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Review

One size will never fit all: the future of research in pediatric transfusion medicine

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There is concern at the National Heart, Lung, and Blood Institute (NHLBI) and among transfusion medicine specialists regarding the small number of investigators and studies in the field of pediatric transfusion medicine (PTM). Accordingly, the objective of this article is to provide a snapshot of the clinical and translational PTM research considered to be of high priority by pediatricians, neonatologists, and transfusion medicine specialists. Included is a targeted review of three research areas of importance: (i) transfusion strategies, (ii) short- and long-term clinical consequences, and (iii) transfusion-transmitted infectious diseases. The recommendations by PTM and transfusion medicine specialists represent opportunities and innovative strategies to execute translational research, observational studies, and clinical trials of high relevance to PTM. With the explosion of new biomedical knowledge and increasingly sophisticated methodologies over the past decade, this is an exciting time to consider transfusion medicine as a paradigm for addressing questions related to fields such as cell biology, immunology, neurodevelopment, outcomes research, and many others. Increased awareness of PTM as an important, fertile field and the promotion of accompanying opportunities will help establish PTM as a viable career option and advance basic and clinical investigation to improve the health and well-being of children.

INTRODUCTION

The Transfusion Medicine/Hemostasis Clinical Trials Network (TMH CTN) was created by the National Heart, Lung, and Blood Institute (NHLBI) in 2002 and is charged with performing trials in children and adults. While there were adequate numbers of pediatric hematologists to put forward ideas for practice-changing studies; it was apparent that there were few investigators focused on PTM. This "failure to thrive" diagnosis in the field of PTM prompted action by the NHLBI in 2005 to form a working group that resulted in curriculum development grants named the Pediatric Transfusion Medicine Academic Awardees (PTMAA) program. Another change that nourished the field of PTM was the American Board of Pathology's move to allow board eligible pediatricians to apply directly into transfusion medicine fellowship after residency. These developments brought awareness to PTM as a field and defined a path for young investigators who wish to focus on PTM.

In 2008, as the PTMAA investigators began to "come of age" they sponsored a working group, with an emphasis strictly on clinical and translational research gaps in the field of PTM. The working group identified three major areas of concern: (i) transfusion strategies; (ii) short- and long-term consequences of transfusion; and (iii) transfusion-transmitted diseases as they relate to neonatal and pediatric patients. In 2009, NHLBI convened a State of the Science Symposium on Clinical Trials in Transfusion Medicine. The aim of this symposium was to identify phase II and III clinical trials that could have a significant impact in advancing transfusion therapies in the next 10 years. Out of the 24 concepts presented, three neonatal/pediatric trials were prioritized within the top ten, selected by an external panel of transfusion medicine experts (1). Since then, NHLBI has initiated less formal meetings where clinical investigators from many disciplines gather to discuss PTM and research gaps within this small field. Future meetings on the State of the Science in transfusion medicine will continue to assess the field of PTM and recommend studies to be performed. The objective of this article is to provide a snapshot of the clinical and translational research thought to be of highest priority by pediatricians, neonatologists, and transfusion medicine specialists.

TRANSFUSION STRATEGIES

Neonatal Transfusions

In the United States, determining the number of transfusions administered to neonates is difficult; however, an estimate of the number of red blood cell (RBC) transfusions given to premature infants has been derived by extrapolating from the University of Iowa's transfusion practices to the US Statistics of Live Births in 2000. According to this estimate, 41,699 of the 56,350 infants of very low birth weight (VBLW, <1,500 g) were transfused with RBCs. The average was three transfusions per

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infant, totaling to >250,000 transfusions in VLBW infants for that year (2). As with RBC transfusions, when platelet transfusion data from the University of Iowa is extrapolated to US neonatal intensive care units admissions, it is estimated that neonates in the United States receive 80,000 platelet transfusions per year (J.A. Widness, personal communication).

Neonatal RBC transfusions. In addition to the paucity of data for transfusions to premature infants there is a lack of consensus on the optimal hemoglobin levels at which neonates should be transfused. The concern is that over-transfusion may pose dangers for the neonate because microvascular blood flow, tissue perfusion, and conversely, oxygenation can be compromised by high hemoglobin levels. Two RBC transfusion threshold trials (3,4) in this population and their subsequent follow up studies (5–7) have been published with discordant results. Both trials independently suggest, but neither proves, that a higher hemoglobin transfusion threshold confers neuroprotection. A definitive randomized clinical trial (RCT), Transfusion of Prematures, or TOP, began in 2013 that will randomize 1,824 extremely low birth weight (ELBW, <1,000 g) infants to a liberal or restrictive RBC transfusion regimen. The thresholds are based on the presence of respiratory support and postnatal age, with the primary outcome of death or significant neurodevelopmental impairment in survivors at 22–26 mo corrected gestational age.

Another high interest RBC transfusion topic in neonates is whether or not age of the RBCs has an effect on clinical outcomes. A Canadian trial, the Age of Red Blood Cells in Premature Infants, was recently completed (8). In Age of Red Blood Cells in Premature Infants, 450 VLBW infants were randomly assigned to either RBCs stored ≤7 d (mean, 5.1 d) or to standard issue RBC with storage ranging from 2 to 42 d (mean, 14.6 d). The primary endpoint was a composite outcome of necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), bronchopulmonary dysplasia, and retinopathy of prematurity at 30 and 90 d. This study suggests that the age of RBCs in VLBW infants studied does not affect common morbidities of prematurity. Nonetheless, there remains interest among US investigators to perform a trial where VLBW infants are randomized to receive RBCs stored ≤7 d or > 14 d (C.D. Josephson, personal communication). A new study would supplement the knowledge gained in Age of Red Blood Cells in Premature Infants by providing a comparison of the differences in RBC processing between Canada and the United States, provide a greater separation of the RBC age in the treatment arms, and examine liberal vs. restrictive transfusion strategies focusing on length and degree of anemia (9).

Neonatal platelet transfusions. There are few evidence-based studies of platelet transfusions in neonates with the association between thrombocytopenia and bleeding remaining unclear. In one study with 58 infants, only two infants with IVH had low platelet counts and prolonged bleeding, and two more had minor decreases in platelet counts but normal bleeding times (10). Another study with 97 subjects found an association between prolonged bleeding times and a platelet count <100,000, but this association was not linear or precise (11). This study agreed with a large (1,283 subjects) study (12) and found a correlation between neonatal thrombocytopenia and higher IVH incidence where almost half of the thrombocytopenic infants had IVH. However, another study with 302 subjects showed no association between platelet count and IVH incidence; more than half the patients had normal platelet counts (13). All these studies focused on preterm infants in the first week of life. Whereas, in a study of 120 full-term infants with alloimmune thrombocytopenia (14), 103 had severe thrombocytopenia. Seventeen of 103 infants developed intracranial hemorrhage, and 13 infants had neurologic sequelae with platelet counts below 50×10^9/l that persisted throughout the 2.5 y follow-up period.

Clearly, important pediatric and neonatal platelet transfusion management questions remain. Among these are (i) should prophylactic transfusions be administered based on platelet count and clinical condition or should they only be administered therapeutically for significant bleeding and (ii) which platelet product(s) are optimal for which clinical thrombocytopenic situations. The only RCT (15); as well as a retrospective review (16) both found that transfusing VLBW with platelet counts of 60×10^9/l or lower in the first week did not reduce the frequency or severity of IVH. As a result, many studies continue to report large variability in the practice of platelet transfusions in the NICU (16–19). Several studies have correlated the number of platelet transfusions with higher morbidity and mortality in NICU patients (18,20–22), but most did not evaluate the severity of illness before platelet transfusion. It remains unclear whether platelet transfusions represent a marker of illness severity or contribute directly to morbidity and mortality. A two-arm RCT to study the incidence of severe bleeding in thrombocytopenic neonates treated with a restrictive vs. liberal platelet transfusion approach has been proposed to address this question (1). The primary endpoint will be the incidence of severe bleeding, as well as the role of prophylactic platelet transfusions.

Pediatric Transfusions

There are 250,000 admissions in 300 US pediatric intensive care units (PICUs) per year and 50% of the children receive at least one RBC transfusion (23). Transfusions in children are given with few evidence-based guidelines and many practices are based on historical norms from adults rather than evidence from pediatric patients. Published surveys have demonstrated that the frequency of RBC transfusion varies widely at children's hospitals in the United States and Canada (24). The results and recommendations for transfusion practice based on trials designed for an adult population should not be automatically adapted to a pediatric population.

Pediatric RBC transfusions. Lacroix and colleagues reported a multicenter RCT (25) testing the hypothesis that liberal and restrictive RBC transfusion programs would be equivalent. Equivalence was defined as a <10% difference in the

development of new or progressive multi-organ dysfunction, the primary outcome of the trial. By intention-to-treat analysis, rates of new or progressive multi-organ dysfunction were 12% in both groups. The investigators concluded that in stable, critically ill children a hemoglobin threshold of 7 g per deciliter for RBC transfusion can decrease RBC transfusion requirements without increasing adverse outcomes. To examine the effect of the age of RBC on outcomes in the pediatric intensive care unit, the Age of Blood in Children in Pediatric Intensive Care Units (ABC-PICU) study (a RCT has been funded by NHLBI and the Canadian Institutes of Health Research) and will begin enrollment in 2014. The study will randomize 1,502 critically ill children to either RBC units stored for <7 d or standard-issue RBC units. The, primary outcome will be new or progressive multi-organ dysfunction at 28 d post-enrollment (26).

Pediatric platelet transfusions. Clinical studies and trials in pediatric platelet transfusion are scarce. A Pub Med search using the terms "pediatric platelet transfusion" yields 564 references published between 1957 and 2014. Of these 564 papers, only ten are focused on platelet transfusions in general pediatric populations. Twenty-three of the papers investigated neonatal transfusions, and 17 reported on platelet transfusions in oncology patients as adjunct to stem cell transplantation or chemotherapy. The most recent disease-agnostic paper from 2007 found that there is wide variability in transfusion practices across the 35 children's hospitals that participated in the study (27). The accompanying editorial emphasized the differences found with respect to the ethnicity of the patients as well as the variations in practice and called for definitive trials to be performed in this area (28).

SHORT- AND LONG-TERM CLINICAL CONSEQUENCES

Pediatric blood recipients have higher post-transfusion survival rates compared to adult blood recipients (29,30). Given longer post-transfusion survival rates and developmental immaturity of the immune system, blood brain barrier, and physiology in general, it is likely that neonatal and young pediatric blood recipients are at greater risk than older transfusion recipients for long-term adverse outcomes from both known and currently unknown adverse effects of transfusion. In this section, three examples are discussed: NEC, RBC alloimmunization, and transfusion-associated microchimerism (TA-MC).

Transfusion Related-Necrotizing Enterocolitis

Approximately 11% of premature infants <29 wk gestation will develop NEC (31) and the mortality rate is 20–30% (32). Infants who survive NEC have long-term complications, including short bowel syndrome, impaired growth, and (neuro-)developmental impairment (33). The disease can frequently progress from early clinical symptoms to intestinal necrosis within hours, limiting the efficacy of therapeutic intervention. Strategies to prevent NEC are a primary focus of research investigations although a limited understanding of the etiology of NEC has slowed these efforts (34). Researchers have identified several factors contributing to the development of NEC including: enteral feeding, gastrointestinal bacterial colonization, gut motility, pro-inflammatory propensity of the immature gut and impaired intestinal blood flow (35–38). The multifactorial nature of the disease suggests that preventative efforts would be more successful if they targeted multiple components, including the emerging risk factor of RBC transfusion and its potential involvement in the pathogenesis of NEC. Investigating the role of RBC transfusion is relevant given the finding that ~25–38% of NEC cases in retrospective studies have been reported to occur within 48 h of RBC transfusion (39–41). A recent meta-analysis of these studies emphasized that RBC transfusion is an independent risk factor for transfusion related NEC and the need for prospective investigations of this devastating clinical entity (42). Future studies should include comparisons of transfusion volumes, irradiated blood products vs. non-irradiated products, age of blood transfused, time of transfusion especially in relation to feeding, and simultaneous feeding vs. withholding of feeding until after transfusion is complete (43).

RBC Alloimmunization

Post-transfusion alloimmunization to minor RBC antigens occurs infrequently in neonates and more commonly in pediatric patients (15,44). RBC alloimmunization arises in certain transfusion recipients exposed to foreign minor RBC antigens such as RhD. Memory lymphocytes induce an immune response that persists throughout life whereas the antibodies that cause the hemolysis often wane over years to undetectable levels by current laboratory methods (a screen which is an indirect antiglobulin test (45)). In females this sensitization can have long-term complications with potential deleterious effects on future pregnancies such as alloimmune hemolytic disease of the fetus and newborn, including fetal demise (46). These children may also experience delayed hemolytic transfusion reactions in the short and long term which can also have fatal consequences. Thus, pediatric patients are especially susceptible to complications of RBC alloimmunization, due to the long lifespan of the patient and the waning detectability of clinically significant antibodies (47).

In addition to identifying who is at the highest risk for clinical consequences from post-RBC transfusion antibody production, it is important to recognize which recipients are more likely to develop antibodies to RBC antigens. One approach to indirectly address this question has been to examine alloimmunization frequencies in patients who received multiple transfusions for hemoglobinopathies (i.e., sickle cell disease (SCD) or thalassemia). RBC alloimmunization rates in these populations are between 16–70% in some studies (48–52). Theoretically, one approach to minimize alloimmunization of susceptible individuals would be to prospectively match recipients for multiple minor RBC antigens. Although the efficacy of this approach has not been rigorously tested, it is practiced in some patient populations, such as patients with SCD. In some medical centers this appears to reduce alloimmunization (53,54). Future research into these questions could lead to individually tailored RBC transfusion therapy, especially

with the use of molecular based testing for minor RBC antigen matching between donor and recipient (55,56).

Transfusion-Associated Microchimerism

Microchimerism (MC) is the persistence and engraftment of <5% of non-self cells circulating in the host. MC occurs naturally during pregnancy with feto-maternal and twinning blood exchange, but MC can also be intentionally induced through hematopoietic stem cell transplantation (57–59). The persistence of donor leukocytes after allogeneic blood transfusion is referred to as transfusion-associated microchimerism (TA-MC) and has been demonstrated in 10–20% of adult patients following transfusion for traumatic injury (60). The development of TA-MC in pediatric blood recipients is important since a majority of transfused children will survive to adulthood. While gamma irradiation may reduce the number of persistent donor leukocytes, there is no universally accepted "standard of practice" for transfusion of gamma irradiated blood in children. In a recent study of 207 adult and 202 pediatric female medical and surgical recipients of leukoreduced and irradiated RBCs and platelets, persistence of TA-MC (Y-chromosome) was not demonstrated at 4- and/or 8-wk after transfusion (61). This suggests that pediatric blood recipients of non-irradiated and shorter storage aged blood components are not at increased risk for development of TA-MC.

Marshall et al. evaluated ten hemoglobinopathy patients for TA-MC; four patients (three SCD and one thalassemia) demonstrated transient MC 22–36 d post-transfusion (62). Further evaluation of persistent MC is warranted in hemoglobinopathy blood recipients to determine whether TA-MC represents a harmful or beneficial consequence of blood transfusion. If persistent TA-MC is demonstrated in a pediatric patient population, the next step is to study the clinical, immunologic, and blood product characteristics associated with the development of TA-MC and to monitor for long-term clinical consequences, e.g., chronic graft-versus-host disease or autoimmune conditions.

INFECTIOUS TRANSFUSION-TRANSMITTED DISEASES

Blood Safety

Surveillance, donor selection, testing, and hemovigilance have led to a low risk for HIV and HCV of approximately one infection in 1–1.5 million units transfused (63). Current blood safety has been maintained by introducing new donor screening questions and new tests are added as new agents and technologies appear (64). To overcome the lack of a formal hemovigilance system, the American Association of Blood Banks and the Centers for Disease Control have recently formed a public-private partnership to implement a Biovigilance Network to include an infectious diseases arm specifically related to the investigation and confirmation of transfusion transmitted infectious diseases (65).

Infectious Diseases in PTM

The efficacy of transfusion in achieving homeostasis must be balanced against the risk of harm. Infants and children have unique issues affecting this balance, including immature immune systems, anatomic issues such as small passages, rapid cell-growth rates, and first exposures. Through adaptive immunity, neonates primarily receive immunoglobulin G passively from their mothers, and although they have a complete repertoire of lymphocyte subpopulations, their cytokine and chemokine production is limited. Infectious diseases of particular concern due to morbidity and mortality implications in neonatal and pediatric populations are cytomegalovirus (CMV), Babesia, and dengue. The current strategy of using CMV seronegative units combined with the removal of latently infected leukocytes is effective, but there are still reports of breakthrough cases of transfusion-transmitted CMV (66), especially in immuno-incompetent premature infants and immunocompromised primary immunodeficiency and oncology/stem cell transplant recipients. Babesia is a tickborne parasite that can be transmitted through transfusion or transplacentally (67). Currently, there is no licensed test to screen blood donors for Babesia; however, the Rhode Island Blood Center is testing the use of serology and nucleic acid testing for neonates and pediatric SCD patients receiving exchange transfusions (68). Lastly, dengue is now endemic in over 100 countries reaching epidemic proportions in some Asian and Latin American countries. There is no vaccine and prevention depends solely on vector control (69).

Pathogen Reduction in PTM

An important strategy to reduce the risk of transfusion-associated bacteremia and other infectious diseases, is pathogen reduction technology. Studies in which platelet components have been cultured at outdate have shown that ~1 in 1,500 apheresis platelet components contain bacteria (70–72). Although the safest and most effective blood products are desired for all patients, pediatric transfusion safety is different in several key respects. Any potential gain in transfusion safety for this population may result in many more disease-free years of life compared to adults. This makes the calculation of cost-effectiveness in quality-adjusted life years gained more compelling for children who receive transfusions.

There are two pathogen reduction methods for cellular products that are under investigation in the United States, and both are effective against bacteria, enveloped and non-enveloped viruses, and parasites. The first of these is a psoralen-based compound that intercalates with the nucleic acid of the pathogen upon activation by ultraviolet light (73). Efficacy was demonstrated in a RCT that allowed enrollment of subjects 6 y and older, but only six subjects aged 16 y or younger were enrolled out of 654 subjects (74). Psoralen treated platelets have been tested in Alsace, France to support pediatric and adult patients with Glanzmann's thrombasthenia (unpublished data). The other technology uses riboflavin and UV light to intercalate with nucleic acids (75). In addition to pathogen reduction, this technology appears to reduce alloimmunization and transplant rejection in an animal model (76) and was effective in an RCT (77). All these methods cause perturbation to the cell membrane raising concerns about introduction

of new chemical entities into the blood, short- and long-term toxicities, and effects on cell quality and transfusion efficacy.

INNOVATIVE STRATEGIES TO PROMOTE RESEARCH IN PEDIATRIC TRANSFUSION MEDICINE

As described herein, recognition of gaps in evidence-based practices for neonatal and pediatric transfusion has stimulated creative strategies to accomplish needed studies. For example, the sub-analysis paper for the platelet dose study (78), which included over 200 children in a 1,350 patient RCT performed by the TMH CTN, yielded information that distinguished the bleeding patterns and amount of bleeding in children as different from adults with hypoproliferative thrombocytopenia. The inclusion of children in this study was important as it revealed the first new information about platelet transfusion in over 30 y within the era of modern chemotherapy and cancer treatment in children. However, platelet dose was not powered to detect differences in the pediatric subpopulation so these findings are still preliminary, but it is clear that a separate study, with a large sample size, is necessary to provide definitive evidence.

One innovative strategy to study large numbers of children is by investigators collaborating with established networks such as the National Institute of Child Health and Human Development Neonatal Research Network which has worked with NHLBI to perform the ongoing TOP trial, deemed in the State of the Science meeting to be one of the most important phase III trials needed in the next decade. Another innovative tact to accomplish large RCTs is by building a grass roots network as the pediatric critical care community has done with the Blood Net program (http://www.bloodnetresearch.org/) and the Pediatric Acute Lung Injury/Sepsis Investigators (http://www.palisi.org/) network. With this infrastructure the network investigators were able to secure funding for enough centers to correctly power different study questions. One tangible result of this collaboration is the ABC-PICU trial described earlier in this review.

Funding for pragmatic RCTs is also now possible through the newly established Federal institute, Patient Centered and Outcomes Research Institute (PCORI) (http://www.pcori.org), which is suitable for some of the research suggested in this review. Specifically, research designs and methodology related to comparative effectiveness, also within PCORI's mission may answer certain questions related to blood utilization's impact on pediatric outcomes; and safe platelet transfusion therapies in premature infants.

In conclusion, this is an exciting time for the field of PTM. Multiple and varied opportunities exist for investigators from various fields to use PTM as a model system to create an evidence-based foundation for clinical decision making while engaging scientific disciplines such as, immunology, cell biology, infectious disease, microbiology, metabolic biochemistry, pharmacology, and epidemiology.

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