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Prenatal ABO/RHD Genotyping: A New Paradigm to Allow for Fresh Whole Blood for Cardiopulmonary Bypass in the Immediate Newborn Period

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Abstract

Compared to standard component therapy, fresh whole blood (FWB) offers potential benefits to neonates undergoing cardiopulmonary bypass (CPB) in the context of open cardiac surgery: decreased blood loss and subsequent risk of volume overload, improved coagulation status, higher platelet counts during and following CPB, circumvention of limited vascular access, and significantly reduced donor exposures. Obtaining FWB, however, entails 2–5 days of preparation, which often precludes its availability for neonates requiring CPB in the immediate newborn period. Using a multidisciplinary approach and molecular ABO/RHD genotyping on amniotic fluid, we developed a protocol to allow procurement of FWB for timed delivery followed by open cardiac surgery. Eligible subjects include patients undergoing genetic amniocentesis following the diagnosis of a fetal cardiac anomaly likely to require open surgical repair in the initial days after

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Statement of Ethics

Informed consent was obtained from the family that participated in this study. The protocol used in this case complies with the guidelines for human studies and animal welfare regulations and is in compliance with Johns Hopkins University's committee on human research.

Disclosure Statement

The authors declare no conflicts of interest.

birth. This protocol has been successfully implemented following prenatal diagnosis of severe fetal cardiac anomalies. Taking advantage of the prenatal time period and the ability to perform fetal blood typing prenatally using molecular genotyping makes possible a new paradigm for the availability of FWB for CPB to improve perioperative, short-term, and long-term outcomes in a population comprised of some of the smallest and sickest patients who will undergo CPB.

Keywords

Congenital heart disease; Cardiopulmonary bypass; Prenatal diagnosis; Red cell genotyping

Introduction

Congenital heart disease (CHD) affects nearly 40,000 newborns per year in the United States. About 25% of neonates with CHD have critical CHD, requiring surgery in the first year of life. The most complex of these critical CHD conditions, including hypoplastic left heart syndrome and transposition of the great arteries, require complex, high-risk cardiac surgeries in the first week of life. Cardiopulmonary bypass (CPB) is required to perform these surgeries and is associated with a significant reduction in the plasma concentrations of coagulation factors particularly for pediatric patients, which can be most profound in neonates. Priming of the CPB circuit can be at least two times the blood volume in these small patients, resulting in hemodilution of coagulation factors and platelets. In order to potentially decrease intraoperative and postoperative bleeding due to coagulopathy, the bypass pump can be primed with fresh whole blood (FWB), which would also be available for transfusion after discontinuation from CPB. Whole blood contains red blood cells, platelets, and coagulation factors and as such is a balanced product. There are several potential advantages to the use of FWB for these neonates, including reduction in blood donor exposure [1], prevention of dilutional coagulopathy that can occur with transfusion of individual component therapy, and decreased incidence of volume overload [2, 3]. Furthermore, as these neonates often have limited venous access, the requirement of transfusing a single blood product as opposed to different individual components (red blood cells, platelets, fresh frozen plasma, and cryoprecipitate in some instances) is also advantageous [4, 5]. However, the limited availability of FWB frequently requires close cooperation with local transfusion therapy services as it is usually not a product maintained in hospitals and often requires 2–5 days to obtain the product, with the time varying greatly depending on patient blood type, day of the week, etc. Unfortunately, these patients often require surgery before the FWB product can be consistently obtained.

Due to the complexity of the cardiac operative procedures these neonates undergo in the first few days of life, at our institution, there was an existing multidisciplinary effort to schedule their deliveries (generally at term) to allow for coordination of care. With the lack of consistent availability of FWB for these cases, we sought to establish a prenatal *ABO/RHD* genotyping protocol, to allow for determination of the fetal blood type by molecular genotyping at the time of genetic amniocentesis. The antenatal blood type result would inform Transfusion Medicine personnel in a timely manner so that fresh whole blood could be ordered and was consistently available for these complex neonatal cardiac surgeries.

Procurement and Transfusion Protocol

A multidisciplinary committee was formed including Maternal Fetal Medicine, Prenatal Genetics, Pediatric Cardiology, Pediatric Cardiac Surgery, Transfusion Medicine, Neonatology, and Pediatric Cardiac Critical Care. The committee worked in conjunction with the American Red Cross and the New York Blood Center Laboratory for Immunohematology & Genetics for provision of molecular *ABO/RHD* fetal blood typing using amniotic fluid. Eligible subjects were identified by criteria which included: patients with a fetal structural cardiac anomaly which required open cardiac surgery in the immediate neonatal period (Table 1), patients planning for continuation of pregnancy, patients undergoing genetic amniocentesis.

An additional aliquot of 10 mL of unspun amniotic fluid was collected at the time of amniocentesis following genetic counseling. This aliquot was submitted for *ABO/RHD* genotyping. A maternal blood sample was also collected to type and screen for maternal antibodies that might cross the placenta. Timing of delivery was established and open cardiac surgery scheduled accordingly. The anticipated date of operative cardiac repair was communicated to the American Red Cross in an effort to have ABO/Rh(D)-identical FWB prepared and available on the specified date. Ideally, the FWB provided would be less than 3 days post-collection to ensure adequate platelet function at the time of transfusion.

The differences in the approach to prenatal and postnatal blood compatibility testing are summarized in Table 2, with the advantages and disadvantages of these products summarized in Table 3. Unlike component therapy, which can be provided in a way that is universally compatible (group O red blood cells, group AB plasma and platelets), only ABO-identical FWB is fully compatible with any recipient. The *ABO/RHD* genotyping protocol provides the transfusion service with a reliable preliminary result of the neonatal blood type, affording time to procure units that are likely ABO/Rh(D)-compatible with the neonate. However, consistent with regulatory standards, the neonatal blood type would be confirmed serologically using an FDA-approved test after delivery, and the test of record would be the serologic blood type. In the event that the genetic blood type and the serologic blood type were ever discordant, the plan for FWB would be abandoned in favor of universally compatible component therapy. Based upon the standard requirements for preoperative priming of CPB pump, and average anticipated intraoperative and postoperative losses, we estimated expected donor exposures for FWB versus standard component replacement therapy (Table 4).

Clinical Case

The protocol was tested for feasibility in a case referred at 34 weeks gestation for prenatally diagnosed fetal D-transposition of the great arteries. Following genetic counseling, the patient elected to undergo amniocentesis for cytogenetic analysis with fetal *ABO/RHD* genotyping. Genotype for *ABO* and *RHD* was sent to a reference lab and returned in 8 days. Her delivery was timed for 37 weeks gestation for cholestasis of pregnancy by repeat cesarean section. Following delivery (birth weight 3 kg), the infant was noted to have significant hypoxia and elevated pulmonary arterial pressure, necessitating urgent balloon

atrial septostomy, which was performed in the neonatal ICU. Pulmonary hypertension was stabilized with nitric oxide and sildenafil treatment, after which the infant underwent open cardiac surgical repair with an arterial switch operation on the fourth day of life. Intraoperatively, the CPB circuit was primed with 300 mL FWB with additional FWB reserved for resuscitation while in the OR (1 donor). Blood loss in first 12 h postoperatively was 79 mL (approximately 2 mL/kg/h); the total amount of FWB transfused was 50 mL (additional 1 donor). This amount of post-op chest tube output and transfusion was well below typical bypass cases where individual components are transfused and thrombocytopenia is more common. Furthermore, the total donor exposure was two allogeneic donors. In comparison, typical donor exposure for individual component therapy transfusion ranges from four to six for these complex cardiac cases (Table 4). Postoperatively, the neonate's platelet count was 155,000/mm³. There was no evidence of coagulopathy. The infant continued to do well and was discharged home on postoperative day 17.

Discussion

Minimizing donor exposure is particularly relevant for pediatric patients undergoing open heart surgery as survival to adulthood is expected in the majority of cases. These patients may require additional cardiac surgeries as in the case of hypoplastic left heart syndrome, and some may become candidates for cardiac transplantation with obvious advantages to limited blood type antibody formation in order to procure a compatible organ donor. For female patients, the avoidance of red cell sensitization is clearly beneficial in terms of anticipated future pregnancies. Operationally for the blood bank, it is generally easier to issue 1–2 units of FWB rather than coordinating universally compatible component therapy.

Education of local maternal fetal medicine specialists, pediatric cardiologists, and prenatal genetic counselors who encounter fetuses with cardiac anomalies (Table 1) will be key for implementation of this protocol. Fetal DNA would need to be procured for *ABO/RHD* genotyping at the time of an amniocentesis performed for prenatal diagnostic evaluation, and patients will need to be counseled regarding the advantages of adding blood genotype testing to their prenatal diagnostic workup.

In conclusion, a protocol that allows for FWB availability for CPB and open cardiac surgical procedures in the immediate newborn period has been developed. This entailed a multidisciplinary team approach that takes advantage of the prenatal time period and capability to perform fetal blood typing prenatally using molecular methodology following the ultrasonographic prenatal diagnoses of severe fetal structural cardiac anomalies. The advances in prenatal diagnosis today permit a new paradigm designed to improve perioperative, short-term, and long-term outcomes in a population comprised of some of the smallest and sickest patients who will undergo CPB.

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Established Facts

- Neonatal patients undergoing open cardiac surgery are at high risk of blood loss and transfusion.
- Fresh whole blood offers benefits of fewer donor exposures, reduced blood loss, and less coagulopathy as compared to standard component therapy for cardiopulmonary bypass.

Novel Insights

- Amniocentesis for *ABO/RHD* genotyping permits prenatal diagnosis and preparation of fresh whole blood to improve perioperative outcomes in neonates undergoing open cardiac surgery in the immediate newborn period.

Table 1.

Congenital heart disease which may require surgical repair in the immediate neonatal period

<i>Cardiac Lesions</i>
D-transposition of the great arteries (D-TGA) with or without VSD
Hypoplastic left heart syndrome (HLHS)
Truncus arteriosus
Univentricular heart syndromes
Left ventricular outflow obstruction lesions
Interrupted aortic arch with VSD
Unbalanced AV canal (some cases)
Shones complex

Table 2.

Summary of the laboratory approach to compatibility testing of whole blood, versus blood components, for neonates

Prenatal laboratory workup: whole blood	Postnatal laboratory workup: blood components
Fetal DNA for determination of likely fetal blood type [*]	Type and screen of cord blood/newborn specimen to determine blood type
Maternal blood sample for red cell antibody evaluation	
Type and screen of cord blood/newborn specimen to confirm DNA test result	

^{*} Added to standard genetic testing (e.g., karyotype, chromosomal microarray).

Table 3.
Summary of the advantages of whole blood, versus blood components, for transfusion to neonates undergoing major surgery

	Whole blood	Blood components
Advantages	Fewer donor exposures Potentially improved coagulation/hemostasis Typically decreased incidence of thrombocytopenia Decreased product and volume replacement needs Complete blood component replacement through a single i.v. access line	Typically readily available
Disadvantages	Requires advanced notice Short shelf life	More donor exposures Potentially inferior coagulation/hemostasis Typically worse thrombocytopenia Sequential replacement of multiple blood products due to limited i.v. access

Table 4.

Donor exposure for neonatal cardiopulmonary bypass/cardiac repair by blood product utilized

	<u>Number of donors</u>	
	fresh whole standard	
	blood	components*
Prime the pump	1	2
Intraoperative loss	0–1	2 or more
Postoperative loss (chest tube output)	1 **	2 or more

* With increased coagulopathy and lower platelet count, blood loss can be greater with standard component therapy.

** This unit is typically from the same donor as the unit from the OR, for total typical exposure of 2 donors.