Title: *In Vivo* Effects of Fibrinogen Concentrate (FC) versus Cryoprecipitate on the Neonatal Fibrin Network Structure after Cardiopulmonary Bypass (CPB)

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Abstract

Like most organ systems in the newborn, the hematologic system is not fully developed at birth. Studies have shown both quantitative and qualitative deficiencies in many pro- and anticoagulation proteins (1). Neonates with congenital heart disease (CHD) often require cardiac surgery and cardiopulmonary bypass (CPB) within a few days of birth. Their immature coagulation system, effects of CPB, and the complexity of the surgical repair contribute to the potential for a substantial coagulopathy following CPB. Transfusion of red blood cells (RBCs), platelets, fresh frozen plasma (FFP), and cryoprecipitate is often needed to restore post-CPB hemostasis. However, excessive bleeding and perioperative transfusions are independent predictors for morbidity and mortality (2-4). Therefore, developing strategies to reduce bleeding and transfusion requirements in neonates after CPB is an urgent need.

The most common hemostatic derangement after CPB is an acute acquired hypofibrinogenemia, which compromises fibrin clot generation and platelet aggregation, resulting in increased bleeding and need for allogenic blood product transfusions. Currently, management of this acquired hypofibrinogenemia is replaced with a blood product, cryoprecipitate. However, fibrinogen concentrate, RiaSTAP (CSL Bering), is a commercially available product that is being used off-label in cardiac and pediatric surgery to reduce bleeding and transfusions. Riastap, made from human plasma, contains purified fibrinogen as a pasteurized, lyophilized powder that has several advantages over cryoprecipitate: 1) small volume; 2) accurate dosing; 3) shorter preparation time since no blood typing or thawing are required; 4) lower risk of infectious transmission; 5) lower risk of allergic and immunologic reactions (5). In addition, recent studies have demonstrated that fibrinogen in neonates exists in a neonatal form that is distinct from the adult form of fibrinogen. Of particular concern, clots formed from a mixture of adult and neonatal fibrinogen, simulating transfusion, took twice as long to degrade as clots containing pure neonatal fibrinogen. These results raise concerns about the ability of the immature neonatal fibrinolytic system to degrade mixed clots and therefore a potential increased risk of adverse thrombotic events after the administration of adult fibrinogen. However, a study specifically targeting neonates undergoing open-heart surgery with CPB has not been done to determine the efficacy of fibrinogen concentrate in this population.

In this study, we plan to complete a randomized, control trial in neonates undergoing cardiac surgery to characterize the *in vivo* effect of transfusion with either cryoprecipitate or fibrinogen concentrate on clot kinetics and degradation. Our secondary goal is to correlate individual clot properties to patient outcomes, specifically the degree of postoperative bleeding, transfusion requirements and signs or symptoms of a postoperative thrombosis.

Introduction and Background

In patients undergoing open-heart surgery (OHS), transfusion of allogenic blood products is independently associated with increased morbidity and mortality (6,7). In response to this, research in adult cardiac anesthesia has focused on blood product usage and developing algorithms to limit blood product exposure. Due to the smaller population, considerable variability in patient characteristics, and wide range of procedures performed in pediatric cardiac patients, the literature regarding blood conservation strategies for pediatric cardiac patients is limited. In children undergoing OHS, there are no published algorithms even though product exposure, particularly in neonates, is significant. The National Heart, Lung and Blood Institute (NHLBI) has determined pediatric transfusion medicine (PTM) to be a high priority for clinical and translational research, specifically targeting transfusion strategies that could reduce short and long-term morbidity and mortality (8).

Congenital heart defects are the most common type of birth defect, affecting approximately 1% of births in the US (9). Up to 20% of these patients require cardiac surgery within the first 30 days of life (9). Neonates are extremely susceptible to the coagulopathic effects of CPB due to an immature coagulation system, massive hemodilution from large circuit primes, and extensive suture lines that accompany complex congenital cardiac repairs (10-11). While all neonates requiring CPB experience bleeding, up to 25% may suffer excessive bleeding requiring massive transfusions (10). Increasing transfusions of allogenic blood products during pediatric cardiac surgery are associated with increased mortality, prolonged mechanical ventilation, prolonged hospital stays, increased risk of infections, kidney injury, and negative immunologic responses (3-4-7). Given the safety concerns and the effects on the immune system associated with blood product administration, the search for alternative options to treat coagulopathy remains an important challenge. Unfortunately, there exists a paucity of adequately studied pharmacologic options to reduce post-CPB bleeding in neonates. Therefore, the aim of this study is to evaluate an effective, yet safe, pharmacologic alternative to blood transfusions for managing post-operative bleeding in neonates after CPB.

Like most organ systems in the newborn, the hematologic system is not fully developed at birth, but the parallel maturation of the coagulation and fibrinolytic systems maintains the hemostatic balance between thrombosis and bleeding. Several

studies have demonstrated that neonates have quantitative and qualitative deficiencies in many pro- and anticoagulation proteins (1, 15-17). Specifically, there is a "fetal fibrinogen" that is qualitatively dysfunctional and exists in a fetal form until approximately one year of age (15). In fact, several *ex vivo* studies have demonstrated improved fibrin network structure in neonatal pediatric cardiac patients (1) with the addition of adult fibrinogen. However, Brown et al (1) demonstrated that adult fibrinogen does not integrate seamlessly with neonatal fibrinogen and may increase clot degradation times, thus increasing the risk of post-operative thrombosis.

In addition to an immature hematologic system, fibrinogen, the final component of the clotting pathway, is one of the earliest coagulation proteins to become depleted during major bleeding and surgery with CPB. Studies have reported worse outcomes in patients with lower fibrinogen levels in multiple clinical settings including trauma, cardiac, and pediatric surgeries (18-22). As a result, an increasing body of literature has demonstrated that targeting higher fibrinogen levels reduced blood loss and transfusions in pediatric patients undergoing craniofacial and scoliosis surgery (19). The available options for fibrinogen replacement are cryoprecipitate and FC. In the United States, cryoprecipitate has been the traditional source of fibrinogen. Cryoprecipitate contains a high concentration of fibrinogen, around 15 g/L (23), and the procoagulant factors vWF, FXIII, and FVIII (23)**)**. Cryoprecipitate is prepared as single units, which are pooled prior to administration with a typical dose being approximately 2-3 units for a neonate. The risk of viral transmission is similar to that of FFP, the fibrinogen concentration is variable, and blood group matching is needed (23). The time to thaw cryoprecipitate is often 45-90 minutes. Cryoprecipitate was withdrawn from most European countries years ago due to safety concerns, but remains available in the USA. In contrast, FC contains purified fibrinogen without other coagulation factors. Viral inactivation during the manufacturing process minimizes the risk of viral transmission (5). The concentration of fibrinogen is standardized, and the administration volume is low (5). In addition, the time to availability is short as it does not require cross-matching or thawing. FC has been effective and well tolerated in various clinical settings. As a result, FC is being used off-label for the treatment of bleeding in pediatric patients. Administration of FC to pediatric patients has proven successful in decreasing major blood loss in the setting of scoliosis and craniosynostosis surgery (19). Galas et al. (20) demonstrated that FC was an effective substitute for cryoprecipitate in a post-bypass transfusion algorithm in older pediatric cardiac patients (ages: 1 month-5 years). However, to date, no study has examined the use of FC in neonates after cardiac surgery with CPB or how FC interfaces with the neonatal clot structure, kinetics, and degradation.

This study represents a shift in the design of hemostatic therapy for neonatal coagulopathy. While many institutions continue to treat post-CPB bleeding in neonates with the transfusion of blood products consisting primarily of platelets and cryoprecipitate, Europe and an increasing number of pediatric institutions are replacing

cryoprecipitate with fibrinogen concentrate. Given the safety risks and immunologic effects associated with blood transfusions, the search for effective alternatives to blood products for managing post-bypass coagulopathy is essential. To date, the studies on neonatal clot structure and kinetics are based on *ex vivo* addition of adult fibrinogen. Therefore, this is the first *in vivo* study to directly compare the effect of cryoprecipitate or FC on the neonatal fibrin network after CPB surgery. Our first hypothesis is that clots formed from FC will display similar structural characteristics as those formed with cryoprecipitate. However, because FC lacks FXIII, an important factor for forming a stable fibrin network but also causing greater resistance to degradation (14), we also hypothesize that clots formed from FC will be degraded faster by the neonatal fibrinolytic system, thus reducing the thrombotic risk associated with transfusion of adult blood products. This work is **significant** because it is the first in vivo study to specifically 1) compare *in vivo* transfusion of cryoprecipitate to FC on neonatal clot structure, strength, and kinetics, 2) assess the degradation rates of two types of adult fibrinogen by the neonatal system, and 3) correlate clot structure and degradation with clinical outcomes.

Significance:

Overall Aim: Determine the effects of post-CBP fibrinogen transfusion on the neonatal fibrin network to develop effective and safe strategies for managing coagulopathies in neonates. This proposal compares the *in vivo* effect of post-CPB administration of FC, a blood product alternative, to cryoprecipitate on neonatal clot properties. Our previous studies using cryoprecipitate suggest that adult fibrinogen does not seamlessly integrate with neonatal fibrinogen and may result in prolonged clot degradation, thus increasing the risk of thrombotic events. It is unclear if this is a direct result of the adult fibrinogen molecule or the other clot stabilizing factors in cryoprecipitate. This is the first *in vivo* study to 1) compare transfusion of cryoprecipitate to FC on neonatal clot structure, 2) assess the degradation rates between the two types of fibrinogen replacement, and 3) correlate clot structure and kinetics with clinical outcomes.

Central Hypothesis: We hypothesize that FC clots will have similar structural characteristics to clots formed from transfused cryoprecipitate, but that FC clots will be degraded faster by the neonatal fibrinolytic system.

Specific Aims:

Aim 1A: Characterize coagulation factors and properties of clot structure, strength, and polymerization kinetics in neonates undergoing CPB surgery after the transfusion of either FC or cryoprecipitate.

Hypothesis 1A: Clots formed after administration of FC will have similar strength and polymerization kinetics as clots formed with cryoprecipitate.

Aim 1B: Compare clot degradation rates in neonates undergoing CPB surgery prior to transfusion of adult fibrinogen, immediately after the administration of FC or cryoprecipitate, upon arrival to the ICU, and at 24hours post-operatively.

Hypothesis 1B: Clots formed after the administration of FC will have faster degradation times at 24hours than those formed with cryoprecipitate.

Aim 2: To complement aims 1A and 1B, we will examine the correlation of clot structure and kinetics to clinical outcomes with specific attention to: 1) transfusion requirements within 24 hours of surgery; 2) 24-hour chest tube output; 3) evidence of adverse thrombotic events.

Hypothesis 2: Patients who have faster clot degradation times will have decreased risk of post-operative thrombotic events, but no difference in transfusion requirements or chest tube output.

Summary: The proposed work represents an innovative and radical shift in managing neonatal coagulopathy. Neonatal CPB patients receive multiple blood products, consisting primarily of platelets and fibrinogen (cryoprecipitate) to reestablish hemostasis after CPB. These procedures are often complicated by excessive bleeding and repeated transfusions, which are associated with increased mortality and morbidity. Blood products are prepared from adult blood, and our recent studies have demonstrated that components of the adult coagulation system, *namely fibrinogen*, do not integrate seamlessly with the neonatal coagulation system. Therefore, developing alternative strategies to reduce blood transfusions and achieve hemostasis are critical. This study proposes to evaluate how two fibrinogen replacement therapies – FC and cryoprecipitate – interface with neonatal fibrin network properties. We will enroll 36 neonates undergoing elective cardiac surgery and randomize them to receive either cryoprecipitate or FC as part of the post-CPB transfusion protocol. We will collect blood at 4 time points 1) baseline; 2) post-transfusion of platelets and either cryoprecipitate or FC; 3) upon arrival to the ICU; 4) 24 hours after surgery. The samples will be used to measure coagulation factors, analyze the fibrin network structure by confocal and scanning electron microscopy, strength parameters by rheology and atomic force microscopy (AFM), polymerization by absorbency and thrombin clotting times, and degradation kinetics by a customs fluidics device.

Experimental Design and Methods:

In this proposal, we aim to compare the effects of post-bypass transfusion of cryoprecipitate to fibrinogen concentrate (FC) on neonatal clot structure, clot kinetics, and clot degradation times. We will further characterize the levels of important procoagulants (thrombin, FXIII, vWF, fibrinogen) throughout the perioperative process to allow for a direct comparison between the current standard of care, cryoprecipitate, and an alternative source of fibrinogen, FC. Additionally, studies have demonstrated increased risk of thrombosis associated with more blood transfusions (24-25) or elevated

levels of vWF (25) in neonates and infants after CPB. As demonstrated by our group, *ex vivo* mixtures of purified neonatal fibrinogen and purified adult fibrinogen (derived from cryoprecipitate) alter clot structure and have prolonged degradation times, increasing the potential for post-transfusion thrombosis. However, these studies were carried out using clots formed *in vitro* from the addition of purified neonatal and adult fibrinogen, which do not fully mimic the structure of clots formed *in vivo*. Therefore, we plan to carry out a prospective, randomized control trial to examine the *in vivo* effects of cryoprecipitate versus fibrinogen concentrate as an alternative treatment for post-CPB coagulopathy in neonates undergoing cardiac surgery (**Figure 6**).

Study Design: This study is a prospective, randomized control trial designed to evaluate fibrinogen concentrate as an alternative therapy to cryoprecipitate in treating post-CPB coagulopathy in neonates undergoing cardiac surgery. After informed consent is obtained, we will randomize infants undergoing cardiac surgery to receive cryoprecipitate (standard of care) or fibrinogen concentrate (FC) (study arm) as part of a post-bypass transfusion algorithm. For patients enrolled in the study, we will follow our standard anesthetic management, cardiopulmonary bypass protocol, and transfusion thresholds in the operating room and ICU. Patient demographics, relevant clinical data, standard laboratory data, and adverse events will be collected for each subject. In addition to our standard of care labs, whole blood samples will be collected from each study patient to analyze various properties of the neonatal fibrin network, including structure, strength, and polymerization and degradation kinetics, after the transfusion of either cryoprecipitate or FC. These studies will allow us to directly compare clotting dynamics in the presence of the current standard of care for treating surgically induced coagulopathy, adult cryoprecipitate, and the potential alternative therapy FC.

Study Population: As discussed above, neonates have unique aspects of their coagulation cascade that warrant specific biological consideration. This study will be a single-center prospective, randomized control trial. After informed and written parental consent, a total of 36 neonates (age less than 30 days) undergoing elective cardiac surgery with cardiopulmonary bypass will be enrolled at Children's Healthcare of Atlanta. The following criteria will be used to determine patient eligibility:

Inclusion Criteria:

1. Full term neonates (36-42 weeks gestational age)
2. Infants $\leq$ 30 days of age at time of surgery
3. APGAR score of 6 or greater at 5 minutes after delivery
4. Neonates undergoing elective cardiac surgery requiring CPB at Children's Healthcare of Atlanta
5. Parents willing to participate and able to understand and sign the provided informed consent

Exclusion Criteria:

1. Preterm neonates (less than 36 weeks gestation)
2. Patients undergoing an emergent procedure or surgery not requiring CPB
3. Patients with personal or family history of a coagulation defect or coagulopathy
4. Parents unwilling to participate or unable to understand and sign the provided informed consent

Recruitment and Enrollment After a patient is deemed eligible for the study, informed and written consent will be obtained from the parents or legal guardian. Only those patients who meet the entry criteria will be approached for study participation. There will be no costs for the participants. At any time, parents and legal guardians will have the option of withdrawing their child from the study.

Randomization As they present to Children's Healthcare of Atlanta, patients will be enrolled consecutively and randomized to receive either FC or cryoprecipitate as part of a standardized transfusion algorithm. Once on CPB, patients will be randomized using block randomization with variable blocks. Patients will be randomly assigned (1:1) to receive either cryoprecipitate (control group) or fibrinogen concentrate (FC) (study group) as part of our post-bypass transfusion algorithm. Ideally, all practitioners would be blinded to the study. However, the dose of fibrinogen concentrate is diluted in normal saline and may be up to approximately 50mls. The use of a placebo would require the administration of up to 50mls of normal saline, 20% of a blood volume in a 3kg neonate. The hemodiluational effects of placebo would potentially confound the results comparing the two groups. Therefore, the anesthesiologist, who will be administrating either cryoprecipitate or FC, will be aware of the group allocation. Parents, surgeons, and critical care physicians will be blinded to the group allocation.

Rationale for Standardized Anesthetic, Cardiopulmonary Bypass and Anticoagulation Management In order to minimize confounding factors, we will follow our standard anesthetic management, cardiopulmonary bypass protocol, and anticoagulation strategy.

Anesthesia Management and Cardiopulmonary Bypass Management
Anesthesia, CPB, and surgery will be performed by the same teams of anesthesiologists, perfusionists, and surgeons. Anesthesia techniques will follow institutional protocol, which include a balanced anesthesia technique and necessary invasive monitoring, including arterial and central pressure monitoring.

Cardiopulmonary Bypass management
Nonpulsatile CPB is performed using a nonheparin-coated system, a Terumo FX-05 hollow-fiber membrane oxygenator (Terumo Cardiovascular Systems, Ann Arbor, MI), and Livanova SMArt-coated neonatal circuit (LIVANOVA USA (Sorin Group USA),

Arvada, CO, USA). All circuits contain a 250-300 ml priming volume, which include 50ml 25% albumin (Grifols), Plasmalyte, 0.5mg/kg mannitol, calcium gluconate, and 8.4% sodium bicarbonate to normalize the pH. Packed RBCs (pRBCs) are added to the circuit as needed to achieve and maintain a hematocrit of 30% throughout the duration of CPB. In accordance with institutional policy, patients having cardiac surgery receive pRBCs that are less than 14 days old. 50ml fresh frozen plasma (FFP) is added to CPB prime and another 50 ml FFP is added upon re-warming. Conventional or zero-balance ultrafiltration is performed throughout the bypass period at both institutions. For all patients, pH-stat blood gas management is used during bypass.

Anticoagulation

Anticoagulation is established with 500units/kg of porcine heparin with 1000 units of heparin added to the CPB prime. Kaolin-activated ACT values greater than 480 seconds are confirmed prior to the initiation of CPB (i-STAT®1 Analyzer; Abbot Point of Care Inc, Abbott Park, IL, USA). Additional heparin is administered as necessary during CPB to maintain an ACT >480 seconds. After completion of surgery, patients are rewarmed to 36^{o}C and weaned from CPB. Protamine (5mg/kg) is used to neutralize heparin upon completion of CPB and confirmed with ACT. Additional protamine is administered if ACT levels are above baseline values. All neonates (patients <30 days of age) will receive anti-fibrinolytic therapy. Tranexamic acid was used: 100mg/kg bolus dose pre-bypass, 100mg/kg bypass dose, and an infusion at 10mg/kg/hr that was terminated at transport to the ICU.

Transfusion Algorithm: In order to minimize confounding factors, a transfusion algorithm and transfusion thresholds will be followed in the operating room and ICU for all patients enrolled in the study. RBCs will be transfused for hematocrit <32% for acyanotic neonates, while cyanotic neonates are transfused RBCs for a goal Hct 35-40%. RBC transfusion is not permitted for hematocrit values higher than 45%.

After enrollment in our study, patients will be randomized to receive either cryoprecipitate or fibrinogen concentrate as part of our transfusion protocol. Our standard transfusion algorithm for neonates includes a quarter to half a unit of platelets and two units of cryoprecipitate, which historically resulted in a median post-operative fibrinogen level of 345mg/dL (258-469) (unpublished data). Using recent studies as well as our historical data, we chose a post-transfusion target fibrinogen level of 300mg/dL. After separation from bypass, patients will receive platelets and *either* cryoprecipitate or FC. For patients randomized to the FC arm, we will calculate the dose of fibrinogen concentrate to achieve a level of 300mg/dL after drug administration using the following equation from Riastap ® Package insert (5):

$$\text{Dose} = \frac{\text{(Target Level-Measured Level)}}{1.7 \times \text{weight(kg)}}$$

In order to account for the hemodilutional effects of the bypass prime on fibrinogen levels, the "measured level" will be the fibrinogen level measured after 10min on CPB. If a patient in either arm continues to have post-bypass bleeding, the anesthesiologist will use point of care testing and the transfusion thresholds outlines in the transfusion algorithm to determine appropriate products for transfusion.

Sample Procurement and Processing– At Children's Healthcare of Atlanta, whole blood samples will be collected from 36 neonates scheduled for elective cardiac surgery requiring CPB. We will collect these samples at four time points: 1) after induction of anesthesia prior to CPB; 2) after termination of CPB and transfusion of platelets and either cryoprecipitate or fibrinogen concentrate; 3) upon arrival to the ICU 4) 24 hours post-operatively in the ICU. These samples will be centrifuged to yield platelet poor plasma (PPP) and stored at -80^{o}C. These samples will be utilized for the clot analysis experiments described in detail below. Samples will be transported according to appropriate regulations to the laboratory of Dr. Brown at North Carolina State University for analysis. All samples will be obtained from an arterial line that is required for the planned surgical procedure.

Analysis of coagulation factors – For each sample, we will measure coagulation factor levels (thrombin, FXIII, vWF, and fibrinogen) using ELISA to elucidate the contribution of these factors present in cryoprecipitate to clot kinetics when compared to FC. Each sample will be analyzed in triplicate to ensure that reproducible and statistically significant data are obtained.

Analysis of clot characteristics - Clots formed from pre-CPB PPP, post-CPB PPP after transfusion of cryoprecipitate or FC, and 24 hours post-operative PPP will be analyzed to compare the effects of FC and cryoprecipitate on clot properties. Using previously described methods, strength parameters will be assessed by rheology and atomic force microscopy (AFM) and polymerization will be determined by thrombin-initiated turbidity/absorbency curves (13). These studies will allow us to directly compare differences between fiber alignment (3-D branching structure), strength, and polymerization differences after the transfusion of FC or cryoprecipitate in neonates after CPB surgery.

Analysis of fibrin fiber alignment – We will quantify clot fiber alignment using an application of an algorithm based on a fast Fourier transform (FFT) that our group has

previously employed (25). Briefly, each confocal image is padded with redundant data; a Gaussian decay (SD=0.25) and tow-dimensional Hann window function are applied to minimize edge effects; and the images are transformed into the frequency domain using a two-dimensional fast Fourier transform. The predominant fiber angle is defined as the angle with the maximum relative intensity, and the alignment index (AI) is defined the fraction of fibers aligned within $\pm 20^0$ of the preferred fiber alignment (PFA) normalized to a random distribution of oriented fibers (1). The higher the AI, the greater alignment of the fibers. Our previous work demonstrated that clots formed from purified neonatal fibrinogen were found to display a significantly higher degree of alignment (i.e. less 3-D structure) compared to those comprised of adult fibrinogen. Although we have not completed *in vitro* studies to analyze the effect of FC on neonatal clot formation, we expect that it will be similar to fibrin alignment in clots formed from purified adult fibrinogen derived from cryoprecipitate. In support of our hypothesis, we expect that both FC and cryoprecipitate will have lower AI than baseline neonatal only clots, but there will likely be no difference between clots formed with FC or cryoprecipitate. Any difference in AI between the two groups will shed light on how pro-coagulant factors present in cryoprecipitate may affect *in vivo* clot formation in neonates after surgery with CPB (13)

Analysis of clot degradation kinetics – To evaluate clot degradation kinetics, we will utilize a previously described custom fluidics-based microscopy approach (1). Our previous data have demonstrated that clots from mixed neonatal and adult fibrinogen degrade slower than neonatal fibrinogen only clots. Given that cryoprecipitate contains both vWF, (essential to the proper functioning of neonatal platelets) and FXIII (important for stabilizing clot strength), it may be that transfusion of adult-derived cryoprecipitate generates a clot that is not as easily degraded by the neonatal fibrinolytic system when compared to FC, which contains fibrinogen only. FC may have faster degradation times when compared with cryoprecipitate, thus potentially reducing post-transfusion thrombotic risk in neonates after cardiac surgery with CPB (1).

Aim 1A (Clot structure, strength, polymerization kinetics between cryoprecipitate and FC) Thirty-six neonates undergoing non-emergent cardiac surgery will be enrolled in this study and randomized to receive either cryoprecipitate or FC as part of post-bypass transfusion algorithm. Blood samples will be collected at four times points: 1) after the induction of anesthesia 2) after transfusion of cryoprecipitate or FC; 3) upon arrival to the ICU; 4) 24 hours after surgery. After samples are collected and processed, they will be transported to Dr. Ashley Brown's lab at NCSU/UNC-CH. Dr. Brown will use ELISA to measure the coagulation factors (thrombin, FXIII, von Willebrand Factor, and fibrinogen) at each time point. Using the previously described methods, strength parameters will be assessed by rheology and atomic force microscopy (AFM); polymerization will be determined by thrombin-initiated turbidity/absorbency curves; fiber alignment using the described fast Fourier transform. Our goal is to characterize how these two sources of adult fibrinogen affect

properties of the neonatal fibrin network including the structure, strength, polymerization kinetics between these two groups.

Aim 1B (Clot degradation kinetics) In addition to the above studies, the blood samples from the same 36 neonates will then be used to perform degradation kinetic studies as described previously. This will be the first study detailing the longitudinal differences in degradation kinetics of neonates undergoing cardiac surgery. After characterizing the coagulation factor levels between patients who receive cryoprecipitate versus FC, we hope to better understand the role of vWF and FXIII in neonatal clot formation and degradation rates and how this may contribute to the risk of thrombotic events.

Aim 2 (Correlation of clot properties to clinical outcomes): Clinical outcome variables will be collected for each patient and correlated to clot properties with specific attention to: 1) transfusions within the first 24 hours of surgery, 2) 24-hour chest tube output, 3) clinical signs and symptoms of postoperative thrombosis. Our multi-site prospective, randomized control trial demonstrated that FC can reduce allogenic blood transfusion in neonates and infants undergoing elective cardiac surgery with CPB when compared to cryoprecipitate. Our proposed laboratory studies use platelet poor plasma to enable us to focus on the contribution of supplemental fibrinogen to neonatal clot kinetics. However, since platelets, particularly the increased dependence on vWF in neonates, also play an important role in post-bypass hemostasis, we plan to correlate clinical characteristics and outcomes with our findings regarding clot kinetics. Clinical outcome variables will be collected for each patient and correlated to clot properties with specific attention to 1) transfusion requirements intraoperatively; 2) transfusion requirements within the first 24 hours of surgery; 3) post-operative bleeding recorded by 24 hour chest tube output; 4) clinical signs and symptoms indicative of postoperative thrombosis; 5) mechanical ventilation time; 6) length of ICU and hospital stay 7) adverse events within seven days of surgery. While our study is not powered to for this outcome, we hope that these data will provide some insight in to the clinical differences between different sources of adult fibrinogen.

Samples Size/Statistical Analysis

Data collected will include demographic data, CBP data, transfusion data, laboratory data and clinical outcomes listed above. As we demonstrated in prior work, neonatal clots prior to transfusion displayed a significantly higher degree of alignment when compared to those composed of adult only or mixed neonatal and adult fibrinogen. Based on our previous data, to detect a difference in degradation times between clots formed with neonatal fibrinogen and clots formed with adult fibrinogen with 80% power, we will enroll at total of 36 patients, with 18 in patients in each arm. All quantitative data will be analyzed using a paired t-test or Wilcoxon rank-sum test to

determine differences between the two groups. Correlation will be determined by either Pearson correlation or Spearman rank correlation. Significance will be defined by a p-value less than or equal to 0.05.

Adverse Events Reporting

Monitoring of Adverse Events (AE) and Serious Adverse Events (SAE) is an important aspect of clinical trials. After enrollment, the research team will collect the adverse events from the medical record. SAEs will be reported to the IRB within the guidelines set by the IRB. Any serious AE will be immediately reviewed by the PI and a senior cardiac anesthesiologist not involved in the study. Quarterly, study subjects will be reviewed on a case-by-case basis by our Quality Improvement Officer and an experienced cardiac anesthesiologist not involved in the study for review of potential adverse events and whether or not the event was related to the study.

Drug reactions including fever, chills, headache, allergic reactions, or thrombosis are potential risks to this study. The most common adverse reactions observed in clinical studies (frequency >1%) with the study drug, fibrinogen concentrate, were fever and headache. The most serious adverse reactions observed are thrombotic episodes (pulmonary embolism, myocardial infarction, deep vein thrombosis) and anaphylactic reactions. These reactions are similar to those of blood transfusions and specifically, cryoprecipitate. The risks are also comparable to cryoprecipitate. Fibrinogen concentrate has been used in Europe and as part of transfusion algorithms around the United States, including our institution with no increased serious adverse events reported. In our preliminary study, we received an IND exemption for the use of fibrinogen concentrate. Thirty patients under one year of age received fibrinogen concentrate instead of cryoprecipitate to treat postoperative bleeding. There was no significant difference in the rate of adverse events, including postoperative thrombosis, between the fibrinogen concentrate versus the cryoprecipitate group. Based on this data and the need to minimize transfusions in the neonatal population undergoing cardiac surgery with CPB, we are seeking an IND exemption for use of this medication in a randomized, control trial.

Patient confidentiality is at risk since protected healthy information is included in the study data. This risk will be minimized by only recording information absolutely necessary to fulfill the study's objectives. Information directly identifying patients will be excluded. Once the samples are collected, a unique study number will be assigned to each sample. This unique number will be used to identify the patient in the research database. The database, along with the code linking the study patient to an assigned number, will be kept in a locked office.

Data Safety Monitoring Plan

A potential risk of this study is related to drug reactions including fever, chills, headache, allergic reactions, or thrombosis. This medication has been used extensively in Europe and as part of transfusion algorithms around the United States. Based on this data and the need to minimized transfusions in the infant population undergoing cardiac surgery with CPB, we have received an IND exemption for use of this medication in this randomized, control trial.

The study subjects will be reviewed on a case-by-case basis at our institution and quarterly by the DSMB. The DSMB will consist of a cardiologist, an anesthesiologist, a hematologist, and a statistician not involved in the study. Serious adverse events, including thrombosis, stroke, sepsis, and death, will be reported to the DSMB within 24 hours of the study team becoming aware of the event. The DSMB will review all safety data, including frequency and severity of AEs.

A second risk of this study is patient confidentiality since protected health information is included in the study data. This risk will be minimized by only recording information absolutely necessary to fulfill the study's objectives. Information directly identifying patients will be excluded (names, addresses, telephone numbers, social security numbers, email addresses, and account numbers). A unique study number will identify the study subjects. The study information will be collected and stored in a password-protected database. Consents, along with the code linking a subject's identity to an assigned number, will be locked in the office of the principal investigator or designee.

The study team will conduct a self-monitoring tool quarterly to ensure data integrity.

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