12.6)	13 (18.8)	0.06
Multiple trauma	1 (0.6)	7 (8.0)	13 (7.1)	1 (1.4)	<0.01
Surgery – no. (%)					
Cardiac	36 (22.9)	13 (14.8)	33 (18.1)	15 (21.7)	0.41
Abdominal	4 (2.5)	3 (3.4)	13 (7.1)	5 (7.2)	0.17
Transplantation	3 (1.9)	0 (0.0)	2 (1.1)	1 (1.4)	0.71
Other surgery	20 (12.7)	16 (18.2)	28 (15.4)	13 (18.8)	0.58
Severity of illness (PRISM score) on day of randomization					
Mean ± SD	3.9 ± 3.8	4.9 ± 4.1	4.5 ± 4.6	5.6 ± 4.8	0.05
Median [IQR]	3.0 [1.0 – 6.0]	4.0 [2.0 – 7.0]	3.5 [0.2 – 7.0]	4.0 [2.0 – 9.0]	0.04

Percentages may not sum to 100 because of rounding.

Abbreviations: SD, standard deviation; IQR, interquartile region; PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality

* Reference group.

† In the restrictive-strategy group of the TRIPICU study, the hemoglobin threshold for transfusion was set at 7 g/dL, with a target range after transfusion of 8.5 to 9.5 g/dL.

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Appendix 1 Definitions of grouped patient characteristics

Table A.1 Grouped patient characteristic definitions

VARIABLE	DEFINITION
Immunomodulatory drugs	chemotherapy, cyclosporine, azathioprine, anti-lymphocyte globulin (ALG), muromonab-CD3 (OKT_3) or anti-thymocyte globulin (ATG), and other immunomodulatory drugs
Comorbidities	inflammatory diseases, hematological problems, and multiple trauma
Inflammatory diseases	systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS)[1]
Hematological problems	hematological dysfunction, disseminated intravascular coagulation, other hematological problems
Surgery	cardiac, abdominal, transplantation, and other surgery defined as neurosurgery, scoliosis, abdominal surgery, and other kinds of surgery

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CHAPTER 5 – LENGTH OF STORAGE OF RED BLOOD CELL TRANSFUSIONS AND HOSPITAL-ACQUIRED INFECTIONS

5.1 Preamble

The results of our scoping review demonstrated that some TRIM mechanisms may be associated with the length of storage of transfused RBC units. Thus, the third study of this thesis aims to evaluate the existence of an association between the transfusion of RBC units stored for different periods of time and HAI incidence rate.

To do so, we have performed a secondary analysis of the TRIPICU database. For this secondary analysis, we included a subset of 257 patients that received only one RBC transfusion during the study period. This allowed us to clearly define the length of storage of transfused RBC units. The original TRIPICU investigators recorded the shelf life for all leukoreduced RBC units given to TRIPICU participants, thus allowing us to research the question regarding age of blood and the incidence rate of HAIs.

5.2 Manuscript 3

Association Between Length of Storage of Transfused Leukoreduced Packed Red Blood Cell Units and Hospital-Acquired Infections in Critically Ill Children: A Secondary Data Analysis of the TRIPICU Study

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Conflicts Of Interest

The authors have no conflicts of interest.

ABSTRACT

Introduction: Red blood cell (RBC) transfusions are commonly administered in critically ill children. Despite their benefits, observational evidence suggests an association between RBC transfusions and hospital-acquired infections (HAIs). One possible mechanism for increased HAI is transfusion-related immunomodulation due to the release of bioactive substances as blood to be transfused ages. We evaluated the association between pre-storage leukoreduced packed RBC storage length and the HAI incidence rate in stable, critically ill children.

Methods: In this secondary analysis of the "Transfusion Requirement in Pediatric Intensive Care Units" (TRIPICU) study, we analyzed a subset of 257 study participants that received only one transfusion. The intervention was separated into the following categories: 1) transfusion of "fresh" leukoreduced RBCs (≤10 days), 2) transfusion of "stored" leukoreduced RBCs (21-34 days), and 3) transfusion of "long-stored" RBCs (≥35 days). These were all compared to a "golden" period (11-20 days), representing the time between "fresh" and "stored". We analyzed the data using descriptive statistics and Quasi-Poisson multivariable regression models to estimate the HAI incidence rate ratio (IRR) and its corresponding 95% confidence intervals (CI).

Results: Of 257 patients, 98 (38%) received fresh blood compared to 51 (20%), 15 (6%), and 93 (36%) receiving "stored", "long-stored", and "golden" blood, respectively. In a multivariable regression model, the association of length of storage time of leukoreduced RBCs was not statistically significant in the "fresh" group (IRR 1.23; 95% CI 0.55, 2.78) and the "stored" group (IRR 1.61; 95% CI 0.63, 4.13) when compared to the "golden" period. However, it was statistically significant in the "long-stored" group (IRR 3.66; 95% CI 1.22, 10.98), showing that "long stored" blood increased the incidence rate of HAI.

Conclusion: Transfusion of leukoreduced RBC units stored for ≥35 days is associated with increased HAI incidence rate in stable, critically ill children.

INTRODUCTION

Red blood cell (RBC) transfusions are a standard supportive measure for critically ill children. It is estimated that 49% of children admitted to 30 North American pediatric intensive care units (PICU) received at least one RBC transfusion during their admission.[1] Transfusing RBCs in critically ill patients is primarily performed to increase low hemoglobin levels and oxygen delivery to better support dysfunctional organs.[2-4] However, observational studies suggest an association between RBC transfusions and the risk of hospital-acquired infection (HAI).[5-9] One proposed mechanism for this association is transfusion-related immunomodulation (TRIM).

RBC units can be stored for up to 42 days. As blood ages, biological and metabolic changes occur, commonly known as the "storage lesion."[10] This happens when aged RBCs undergo hemolysis, which leads to decreased oxygen affinity and release of bioactive substances, e.g., including many pro- and anti-inflammatory substances like cytokines and extracellular vesicles.[11] The release of the aforementioned bioactive substances has been shown to potentially lead to immunosuppression of the transfusion recipient through TRIM.[12-16]

Due to the potential problems associated with stored RBCs, it was hypothesized that transfusion of fresh blood, i.e., blood that has been recently donated, could mitigate the immunological changes associated with stored blood. Randomized controlled trials (RCT) have studied the association between the length of storage of RBCs and HAIs in critical patients, but none could prove that fresh blood was superior to stored blood regarding the risk for HAIs.[17-23] In fact, some RCTs have shown an increase in HAI for patients who were transfused fresh blood.[18-20,22]

We hypothesize that both fresh (≤10 days) and stored (≥ 21 days) leukoreduced RBC units would be associated with an increase in the incidence rate of HAI. A study by Mack et al. suggested that the effect of storage age is nonlinear.[24] Our study aims to determine the association between length of storage of transfused leukoreduced packed RBCs and the overall HAI incidence rate in critically ill children. To do so, we performed a secondary analysis of the Transfusion Requirements in Pediatric Intensive Care Units (TRIPICU) study.[25]

METHODS

Study Design

The TRIPICU study was a non-inferiority RCT performed in 19 PICUs across Canada, Belgium, the United Kingdom, and the United States from 2001 to 2005.[25] For this secondary analysis, data were analyzed as an observational study.

Participants

TRIPICU included 637 hemodynamically stable critically ill children between the ages of 3 days and 14 years who had at least one hemoglobin concentration of ≤ 9.5 g/dL within the first 7 days of admission to the PICU. Hemodynamic stability was defined as mean systemic arterial pressure not < 2 standard deviations below the normal mean for age and no increment of cardiovascular treatments and fluid administration at least 2 hours before enrolment. Children were excluded if they were expected to stay < 24 hours in the PICU, patient's study participation was not approved by treating physician, had acute blood loss, weighed < 3.0 kg, had cardiovascular problems, were never discharged from neonatal ICU, had hemolytic anemia, or were enrolled in another study.

This secondary analysis focused on a subset of 257 patients that received only one RBC transfusion during the study period. The shelf life for all RBC units given to TRIPICU participants had been recorded. Using patients who received one RBC transfusion only, we were able to clearly define the length of storage of transfused RBC units and avoid confounding by indication and multiple transfusions. Patients with previous blood transfusions during the hospital stay that led to study enrollment were excluded to ensure that there were no residual effects from transfusions received before the trial. We also excluded patients for whom the study protocol had been temporarily suspended to avoid selection bias, as suspensions primarily occurred due to emergencies (e.g., acute respiratory distress syndrome, worsened shock, or increased bleeding) whose management could have increased the patient's risk for HAI.

Interventions

In this secondary analysis of patients that received only one transfusion, the intervention was categorized into three categories: 1) the transfusion of "fresh" leukoreduced RBC units, 2)

the transfusion of "stored" leukoreduced RBC units, and 3) the transfusion of "long-stored" leukoreduced RBC units. These interventions were compared to a "golden" period. Storage age was defined as the number of days between blood donation and blood transfusion.

For the purpose of this study, "fresh" RBC was defined as RBC units whose shelf life was ≤ 10 days, as white blood cells and dendritic cells present in the RBC units after leukoreduction are still viable during this period, which may increase the risk for TRIM.[26-28] "Stored" RBC was defined as RBC units whose shelf life was 21-34 days and "long-stored" RBC, as RBC units with a shelf life of ≥ 35 days. Literature shows that the major effects of TRIM mechanisms associated with stored blood are more evident after 3 weeks of storage.[29,30] In addition, studies have demonstrated an association between harmful outcomes and RBC units transfused after 35 days of storage.[31,32] Finally, we used leukoreduced RBC units stored between 11 days and 20 days ("golden" period) as the reference category; the reference category was determined *a priori* based on storage time associated with the potential aforementioned TRIM mechanisms.

Outcomes

The primary outcome of this secondary analysis was the overall incidence rate of HAIs. We accounted for all HAI episodes that happened after randomization as the HAI incidence rate numerator. Our denominator was the number of patient-days in the PICU after randomization, defined as the number of days between the randomization date and PICU discharge date or date of death during PICU stay. For patients who had long PICU stays, follow-up was truncated at day 28 after randomization.

HAIs during the TRIPICU study were diagnosed by medical teams and/or infection control teams. They were validated by TRIPICU site investigators and/or infection control teams. Medical teams were not blinded for the intervention. The HAIs included central line-associated bloodstream infections (CLABSI), bloodstream infection, sepsis, pneumonia, tracheitis, upper respiratory tract infection, catheter-associated urinary tract infection (CAUTI), urinary tract infection, surgical site infection (SSI), meningitis, ventriculoperitoneal shunt infection, mediastinitis, conjunctivitis, and otitis media.[33] In TRIPICU, HAIs were defined according to the Centers for Disease Control and Prevention (CDC, 1988) definitions, as they were considered the gold standard at the time.[33] However, to increase the specificity of CLABSI and CAUTI, the

research team used a modified definition that required the presence of positive bacterial cultures.[34] Importantly, these definitions are very similar to the CLABSI and CAUTI definitions currently recommended by CDC in 2020.[35]

Data Collection

Data was collected in the TRIPICU study by medical teams using patient data collection forms. We utilized data on demographics (age, weight, sex), length of stay in PICU before randomization, TRIPICU study group assignment, use of immunomodulatory drugs, comorbidities, surgery, and severity of illness on the day of randomization. Immunomodulatory drugs were defined as chemotherapy, cyclosporine, azathioprine, anti-lymphocyte globulin (ALG), muromonab-CD3 (OKT_3) or anti-thymocyte globulin (ATG), and other immunomodulatory drugs, including steroids. Comorbidities included inflammatory diseases, hematological problems, and multiple trauma. Inflammatory diseases were defined as systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS).[36] Hematological problems were defined as hematological dysfunction, disseminated intravascular coagulation, other hematological problems. Surgery included cardiac, abdominal, transplantation, and other surgery defined as neurosurgery, scoliosis, abdominal surgery, and other kinds of surgery. Pediatric Risk of Mortality (PRISM) score was used to determine the severity of illness on the day of randomization.[37]

Statistical Analysis

We used descriptive statistics to summarize the characteristics of the study groups. Data were analyzed using means (standard deviation [SD]) or medians (interquartile ranges [IQR]) for continuous variables and proportions for categorical variables. As the descriptive statistics data were not normally distributed, we used the Kruskal-Wallis test to compare continuous variables. Categorical variables were analyzed using the chi-square test or Fisher's exact test.

Our study outcome measure of effect was the incidence rate ratio (IRR) of HAI and its 95% confidence interval (CI). As study data did not follow a Poisson distribution, we used multivariable Quasi-Poisson regression models to analyze the association between length of leukoreduced RBC storage and HAI incidence rate. An offset of log(ICU patient-days) was used

to account for the patients' varying follow-up days. Analyses were conducted using R version 4.0.0.[38]

RESULTS

Of the original 637 patients randomized in the TRIPICU study, 257 (40.3%) received only one RBC transfusion and were included in this secondary analysis. Table 1 shows the baseline demographic and clinical characteristic variables for participants. There were significant differences between the groups regarding patient age, sex, multiple trauma, cardiac surgery, other surgery, PRISM score on the day of randomization, and the use of immunomodulatory drugs. Table 2 shows a summary of the observed HAIs in each group.

Compared to the reference category, defined as patients who were transfused RBCs stored from 11 to 20 days ("golden period"), the HAI incidence rate for patients who received "fresh", "stored", and "long-stored" RBCs were higher (Table 1). The results of the adjusted quasi-Poisson multivariable model (Table 3) were not statistically significant for transfusion of "fresh" (IRR 1.23; 95% CI 0.55, 2.78) or "stored" (IRR 1.61; 95% CI 0.63, 4.13) RBCs. The transfusion of "long-stored" RBCs was associated with an increase in the HAI incidence rate (IRR 3.66; 95% CI 1.22, 10.98).

DISCUSSION

This secondary analysis of the TRIPICU study aimed to evaluate the association between length of storage of transfused leukoreduced RBCs and the overall HAI incidence rate in critically ill children. The transfusion of leukoreduced "long-stored" RBCs (i.e., RBC units stored for ≥35 days) was associated with an increase of the HAI incidence rate in stable, critically ill children. In addition, a trend towards increased HAI incidence rate after transfusion of "fresh" and "stored" RBCs was observed.

These results supported the hypothesis that the transfusion of "long-stored" RBCs increases the risk of HAIs. Historically, it was theorized that stored leukoreduced blood was harmful to patients due to RBCs' physiological changes during storage, resulting in TRIM.[39-41] Several studies have shown an increase in bioactive RBC-derived microparticles and

microvesicles as storage time increases.[15,16,42] RBC membranes consist primarily of lipids, and free lipid particles have been demonstrated to increase after 2 to 3 weeks of storage.[43] Such microparticles have been shown to inhibit the release of important cytokines, including interleukin (IL)-8, IL-10, and tumour necrosis factor-alpha.[42] Additionally, the number of RBC extracellular vesicles increases during RBC units' storage, ultimately modifying T cell responses and increasing pro-inflammatory capability through various cytokines.[15,16] Based on these multiple studies, RBC-derived microparticles and extracellular vesicles can result in immunosuppression or increased inflammation, thus altering the response to bacterial pathogens depending on the bioactive substance released.

Given the potential increased risk for HAI after transfusion of older blood, multiple studies evaluated the effect of transfusing fresher RBC units. The hypothesis behind these studies was that the transfusion of fresher blood might result in better patient outcomes, including lower mortality.[17-23,44,45] However, not only several RCTs showed that transfusing fresh blood does not improve patient outcomes as previously expected, but they also reported inconclusive results regarding the potential effect of fresh blood transfusion on the risk for HAIs.[17-23]

Five RCTs studied the effect of fresh blood in adult critically ill patients, and all except one used leukoreduced RBCs.[17,19-21,23] A study by Cooper et al., including 4,919 patients, reported an odds ratio (OR) of 0.90 (95% CI 0.57, 1.42) associated with the transfusion of fresh blood (freshest available).[17] Lacroix et al. found a higher incidence rate of HAIs in critically ill adults who received fresh (<8 days) vs. stored RBC units (34.1% vs. 31.3%; absolute risk reduction in the standard delivery group of 2.8%, 95%CI –0.9, 6.5).[19] Two studies in adult ICU surgical patients demonstrated that HAIs were diagnosed more frequently in patients who received stored blood than in the fresh blood group.[21,23] Spadaro et al. was the only study that did not use leukoreduced RBCs, and they found a higher risk of HAIs in the stored group compared to the fresh group (≤14 days; relative risk [RR] 1.17; 95% CI 0.71, 1.93).[21] Similarly, Steiner et al. found that the proportion of HAIs was 8% in the fresh (≤10 days) group compared to 9% in the stored (≥21 days) group (p = 0.59).[23] However, when Schreiber et al. studied adult ICU trauma patients, they observed a higher proportion of patients diagnosed with HAIs in the fresh leukoreduced RBC group (30% fresh [≤14 days] vs. 25% stored, p = 0.77).[20]

In the pediatric critical care population, Spinella et al. reported an absolute risk difference for HAIs of 0.1% (95% CI -1.6, 2.0) when comparing patients who were transfused fresh (≤7

days) vs. stored RBCs.[22] Furthermore, Fergusson et al. showed that the risk of HAIs in neonatal intensive care unit (NICU) patients receiving fresh (≤7 days) RBC transfusions were 2% higher than the risk of premature babies receiving stored blood; however, this result was not statistically significant (RR 1.02; 95% CI 0.88, 1.19).[18]

One possible explanation for these contradicting results is that both fresh and stored leukoreduced blood increase the incidence of HAIs. It was initially hypothesized that leukoreduction could eradicate the risk for TRIM since the most well-studied TRIM mechanisms are related to the presence of white blood cells in transfused blood.[41] However, it is important to note that leukoreduction does not completely remove white blood cells from RBCs units, as up to $5x10^6$ can be found in each leukoreduced RBC unit.[46] Consequently, it is possible that the remaining immunologically active white blood cells present in transfused blood would lead to a downregulation of the transfusion recipient's immune system. This is of particular interest for fresh blood, as studies showed that the remaining white blood cells are immunologically active, with their apoptosis peaking between 7 and 14 days of storage.[26]

A study by Reed et al. showed that the freshness of stored blood influenced the survival and function of white blood cells, as microchimerism, defined as the persistence of donor cells in recipients years after blood transfusions that occur in a state of reciprocal tolerance, could not be detected in adult trauma patients who received blood stored for more than 12 days.[47] Blood donor's dendritic cells that are human leukocyte antigen (HLA)-DR matched to the transfusion recipient can present antigens to recipient T cells through a process called direct allorecognition.[48] The interaction results in mononuclear cells being able to persist in low levels in the recipient (microchimerism).[49] Markowicz and Engleman showed that dendritic cells are only viable for up to one week after blood is donated.[20] This may explain why Reed et al. found that microchimerism was associated with fresh blood, as one mechanism by which microchimerism can occur is through the interaction mentioned above involving dendritic cells.[47] The clinical consequences of microchimerism for transfused patients could include graft-vs.-host or auto-immune effects. Thus, based on the aforementioned studies, it is plausible that the transfusion of both fresh blood and stored blood may lead to immunosuppression by different mechanisms.[50]

Our study has limitations. The date when the HAI was diagnosed was not recorded in the TRIPICU study. However, all events happened after patient randomization. Furthermore, the

original study did not collect device days, except for mechanical ventilation, which prevented us from calculating the incidence rate for different HAIs. Nevertheless, this did not impact the overall HAI incidence rate calculation as its denominator is PICU-patient days. The follow-up period for HAIs ended on the day of PICU discharge, which made it impossible to capture HAIs occurring within 24 hours after PICU discharge. According to the CDC's infection control definitions, such HAIs would still be attributable to the PICU.[35] An additional limitation would be the risk of bias if HAIs happened after randomization but before blood transfusion. However, this risk is low as TRIPICU participants' time from randomization to transfusion was 0.1 days in the liberal-strategy group and 1.7 days in the original restrictive-strategy group. Finally, there may be potential for uncontrolled confounding factors and a lack of adjustment. As there is a large imbalance between the groups concerning specific variables, including age, PRISM score, and type of surgery, there may be the possibility that the model fit is incomplete.

Nevertheless, our study has several strengths. The TRIPICU study had HAIs as a secondary outcome and consequently recorded high-quality granular data on HAI risk factors and blood transfusions. Also, the HAI definitions used in the TRIPICU study followed the CDC 1988 definitions, and some were modified to include the presence of positive bacterial cultures.[33,34] The use of laboratory-confirmed HAI definitions aligns well with the current HAI definitions proposed by the CDC.[35] Another strength of our study is the use of a "golden" period as the comparison group. Its definition was based on biological data on potential TRIM mechanisms associated with the length of RBC storage.[26-28] Additionally, our study used incidence rate ratios as our outcome measure; this allowed us to account for patients with varying times at risk for developing HAI due to different PICU length of stay. Lastly, there is the risk for unmeasured confounders as the database used, despite being very comprehensive, did not include data on all the risk factors for HAI.

Understanding the association between age of blood is essential to provide the best and safest care for pediatric patients. Our finding that RBCs stored for ≥35 days are associated with a higher HAI incidence rate may be used to inform blood transfusion protocols for severely ill children. This further highlights the need for more high-quality research to determine the association between RBC length of storage and HAIs.

Table 1 Clinical Characteristics of the patients – length of storage of blood

Variable (total N= 257)	Fresh Group (N = 98)	Golden Period Group * (N = 93)	Stored Group (N = 51)	Long Stored Group (N = 15)	P Value
Length of storage of RBC – days	0 to 10	11 to 20	21 to 34	35 to 42	–
Demographics					
Age – months					
Mean ± SD	17.6 ± 31.6	32.4 ± 43.7	42.7 ± 48.6	70.5 ± 54.0	<0.01
Median [IQR]	4.0 [2.0 – 17.8]	11.0 [5.0 – 41.0]	22.0 [4.5 – 71.5]	92.0 [14.5 – 104.0]	<0.01
Weight – kilograms					
Mean ± SD	9.5 ± 9.9	12.0 ± 10.0	16.2 ± 15.0	22.7 ± 15.4	<0.01
Median [IQR]	6.0 [5.0 – 11.0]	9.0 [6.0 – 14.0]	10.0 [6.0 – 21.0]	23.0 [10.0 – 27.5]	<0.01
Male sex – no. (%)	59 (60.2)	70 (75.3)	30 (58.8)	9 (60.0)	0.10
Length of stay in PICU before randomization – days					
Mean ± SD	2.1 ± 1.6	2.1 ± 1.5	2.4 ± 1.7	2.0 ± 1.4	0.62
Median [IQR]	2.0 [1.0 – 3.0]	2.0 [1.0 – 3.0]	2.0 [1.0 – 4.0]	1.0 [1.0 – 2.5]	0.64
Restrictive strategy group – no. (%) †	31 (31.6)	30 (32.3)	15 (29.4)	5 (33.3)	0.98
Immunomodulatory drugs – no. (%)	23 (23.5)	18 (19.4)	11 (21.6)	2 (13.3)	0.82
Comorbidities – no. (%)					
Inflammatory diseases	61 (62.2)	48 (51.6)	35 (68.6)	9 (60.0)	0.21
Hematological problems	12 (12.2)	8 (8.6)	7 (13.7)	2 (13.3)	0.72
Multiple trauma	2 (2.0)	5 (5.4)	6 (11.8)	3 (20.0)	0.01
Surgery – no. (%)					
Cardiac	20 (20.4)	21 (22.6)	5 (9.8)	1 (6.7)	0.17
Abdominal	8 (8.2)	4 (4.3)	2 (3.9)	0 (0.00)	0.61
Transplantation	1 (1.0)	0 (0.0)	0 (0.0)	1 (6.7)	0.15
Other surgery	12 (12.2)	16 (17.2)	9 (17.6)	5 (33.3)	0.21
Severity of illness (PRISM score) on day of randomization					
Mean ± SD	4.9 ± 4.6	4.1 ± 4.1	3.8 ± 3.5	6.7 ± 5.2	0.06
Median [IQR]	4.0 [1.0 – 7.0]	3.0 [1.0 – 6.0]	3.0 [1.0 – 5.0]	7.0 [2.5 – 10.0]	0.14

Percentages may not sum to 100 because of rounding.
Abbreviations: SD, standard deviation; IQR, interquartile region; PICU, pediatric intensive care unit; PRISM, Pediatric Risk of Mortality.
* Reference group.
† In the restrictive-strategy group of the TRIPICU study, the hemoglobin threshold for transfusion was set at 7 g/dL, with a target range after transfusion of 8.5 to 9.5 g/dL.

Table 2 Summary of observed hospital-acquired infections (HAI) in the groups

Variable (total N= 257)	**Fresh Group (N = 98)**	**Golden Period Group * (N = 93)**	**Stored Group (N = 51)**	**Long Stored Group (N = 15)**
Length of storage of RBC – days	0 to 10	11 to 20	21 to 34	35 to 42
Type of Hospital-acquired Infection (HAI)				
Catheter-related site infection	0	0	0	0
Catheter-related bacteremia	0	1	1	0
Catheter-related sepsis	1	0	0	0
Nosocomial bacteremia	1	1	2	0
Nosocomial urinary tract infection	1	3	0	2
Nosocomial wound infection	0	0	2	0
Nosocomial sinusitis	0	0	0	0
Nosocomial upper respiratory tract infection	2	1	0	1
Nosocomial pneumonia	5	4	1	2
Nosocomial lower respiratory tract infection, excluding pneumonia	1	2	1	1
Other nosocomial infections	1	0	1	0
Total HAI	12	12	8	6

* Reference group

Table 3 Adjusted and Non-Adjusted Incidence Rate Ratios

Length of Storage (Days)	Number of Patients	Number of Hospital-acquired Infections	Patient days (per 1000-patient days)	IR (95% CI)	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)*
Less or equal to 10 days	98	12	603	19.90 (10.28, 34.76)	1.13 (0.49, 2.61)	1.23 (0.55, 2.78)
11-20 days (reference)	93	12	679	17.67 (9.13, 30.87)	1.00 (reference)	1.00 (reference)
21 days to 34 days	51	8	296	27.03 (11.67, 53.25)	1.53 (0.60, 3.92)	1.61 (0.63, 4.13)
35 days or more	15	6	102	58.82 (21.59, 128.03)	3.33 (1.19, 9.33)	3.66 (1.22, 10.98)

Abbreviations: IR, incidence rate; IRR, incidence rate ratio; CI, confidence interval

*Quasi-Poisson model adjusted for age of patient, sex, multiple trauma, cardiac surgery, other surgery, immunomodulatory drugs, PRISM score at randomization, and TRIPICU RCT study group

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CHAPTER 6 – SUMMARY AND CONCLUSIONS

This thesis evaluated the association between blood transfusions and HAI incidence rate while specifically focusing on a cohort of critically ill children in PICUs. Our first manuscript aimed to provide an overview of the existing literature on TRIM mechanisms to identify knowledge gaps and explain how RBC transfusions may increase the HAI incidence rate. We found that TRIM mechanisms can be categorized into four groups: 1) effects related to the presence of allogeneic WBCs in transfused RBCs, 2) apoptosis of allogeneic WBCs, 3) effects related to allogeneic transfused RBCs, and 4) hemolysis of allogeneic RBCs. Additionally, different TRIM mechanisms depending on the blood's length of storage may occur if the blood is "fresh" compared to "stored," both of which were found to lead to immunosuppression.

Our second manuscript aimed to evaluate the association between RBC transfusions and the HAI incidence rate in critically ill children. Our *post hoc* secondary analysis of the TRIPICU RCT demonstrated that exposure to ≥3 RBC transfusions was associated with an increased HAI incidence rate. We observed a dose-response trend regarding the number of RBC transfusions and their volume, suggesting that children who receive multiple transfusions and/or higher volume may be at a higher risk for HAI.

Our third manuscript aimed to evaluate the association between length of storage time of RBCs transfused and the incidence rate of HAIs in critically ill children. We found that RBCs stored for ≥35 days are associated with a higher HAI incidence rate in severely ill children. Furthermore, we showed a trend towards increasing HAI incidence rate with the transfusion of both fresh and stored RBC units.

The discussions of our studies' results were already presented in the three manuscripts (Chapter 3, Chapter 4, Chapter 5). We will, therefore, focus on the interpretations and implications of our findings in this chapter.

Until now, the vast majority of papers study the association between blood transfusions and HAI using incidence proportion and not incidence rate as the outcome. Incidence proportion is calculated by dividing the number of new cases during a specified time interval by the total number of people at risk at baseline. Whereas incidence rate uses the same numerator as incidence proportion, but the denominator is the total amount of time risk experienced by all persons under observation for a specified time period. Incidence proportion describes the average

risk of developing a given outcome among a group of people during a specified observation period. Comparatively, the incidence rate is used to describe the rate of occurrence of new cases at a single instant in time. It can show how quickly disease occurs in a population.

A disadvantage of using incidence proportion is that it can only be interpreted when the time period it applies to is known. Additionally, the incidence proportion assumes that all persons under observation at baseline are followed for the entire observation period. As following all study participants for the entire study period is not usually feasible in studies due to withdrawing from the study, lost to follow-up, or competing risks, the incidence proportion may not be representative of the study. Therefore, one of the advantages of using the incidence rate, instead of incidence proportion, is that it accounts for people entering and leaving the study, as person-time is calculated for each study participant. Thus, it accounts for those study participants who are lost to follow-up or who die during the study. It also allows participants to enter the study at various time points. The original TRIPICU study used the proportion of patients that had HAIs as their secondary outcome. However, in our secondary analyses, we used the incidence rate of HAIs. This allowed us to account for patients with varying times at risk for developing HAI due to different PICU stay lengths. Using incidence rates also allowed us to account for repeatable events, as the patients' follow-up continues as long as a person remains at risk. In our Poisson regression models, the variables that were included in the models were determined by biological plausibility or by assessing collinearity between them.

Past studies have counted patients as infected or not, without acknowledging the total number of HAI episodes per patient. As mentioned above, we used all HAI episodes by including repeatable events to better understand the magnitude of the association between RBC transfusions and HAI. If we only counted the incident infection per patient, such as what incidence proportion captures, we could have underestimated the association of RBC transfusion. Our rationale to do so is based on our hypothesis that TRIM would increase the risk for all HAIs, independently of their type or pathogen. We also assumed that the association between transfusing leukoreduced RBCs and HAIs could lead to patients developing more than one HAI during their PICU admission.

Our secondary analyses used a database with a pre-determined number of patients. The original TRIPICU study included 637 participants. To minimize bias, we excluded patients who had a protocol suspension to avoid selection bias, given that suspensions primarily occurred due

to emergencies whose management could have increased the patient's HAI risk. We also excluded patients who had had previous blood transfusions during the hospital stay to ensure that our results would not be biased by the presence of residual effects from pre-trial transfusions. When specifically analyzing the association of length of storage, we restricted our analysis to patients who received only one RBC transfusion. This allowed us to better define the length of storage of transfused RBC units and avoid confounding by indication and from receiving multiple units of differing storage lengths. As a result of using a subset of the original study, it is possible that we were underpowered to detect statistically significant associations. Nevertheless, TRIPICU was a RCT that used a very strong methodology and the original investigators collected very granular data on RBC transfusions and HAIs. The hypotheses that we generated using such robust data are important for the field of transfusion medicine.

Future studies are warranted to confirm the results found in this thesis. We are currently performing an individual patient data meta-analysis investigating the association between age of blood and the incidence of HAIs. We expect that this study will be able to answer the length of storage question as it consists of individual patient data from 7 RCTs (10,788 patients)[46,49,103-107] conducted in neonatal, pediatric, and adult ICU patients that assessed the effect of length of storage time of transfused RBC units, and that collected data on HAIs.

In conclusion, in this thesis, I presented a scoping review focusing on TRIM mechanisms and described that the mechanisms could be groups into four overarching categories. In addition, through a secondary analysis of the TRIPICU RCT, I demonstrated that the transfusion of ≥3 units of leukoreduced red blood cell transfusions could lead to an increased rate of hospital-acquired infections in critically ill pediatrics. I also showed that severely ill children who are transfused RBCs stored for ≥35 days have a higher incidence rate of HAIs. Importantly, the results of this thesis will be used to inform transfusion practices and medicine transfusion research in the pediatric critical care setting, with the ultimate goal of reducing the HAI incidence rate in this susceptible population.

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