Transfusion-related risks associated with intraoperative immunoadsorption compared to plasma exchange transfusion in neonatal patients undergoing ABO-incompatible heart transplantation

Sienna Cole[1]

[1] Michener Institute of Education at UHN, Toronto, ON Canada

E-mail: 20sc7@michener.ca

Received 01/07/2021
Published 04/10/2021

Recommended by Brian Hyndman.

1. Introduction

Congenital heart defects (CHDs) are present in an average of 40,000 births per year in the United States, accounting for almost 1% of total births per year (1). CHDs are responsible for about 4.2% of all neonatal deaths, and one in four babies with CHD are classified as having a critical CHD (1). Most infants with critical CHDs will require surgery in their first year of life, emphasizing the urgency and significance of neonatal heart transplantations (1).

The invention of ABO-incompatible heart transplantations for neonates has been monumental in the field of cardiac surgery. Compared to any other age groups, neonates requiring a heart transplant are at a greater risk of waiting-list mortality for a compatible donor organ (2). In the neonatal stage of development serum anti-A or anti-B antibodies (i.e., isohemagglutinins) are not present in sufficient quantities to elicit an immune response; therefore, neonates can accept organ transplantations from donors with an incompatible blood type from their own (3). This discovery significantly reduced the waiting times for neonates to receive heart transplantations via elimination of the requirement for donor and recipient compatibility, and decreased mortality during this sensitive time period (4).

Plasma exchange transfusion (PET) before cardiopulmonary bypass (CPB) surgery is currently implemented during ABO-incompatible neonatal heart transplantations to lower circulating anti-A or anti-B antibody levels (5). Although PET is effective in decreasing the risk of acute organ rejection in neonatal patients, patients were exposed to large amounts of blood products, and immunologically incompatible blood from genetically diverse donors (5). The addition of blood products and the process of red blood cell (RBC) transfusions can drastically influence clinical outcomes (6). RBC transfusions expose patients to risks such as transfusion-related lung injury, hemolytic transfusion reactions, and transfusion-related circulatory overload, and have been strongly associated with increased rates of postoperative morbidity and mortality (6,7).

Intraoperative immunoadsorption was first reported to effectively reduce isohemagglutinin levels in neonates undergoing ABO-incompatible heart transplantation in 2018, while decreasing the requirement of blood products (8). This procedure has only been investigated by one team of researchers at the Great Ormond Street Hospital in London, United Kingdom. Thus, it is unclear if this has generalizability to a larger neonatal patient population. Furthermore, there have been no reports on the relationship between intraoperative immunoadsorption and the risk of postoperative mortality and morbidity in patients, or other transfusion-related risks vs. PET.

The aim of the proposed pilot study is to evaluate the transfusion-related risks associated with intraoperative immunoadsorption compared to PET in neonatal patients who undergo ABO-incompatible heart transplantation

2. Literature Review

2.1 Clinical Relevance Neonatal heart transplantations were invented in the 1980's for infants with CHD unamenable to any other corrective therapy (2). Neonatal patients who underwent heart transplantation were proven to have extremely successful long-term survival outcomes in contrast to other forms of solid organ transplantation (9). However, the limited donor pool has had severe consequences for neonates in need of a heart transplant. Infants who require heart transplantation experience an average wait time of approximately 110 days and have a waiting list mortality of 34%, the highest waiting list mortality compared to all other age categories (2).

Sadly, the number of infants placed on the waiting list has gradually increased each year, whereas the number of donors has remained relatively constant (2). Indications for neonatal heart transplants have also been restricted in efforts to address the high demand yet low supply of neonatal donors (2). Currently the indications for newborn heart transplantation include severe cardiomyopathy involving primary tumors, single ventricle physiology with decreased ventricular function, pulmonary atresia-intact ventricular septum with right ventricle dependent coronary circulation, and severe Ebstein's anomaly (2). Therefore, strategies to increase donor availability are of the utmost importance to ameliorate the crucial issues surrounding neonatal heart transplantation.

2.2 Evolution of Neonatal Heart Transplantation In 1967, Adrian Kantrowitz attempted the first neonatal heart transplantation on a 19 day-old infant who suffered from Ebstein's anomaly (10). The surgery was performed, but the patient died 6.5 hours after the procedure due to cardiac arrest (10). In 1978 the topic of early immunity was researched and heart transplantation in neonates was reevaluated for treatment of hypoplastic left heart syndrome (HLHS) (11). During this time, immature baboons were explored as potential clinical donors (12). In July 1984, an 11-day old baby with HLHS underwent a cardiac allotransplantation in London, however several postoperative complications resulted in her death 18 days later.

Following the allotransplantation, in October 1984, an orthotopic cardiac xenotransplantation was performed on a 12-day old human neonate with HLHS known as 'Baby Fae' (12). A female infant baboon was selected as the donor, unfortunately the recipient died 20 days after the surgery (12). After multiple attempts and challenges, the first successful human neonatal heart transplant occurred on November 20, 1985 and became a routine surgical technique in the following decade (2).

2.3 Neonatal Immunity Solid organ transplantations between incompatible blood groups were previously futile. A retrospective observational study of 4895 heart transplants across 66 centers globally in 1990 revealed that eight patients had received ABO-incompatible hearts. Five of these eight transplant cases were subjected to lethal outcomes, caused by a hyper-acute rejection instigated by recipient isohemagglutinins (13). These proteins bind to their corresponding carbohydrate blood group antigens on endothelial cells of the donor organ and initiate the complement cascade, triggering immediate thrombosis in the graft vasculature (14). Infants and neonates were found to have insufficient anti-A or anti-B titres until they reached 12-14 months of age (3). Thus, it was concluded that neonatal immune systems are incapable of eliciting a robust immune response against carbohydrate antigens, preventing the initiation of the complement cascade and hyper-acute rejection against foreign tissue.

The reasons for neonatal tolerance of transplants are still relatively unknown. It has been suggested that the deletion of autoreactive T-cells plays a role, as well as the presence of an immature antigen-presenting system, low cellular chimerism, and the regulatory effects of the neonatal immune response influenced by donor and host cell interactions (4).

2.4 ABO-Incompatible Heart Transplantation In 2001, ABO-incompatible heart transplants were performed on 10 infants for the first time at the Hospital for Sick Children in Toronto, Canada (15). This procedure was revolutionary, due to the previous detrimental effects of incompatible organ transplants.

Issitt *et al.* concluded that a PET performed before the initiation of CPB additionally lowered the circulating isohemagglutinin titres in infants (5). In 2012, 21 patients from ages 3-44 months were subjected to a "3 times" PET prior to ABO-incompatible heart transplantation (5). Packed red blood cells (PRBCs) of the recipient blood group and donor compatible fresh frozen plasma (FFP) were used to exchange patient blood volume (5). To achieve a "3 times" PET the pump prime was mixed to have an FFP: PRBC ratio of 1:1, allowing a prime volume of 3 times the circulating volume at a hematocrit of 30% to be administered and exchanged with the patient's circulating blood volume (5). CPB using an adult reservoir, pediatric oxygenator, ultrafiltration pump, extracorporeal circuit, and "3 times" PET resulted in an ideal reduction in patient isohemagglutinins, confirmed by a quick spin antibody test (5). The "3 times" PET produced hemodynamic stability in patients, accompanied by no signs of organ rejection (5).

Although PET is an effective technique to facilitate ABO-incompatible heart transplants, the use of blood products during CPB has been associated with negative postoperative outcomes in patients (7). In a retrospective cohort study performed in the United Kingdom, three databases were used to analyze adult patients who underwent cardiac surgery (7). The PATS database, blood bank database of blood products administered, and hematology database with blood test results were reviewed and two outcomes of interest were evaluated (7). A composite ischemic outcome defined as patients who experienced stroke, renal

complications, or myocardial infarction, and composite infection outcome such as respiratory infection, septicemia, or wound infection were the two primary outcomes implemented in this multivariable logistic regression model (7). It was determined that RBC transfusion was highly associated with composite infection and ischemic outcomes (7). In addition, there was an association between transfusion and increased morbidity resulting in increased admission costs, as well as ICU and total postoperative hospital stays (7). Furthermore, patients who received transfusions had a hazard of death almost six times higher in the first 30 days than patients who did not receive transfusions (7). Thus, transfusions were found to be associated with higher risks of morbidity and mortality in adult cardiac surgery patients.

An analysis of hemovigilance records from Quebec, Canada in 2007, and from France, the United Kingdom, and the United States in 2009 revealed that transfusion-related lung injury, hemolytic transfusion reactions, and transfusion-related circulatory overload were the three primary causes of transfusion-related mortalities during CPB (6).

Intraoperative immunoadsorption was implemented in the CPB circuit during neonatal heart transplantations in 2018 and effectively reduced isohemagglutinin levels in ABO-incompatible neonates, while decreasing the requirement of blood products (8). The modified intraoperative immunoadsorption circuit functions to separate plasma from the circulating volume (8). A plasma separator (Asahi Kasei PS-03; LINC Medical Systems Ltd, Leicester, UK) was placed in parallel to the hemofilter (HF-06; LivaNova) combined with a positive screw locking (POS lock)-ended wye connector to the hemofiltration line (8). A second POS lock-ended wye connector was placed distally from the hemofilter and plasma separator to combine the two flows (8). From this point a wye connector was added to direct flow to the venous reservoir and the 1/8” line to the right atrium (8). The differences between the intraoperative immunoadsorption and PET circuits are shown in Figure 1. The intraoperative immunoadsorption circuit introduced the immunoadsorption pump in addition to the ultrafiltration pump incorporated in the PET circuit.

The first clinical case that documented the use of this technique administered two units of PRBCs and one unit of plasma, a total volume of 720mL to the patient (8). Alternatively, if PET was used the patient would have received eight units of PRBCs and ten units of plasma, a volume equivalent to 4000mL (8).

2.5 Contribution to Knowledge Base Although intraoperative immunoadsorption has been performed during heart transplantation in ABO-incompatible neonates and yielded successful results, there has been little research to compare this technique with PET, the current standard to reduce circulating isohemagglutinins prior to CPB. Due to the small patient population, PET and intraoperative immunoadsorption have been studied using retrospective or case study models. This investigation would be the first randomized control trial to directly compare transfusion-associated risks, including postoperative morbidity and mortality, as well as ischemic and infection outcomes in neonates. Previous studies that examined the effectiveness of PET and intraoperative immunoadsorption reported the risk of postoperative mortality among patients, however endpoints to assess the wide range of transfusion-associated risks were not measured.

One key limitation to evaluating this relationship and comparing the effectiveness of these two procedures would be the small population size of neonates who require heart transplants. As the donor pool is low, this would pose a limitation as to how many patients can be included in the sample and could affect the ability to make statistically significant conclusions from the collected data.

This study has meaningful clinical relevance, as the findings from this experiment could be used to support practices and decisions that decrease the risk of mortality of neonates waiting for a compatible donor heart prior to transplantation. Transfusion-related risks pose a large concern in regard to patient ischemic and infection outcomes, as well as postoperative morbidity and mortality (6,7). By investigating how intraoperative immunoadsorption and PET impact variables associated with transfusions, ABO-incompatible surgery can be improved to deliver the highest quality of care to neonatal cardiac patients. In the future, techniques used to optimize ABO-incompatible heart transplantation could increase generalizability leading to more reliable evidence-based decision making regarding the use of incompatible donor organs in other organ transplant surgeries.

2.6 Ethical Issues and Mitigation Strategies

Recruiting patients will be difficult not only due to the small population of neonates who require heart transplantation, but also because of the many challenges associated with approaching parents at this emotionally stressful time when their baby is ill (16). This study and the potential risk of adverse outcomes requires informed consent to be given quickly, as the timescale for making a decision to proceed with a neonatal transplant is often short. The attitudes of parents with respect to enrolling their neonate in clinical trials is the main ethical issue that needs to be addressed prior to carrying out this proposed RCT.

A systematic review conducted in 2015 revealed the motivation of parents who declined or consented to their neonate’s participation in clinical trials, raising important ethical considerations (16). Parents consented to participation in research because of altruistic motives that the given clinical trial would benefit other babies, parents, or society at large, and that their own baby would benefit themselves (16). In contrast, parents who declined to participate in research reported that it was inconvenient or a burden for their neonate and conveyed their apprehension about the potential

risks and harms that may ensue as a result of participation in the study (17).

Feelings of dread, fear, vulnerability, and confusion have been noted by parents who have the opportunity to make a decision on behalf of their neonate and can influence their willingness to consent to medical research (18). In previous studies, parents reported feeling pressured or coerced into participation, where some were unaware of their right to refuse participation under the premise of voluntary consent (19).

Clinicians have been found to respect parent authority and are proponents of the informed consent process (20). Clinicians who argue against obtaining informed consent from the parents of neonates believe that ultimately the clinician is the best decision maker for sick neonates (21). Parents and clinicians may have conflicting opinions on the optimal decision that benefits the neonate, which may compromise parent authority.

In order to give valid consent, parents of neonatal patients must be of sound mind and mentally capable of making a decision on their infant's behalf (16). The competence and capacity for a parent to make this decision may be influenced by many factors including the degree of understanding achieved after explanation of the proposed study, emotional state, and time available before the decision must be confirmed (16). Studies to evaluate the emotional state of parents who gave informed consent revealed that parents experienced a range of emotions such as anxiety and stress; the extent to which these emotions impaired their ability to make an informed decision was shown to vary (19).

The understanding of parents about an intended research study has also been reported to be variable (19). Some parents exhibited a clear comprehension of a proposed trial, however many parents displayed little or no understanding of the trial (19,22). When comparing the researcher's particular classification of potential risks, it was evident that some parents did not comprehend the risks correctly (16).

Time may also heavily influence the process of informed decision making due to the constraints it poses on individuals to process information (16). Two observational studies evaluated clinical trials with different timescales and levels of risk to determine the impact of time on decision making in parents with neonates (19,23). It was concluded that although a majority of parents believed they had adequate time, there was a subset of parents who felt that they did not have ample time to make their decision (19,23).

The ethical considerations discussed that relate to informed consent such as motivation for enrollment, emotional state, mental capacity, and time constraints may potentially affect the proposed RCT involving neonates undergoing ABO-incompatible heart transplantation (16). Clinicians involved in this RCT will provide parents of neonatal patients with information required to make an informed decision. Surgeons and primary investigators will outline the details of the study verbally and provide a written copy as a summary for prospective subjects. Mitigating these ethical issues is crucial to maintain the four ethical tenants autonomy, beneficence, justice, and non-maleficence that govern experimental research and protect the patient from harm (24).

3. Methodology

3.1 Study Design A pilot experimental randomized control trial (RCT) will be used to compare the effectiveness of intraoperative immunoadsorption vs PET in neonates undergoing ABO-incompatible heart transplantation. A pilot RCT will allow for the feasibility of the intervention and study design to be assessed, in addition to providing estimates of the effect sizes for sample size calculations before a full-scale RCT is conducted (25). An RCT will allow for direct comparison of intraoperative immunoadsorption and PET, and aid clinicians in their goal to achieve evidence-based practice (26). An experimental study can be used to determine an outcome as a direct result of a particular exposure (26). In contrast, observational studies provide insight as to whether or not a given exposure is associated with a specific outcome; therefore, an RCT is superior for this proposed investigation in order to make inferences about cause and effect relationships (26).

In order to conduct an RCT a sample from a larger target population is randomly assigned to different groups; typically a standard treatment or placebo group is compared to a new treatment group (26). The effects of each treatment at predetermined time points are measured and classified as endpoints (26). The design of an RCT begins with the development of a clinically significant research question and formation of a hypothesis (26). This research question includes the criteria population, intervention, control, and outcomes (PICO) (27).

Internal and external validity are two parameters that are related to the quality of a proposed RCT (26). When an RCT has high internal validity, conclusions and differences observed between the two groups involved in the study can be attributed to the intervention (26). Thus, no other factors such as comedications, i.e. the use of multiple medications for the same indication, age, ethnicity, etc. can be associated with the outcome aside from the intervention that was tested (26, 28).

Internal validity can be prone to bias and random error (26). Bias is a form of systematic error that results in a consistent deviation of results from the truth, where the true distinction between the two groups is over- or underestimated; bias can arise due to design flaws, misreporting of the trial, or errors in the conduction of the experiment (26). Sources of bias are commonly detected during data collection, statistical analysis, or overall interpretation of the data (26).

External validity is an indicator of how well the results of an RCT can be generalized to the larger population and incorporated into clinical practice (29). High internal validity is a requirement in order for a study to achieve a high external validity (30). If the data is flawed or misleading, it is not possible to apply findings to a greater number of individuals (30). An RCT that is proven to have high internal and external validity is advantageous to determine causal relationships between an intervention and outcome; this is a main strength of constructing and applying a rigorous RCT design to a clinical research question of interest (26).

With regard to bias, there are four primary sources of bias that commonly affect RCT studies: selection bias, attrition bias, detection bias, and performance bias (29,31). Selection bias can be minimized with randomization, to ensure that each participant has an equal chance of being allocated to either group (26). Selection bias may also affect the ability to generalize results to the larger population, if the patients selected for the trial do not accurately resemble the true patient population of interest (26). Attrition bias may occur when there are methodological differences in the number of individuals that drop out of the study from the two assigned groups (32). Participants can choose to leave an RCT at any time and may drop out due to withdrawal of informed consent, violation of treatment protocol, or subjects may cease contact with the investigators all together (26). Detection bias can be attributed to a difference in how outcomes are assessed in each group (26). Performance bias is closely related to detection bias and is largely influenced by the investigator's individual perception about the interventions being studied (33). Furthermore, performance bias can be exacerbated by the exposure of factors unrelated to the intervention that differ between the two groups (33).

The proposed pilot study to investigate the effectiveness of intraoperative immunoadsorption vs PET in neonates undergoing ABO-incompatible heart transplantation in reducing transfusion-related risks has the potential to be affected by selection bias. Selection bias is a concern to this experiment as only one center will be used to gather participants; however, groups of neonates will be similar in terms of age, sex, and disease severity. Typically, these variables can be confounders to an RCT; the purpose of an RCT is to measure the efficacy between two interventions, therefore when sample groups are not similar it is challenging to determine whether the intervention was responsible for a change in the outcome measurement or whether the change in outcome was due to another variable (26). When samples have similar characteristics, differences between the two groups can be attributed to the intervention (26).

In addition to validity, blinding is an important consideration in the implementation of an RCT and plays a fundamental role in mitigating potential performance bias (26). Blinding can be single-blind where the assessor or patient is unaware of the group assignment, double-blind in which both the assessor and patient are unaware of the group assignment, or triple-blind where in addition to the assessor and patient the individual who conducts the statistical analysis does not have any information about the group assignment (26). In an ideal situation five predominant groups should be blinded to the assignment of patients to either control or treatment groups; patients, clinicians, data analysts, data collectors, and adjudicators of outcome measurements (34).

One main limitation of an RCT is the significance of loss to follow-up bias (35). It has been reported that patients lost to follow-up tend to have a different prognosis than patients who complete the entire study (35). An asymptomatic outcome after receiving treatment in an RCT can motivate patients to withdraw; conversely, patients may be lost to follow-up due to either an adverse complication or death (35). Estimates have shown that less that 5% loss to follow-up results in minimal bias, whereas over 20% loss to follow-up poses a serious threat to validity (35). Ideally an RCT would aim to recruit more participants than required to draw statistically significant conclusions from results and maintain a high level of internal and external validity. In the proposed RCT involving neonates, a small population size limits the ability to recruit more individuals to compensate for suspected dropouts.

Thus, this proposed RCT will aid in determining if there is a causal relationship between intraoperative immunoadsorption and PET in reducing transfusion-related risks to neonates undergoing ABO-incompatible heart transplant surgery. This study will be most prone to selection bias, as well as loss-to follow up bias, however the advantages of this RCT design in terms of making causal conclusions about a specific intervention and outcome support the use of this study design as opposed to an observational model.

3.2 Data Collection

To achieve a high external validity, specific inclusion and exclusion criteria will be implemented in order to generalize findings to the larger neonatal population. Two groups of ten neonates less than or equal to 28 days old requiring heart transplant surgery will be recruited over the year 2022 for this proposed study and will be enlisted from the Hospital for Sick Children in Toronto, Canada (36). The sample size for this experiment is limited by the number of neonates who undergo heart transplantation annually, as well as the restrictions to the current indications for this procedure due to the low number of neonatal donor hearts available (2). From 2000-2001, only 19 heart transplants were performed in children aged 0-18 years old at the Hospital for Sick Children (37). Therefore, recruiting 20 neonates for this experiment within one year may not be feasible given the circumstances and nature of neonatal research. This will influence the external validity of the proposed RCT and possibly have implications for generalizing results to the larger population.

The inclusion criteria for patient recruitment will be based on the current indications for newborn heart transplantation; eligible neonatal patients who are able to participate in this study may have severe cardiomyopathy involving primary tumors, single ventricle physiology with decreased ventricular function, pulmonary atresia-intact ventricular septum with right ventricle dependent coronary circulation, or severe Ebstein's anomaly (2). Severity of neonatal heart disease will also be considered when recruiting patients for this study to ensure that this is not a confounding variable that affects transfusion-related risks observed after each intervention.

Exclusion criteria comprise patients who have congenital heart disorders amenable to other therapeutic interventions, such as medications or pharmaceuticals, as well as infants who are not within the age category to be classified as a neonate. Neonates of any blood type can be included in this study, with consent and acknowledgement to receive a donor heart incompatible to their own blood type.

Informed consent will be obtained from the patients' parents/caregivers after the primary research investigators and surgeons provide a clear overview about the two procedures intraoperative immunoadsorption and PET. A written summary of the details of the study, as well as the possible risks and unintended harm will be provided to the parents of neonatal patients to aid them in making an uncoerced decision to enroll in the experiment. As this RCT poses unique challenges due to the surgical setting, patients can withdraw prior to surgery and surgeons will use their discretion if they believe the research is at risk of compromising the health status of the neonate. The narrow age range of neonates who can participate in this experiment warrants a brief time period to obtain informed consent from parents; parents will have 4-5 days to make their decision when all other criteria such as availability of a donor heart, blood products, and clinicians are accounted for.

The proposed study involving neonates undergoing ABO-incompatible heart transplantation was designed with the intention of minimizing systematic bias. Patients will be randomly assigned to one of the two interventions i.e., CPB with intraoperative immunoadsorption or CPB with PET via a random number generator. The surgeon, perfusionist, and anesthetist will receive an opaque envelope with the assigned procedure on the day of the surgery, reducing any potential for selection bias. The opaque envelope functions to blind surgical team members from the intervention. The goal of this study is to blind individuals from the five main groups involved in an RCT, i.e., patients, clinicians, data analysts, data collectors, and adjudicators of outcome measurements. The perfusionist will set up the circuit after confirmation of the assigned technique and ensure the amount of blood products required for each procedure are in the operating room upon initiation of bypass. To combat attrition bias, this experiment will be analyzed in terms of the intention-to-treat (ITT) method (26). This approach includes all randomized participants in the final evaluation, irrespective of participants who did not complete the study (26). The perfusionist, anesthetist, and surgeon have a duty to report any adverse effects seen patients while on bypass, primarily observing qualitative and quantitative effects of administering blood products to neonates. A detailed surgical log of hemodynamic variables and patient monitoring data will be recorded to assess the condition of the neonate in terms of transfusion-related risks.

The proposed experiment will evaluate the effectiveness of intraoperative immunoadsorption and PET in decreasing transfusion-related risks during ABO-incompatible heart transplantations during CPB. Two main outcomes of interest will be measured. The first outcome will be a composite infection outcome, observed as a wound infection, respiratory infection, or septicemia. The second outcome will be a composite ischemic outcome that will be defined as a permanent or transient stroke, myocardial infarction, or renal complication where creatinine is reported to be over 200 mmol/L. Composite ischemic and infection outcomes are two indicators that will be used to determine how postoperative morbidity in each group differs. These two outcomes will be confirmed by the surgeon, perfusionist, and anesthetist, reflected by hemodynamic monitoring, bloodwork, and symptoms for each condition.

Mortality will be assessed during three distinct time periods: 0-30 days post-operative, 30 days to one-year post-operative, and more than one-year post-operative. Patients will be followed up yearly up to a maximum of seven years after heart transplantation to reassess mortality due to any cause.

3.3 Data analysis A multivariable logistic regression model will be used as the primary method of data analysis. This type of regression differs from a multiple linear regression model because the dependent variables or outcome variables are dichotomous, for example diseased or not diseased (38). This model aims to illustrate the relationship between a specific outcome and a set of predictors (38). The independent variables in a multivariable logistic regression model are known as covariates (38). Predictor variables can belong to any classification of data such as continuous, ordinal or categorical (38). An advantage to using this type of statistical analysis is the ability to evaluate multiple predictor variables in efforts to ascertain which variable most accurately predicts a particular outcome (38).

In the proposed investigation, intraoperative immunoadsorption and PET will serve as the two covariates. This regression model will be used to compare these two independent variables and their relationship with multiple outcomes of interest including composite infection outcome, composite ischemic outcome, and mortality. Each outcome is categorical and will be expressed as a cumulative score, where the patient will either experience or not experience the outcome regardless of severity (38). An infinite range of values will not be used to evaluate the relationship between

each technique and transfusion-related risks, therefore a multivariable logistic regression model is advantageous in contrast to a linear regression model which is optimal for continuous outcome data (38).

All data analyses will be performed in STATA9.2 (Stata Corp, College Station, Tex). Confidence intervals equal to 95% for a causal effect will be used for statistical procedures; a 95% confidence interval provides an estimation that the true effect of the results of an experiment are within at least 95% of the intervals calculated when the exposure or independent variable is randomized, and the data is randomly sampled from a larger target population (39). Confidence intervals represent the uncertainty of the results being true due to random error (39).

Another parameter defined with respect to data analysis is statistical significance. Statistical significance is a measure used to assess the probability of the null hypothesis being true in comparison to an appropriate level of uncertainty associated with discerning the true answer of an experiment (40). The significance level is designated by the Greek letter alpha and it is equal to the probability of the results being incorrect (40). The chosen significance level for this RCT will be 0.05, establishing that there is a 5% level of uncertainty that the outcome of this experiment is true (40). A p-value known as the probability that the null hypothesis is true given a specific set of collected data was chosen to be 0.05 (40). Thus, any analysis of the data that computes a p-value of less than 0.05 will be deemed to be statistically significant, where there is less than a 5% chance of the null hypothesis being correct (40). In the current proposed RCT, the null hypothesis would indicate that there is no difference between the two interventions intraoperative immunoadsorption and PET during ABO-incompatible neonatal heart transplantation when associated with the outcome measures of interest.

4. Conclusion

The field of neonatal cardiac surgery was transformed by the success of ABO-incompatible heart transplantations, however this procedure continues to expose neonatal patients to inherent risks related to blood transfusions. The proposed pilot study aims to evaluate the effectiveness of the two techniques intraoperative immunoadsorption and PET in minimizing transfusion-associated risks due to the administration of blood products during neonatal cardiac heart transplant surgery, with the ultimate goal of reducing waiting list mortality of neonates who require a heart transplant. There are main limitations and ethical issues concerning this research trial, which include the small population size of neonates who meet the specific inclusion criteria required to participate in this experiment, and anticipated challenges with obtaining informed consent for enrollment from parents on behalf of their neonate.

The findings from this experiment could improve neonatal outcomes in ABO-incompatible heart transplantations and may also pertain to the future use of incompatible donor organs in a variety of organ transplant surgeries. The clinical significance of this investigation is of the utmost importance to clinicians and their decision-making process, in efforts to deliver an exceptional standard of care to neonatal cardiac surgery patients.

References

1. Learn about congenital heart defects [Internet]. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention; 2020 [cited 2021Feb14]. Available from: https://www.cdc.gov/ncbddd/heartdefects/index.html
2. John M, Bailey LL. Neonatal heart transplantation. Ann Cardiothorac Surg. 2018 Jan;7(1):118–25.
3. Fong SW, Qaqundah BY, Taylor WF. Developmental patterns of ABO isoagglutinins in normal children correlated with the effects of age, sex, and maternal isoagglutinins. Transfusion (Paris). 1974 Nov 12;14(6):551–9.
4. West LJ. Neonatal tolerance: applicability to solid organ transplantation. Curr Opin Organ Transplant. 2016 Feb;21(1):66–73.
5. Issitt R, Crook R, Cross N, Shaw M, Robertson A, Burch M, et al. Incompatible ABO-plasma exchange and its impact on patient selection in paediatric cardiac transplantation. Perfusion. 2012 Nov;27(6):480–5.
6. Lavoie J. Blood transfusion risks and alternative strategies in pediatric patients: pediatric transfusion risks and alternatives. Pediatr Anesth. 2011 Jan;21(1):14–24.
7. Murphy GJ, Reeves BC, Rogers CA, Rizvi SIA, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation. 2007 Nov 27;116(22):2544–52.
8. Issitt R, Crook R, Shaw M, Robertson A. The great ormond street hospital immunoadsorption method for ABO-incompatible heart transplantation: a practical technique. Perfusion. 2021 Jan;36(1):34–7.
9. Copeland H, Razzouk A, Chinnock R, Deming D, Hasaniya N, Bailey L. Pediatric recipient survival beyond 15 post-heart transplant years: a single-center experience. Ann Thorac Surg. 2014 Dec;98(6):2145–51.

10. Kantrowitz A, Haller JD, Joos H, Cerruti MM, Carstensen HE. Transplantation of the heart in an infant and an adult. Am J Cardiol. 1968 Dec;22(6):782–90.

11. Bailey LL. The evolution of infant heart transplantation. J Heart Lung Transplant. 2009 Dec;28(12):1241–5.

12. Bailey LL. Baboon-to-human cardiac xenotransplantation in a neonate. JAMA J Am Med Assoc. 1985 Dec 20;254(23):3321.

13. Cooper DK. Clinical survey of heart transplantation between ABO blood group-incompatible recipients and donors. J Heart Transplant. 1990 Aug;9(4):376–81.

14. Paul LC, Baldwin WM. Humoral rejection mechanisms and ABO incompatibility in renal transplantation. Transplant Proc. 1987 Dec;19(6):4463–7.

15. West LJ, Pollock-Barziv SM, Dipchand AI, Lee KJ, Cardella CJ, Benson LN, et al. ABO-incompatible heart transplantation in infants. N Engl J Med. 2001 Mar 15;344(11):793–800.

16. Wilman E, Megone C, Oliver S, Duley L, Gyte G, Wright JM. The ethical issues regarding consent to clinical trials with pre-term or sick neonates: a systematic review (framework synthesis) of the empirical research. Trials. 2015 Dec;16(1):502.

17. Baker L, Lavender T, Tincello D. Factors that influence women's decisions about whether to participate in research: an exploratory study. Birth. 2005 Mar;32(1):60–6.

18. Tooher RL, Middleton PF, Crowther CA. A thematic analysis of factors influencing recruitment to maternal and perinatal trials. BMC Pregnancy Childbirth. 2008 Dec;8(1):36.

19. Burgess E. Consent for clinical research in the neonatal intensive care unit: a retrospective survey and a prospective study. Arch Dis Child - Fetal Neonatal Ed. 2003 Jul 1;88(4):280F – 286.

20. Albersheim SG, Lavoie PM, Keidar YD. Do neonatologists limit parental decision-making authority? A Canadian perspective. Early Hum Dev. 2010 Dec;86(12):801–5.

21. Paulmichl K, Hattinger-Jürgenssen E, Maier B. Decision-making at the border of viability by means of values clarification: a case study to achieve distinct communication by ordinary language approach. J Perinat Med [Internet]. 2011 Jan 1 [cited 2021 Mar 22];39(5). Available from: https://www.degruyter.com/document/doi/10.1515/jpm.2011.066/html

22. Marc-Aurele KL, Steinman SL, Ransom KM, Finer NN, Dunn LB. Evaluation of the content and process of informed consent discussions for neonatal research. J Empir Res Hum Res Ethics. 2012 Jul;7(3):78–83.

23. Hoehn KS, Nathan A, White LE, Ittenbach RF, Reynolds WW, Gaynor JW, et al. Parental perception of time and decision-making in neonatal research. J Perinatol. 2009 Jul;29(7):508–11.

24. Page K. The four principles: can they be measured and do they predict ethical decision making? BMC Med Ethics. 2012 Dec;13(1):10.

25. Feeley N, Cossette S, Côté J, Héon M, Stremler R, Martorella G, et al. The importance of piloting an RCT intervention. Can J Nurs Res Rev Can Rech En Sci Infirm. 2009 Jun;41(2):85–99.

26. Siepmann T, Spieth PM, Kubasch AS, Penzlin AI, Illigens BM-W, Barlinn K. Randomized controlled trials-a matter of design. Neuropsychiatr Dis Treat. 2016 Jun;1341.

27. Aslam S, Emmanuel P. Formulating a researchable question: a critical step for facilitating good clinical research. Indian J Sex Transm Dis AIDS. 2010 Jan;31(1):47–50.

28. Möller H-J, Seemüller F, Schennach-Wolff R, Stübner S, Rüther E, Grohmann R. History, background, concepts and current use of comedication and polypharmacy in psychiatry. *Int J Neuropsychopharmacol*. 2014 Jul;17(7):983-996.

29. Rothwell PM. External validity of randomised controlled trials: to whom do the results of this trial apply? The Lancet. 2005 Jan;365(9453):82–93.

30. Akobeng AK. Assessing the validity of clinical trials. J Pediatr Gastroenterol Nutr. 2008 Sep;47(3):277–82.

31. Juni P. Systematic reviews in health care: assessing the quality of controlled clinical trials. BMJ. 2001 Jul 7;323(7303):42–6.

32. Berkman ND, Santaguida PL, Viswanathan M, Morton SC. The empirical evidence of bias in trials measuring treatment differences [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2014 [cited 2021 Mar 21]. (AHRQ Methods for Effective Health Care). Available from: http://www.ncbi.nlm.nih.gov/books/NBK253181/

33. Schulz KF, Grimes DA. Blinding in randomised trials: hiding who got what. The Lancet. 2002 Feb;359(9307):696–700.

34. Karanicolas PJ, Farrokhyar F, Bhandari M. Practical tips for surgical research: blinding: who, what, when, why, how? Can J Surg J Can Chir. 2010 Oct;53(5):345–8.

35. Dettori J. Loss to follow-up. Evid-Based Spine-Care J. 2011 Feb;2(01):7–10.

36. Mazhar A, Rehman A, Sheikh MA, Naeem MM, Qaisar I, Mazhar M. Neonates-a neglected paediatric age group. JPMA J Pak Med Assoc. 2011 Jul;61(7):625–8.

37. Government of Ontario, Ministry of Health and Long-Term Care. [Internet]. specialized pediatric services review - ministry reports - publications - public information - MOHLTC. Government of Ontario, Ministry of Health and Long-Term Care; 2002 [cited 2021Feb23]. Available from: https://www.health.gov.on.ca/en/common/ministry/publications/reports/pediatric_services/pediatric_services.aspx

38. Brunner HI, Giannini EH. trial design, measurement, and analysis of clinical investigations. In: Textbook of Pediatric Rheumatology [Internet]. Elsevier; 2011 [cited 2021 Mar 21]. p. 127–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9781416065814100007X

39. Greenland S. Interval estimation by simulation as an alternative to and extension of confidence intervals. Int J Epidemiol. 2004 Dec 1;33(6):1389–97.

40. Tenny S, Abdelgawad I. Statistical significance. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [cited 2021 Mar 21]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK459346/