


The Developing Balance of Thrombosis and Hemorrhage in Pediatric Surgery: Clinical Implications of Age-Related Changes in Hemostasis

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Abstract

Bleeding and thrombosis in critically ill infants and children is a vexing clinical problem. Despite the relatively low incidence of bleeding and thrombosis in the overall pediatric population relative to adults, these critically ill children face unique challenges to hemostasis due to extreme physiologic derangements, exposure of blood to foreign surfaces and membranes, and major vascular endothelial injury or disruption. Caring for pediatric patients on extracorporeal support, recovering from solid organ transplant or invasive surgery, and after major trauma is often complicated by major bleeding or clotting events. As our ability to care for the youngest and sickest of these children increases, the gaps in our understanding of the clinical implications of developmental hemostasis have become increasingly important. We review the current understanding of the development and function of the hemostatic system, including the complex and overlapping interactions of coagulation proteins, platelets, fibrinolysis, and immune mediators from the neonatal period through early childhood and to young adulthood. We then examine scenarios in which our ability to effectively measure and treat coagulation derangements in pediatric patients is limited. In these clinical situations, adult therapies are often extrapolated for use in children without taking age-related differences in pediatric hemostasis into account, leaving clinicians confused and impacting patient outcomes. We discuss the limitations of current coagulation testing in pediatric patients before turning to emerging ideas in the measurement and management of pediatric bleeding and thrombosis. Finally, we highlight opportunities for future research which take into account this developing balance of bleeding and thrombosis in our youngest patients.

Keywords

blood coagulation factors, cardiopulmonary bypass, clot structure, coagulation, pediatric thrombosis, hemostasis

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Background

Bleeding and thrombosis in infants and children are not typically regarded as significant clinical problems, since the overall incidence is low relative to the adult population.¹ Management strategies to date have been largely extrapolated from adults.² However, as we increasingly manage critically ill children undergoing major surgical interventions, the incidence of bleeding and thrombosis in this population has also increased,³ and strategies developed for the adult hemostatic system may not be effective in pediatric patients. More research is needed to better characterize the biology of the developing hemostatic

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system in normal and critically ill children, define clinically meaningful criteria and tests for assessment, and identify effective treatment strategies.

In this review, we examine our current understanding of the developing hemostatic systems in pediatric patients from infancy to adulthood, a concept referred to as “developmental hemostasis,” and discuss emerging concepts of immune cross talk in pediatric hemostasis compared to adults. We then examine its impact on pediatric surgery and critical care, describing clinical scenarios in which our inability to effectively assess and treat pediatric hemostatic derangements is particularly apparent. We describe available tests to evaluate the hemostatic system and discuss the pitfalls inherent in the use of traditional coagulation measures and targets in this population. Finally, we suggest future directions for basic, translational, and clinical research in this important field.

Developmental Hemostasis

Our current understanding of coagulation has evolved from a linear cascade of enzymatic reactions to a shifting network of interactions among plasma proteins, endothelium, foreign surfaces, and inflammatory cells. The responses to tissue or endothelial injury or exposure to foreign surfaces are complex and dynamic, making timely, accurate, and complete hemostasis assessment difficult in the clinical environment. In the pediatric population, age-related changes in hemostatic components add additional complexity, since levels, characteristics, and interactions of pro- and anticoagulant factors vary from infancy to adulthood. Changes in the developing hemostatic system were first described by Andrew et al in the 1980s and have been called “developmental hemostasis.”⁴⁻⁶

Classical Coagulation Cascade Model

The classical coagulation cascade model, first described in the 1960s, describes a series of enzymatic reactions and interactions among proteins and clotting factors via the contact activation (intrinsic) and tissue factor (extrinsic) pathways that converge on a final common pathway that culminates in thrombin formation (Figure 1A).¹² However, this model was found not to adequately predict various coagulopathic conditions or responses to treatment in the clinical setting,¹³ and a model incorporating the complexity of the interplay between cellular elements and circulating coagulation factors emerged.⁸

Cell-Based Model of Hemostasis

The cell-based model of hemostasis gives a more complete and integrated view of coagulation *in vivo*. Although the classical coagulation cascade model accounts for the activity of soluble clotting factors, this model does not account for the important contributions of the cells and tissues where coagulation occurs. In particular, the contribution and importance of tissue factor-bearing cells and platelets are not incorporated. In their seminal article presenting this cell-based model of hemostasis, Hoffman and Monroe describe 3

phases of coagulation in response to injury: *initiation*, *activation*, and *propagation*.⁸ Initiation occurs via binding of circulating factor VII to exposed tissue factor-bearing cells, which initiates the formation of thrombin and factors Xa and IXa. Amplification follows as platelets activate and aggregate. Finally, propagation of the response leads to clot formation, as platelet surface factors and active proteases combine to form thrombin and fibrin polymers (Figure 1B).¹² While the details of the cell-based model are beyond the scope of this review, a few points warrant attention in the context of developmental hemostasis. First, in contrast to the classical coagulation cascade, the cell-based model of hemostasis incorporates and emphasizes the location of clot formation and inciting cell or factor. For example, membrane phospholipids—in particular, on platelets—are important cofactors in thrombin generation, and coagulation therefore varies by location. Additionally, the cell-based model emphasizes that “tenase” (factor VIIIa-factor IXa) complex formation during the propagation phase can occur due to a variety of stimuli and ultimately leads to thrombin generation sufficient for clot formation.⁸

Differences in the pediatric hemostatic system, where levels of coagulation factors differ significantly from adults but do not result in abnormal hemostasis, are therefore more readily understood within the complex interplay of cellular and circulating factors included in the cell-based model of hemostasis. If one were to seek to describe pediatric hemostasis purely based on the coagulation cascade model, one would find that through the first years of life, children are “deficient” in all relevant factors, save FVIII and fibrinogen, and have increased circulating von Willebrand factor (vWF; Figure 2).⁴⁻⁶ Laboratory values for traditional coagulation measurements such as Partial Thromboplastin Time (PTT) reflect these deficiencies even in normal children, but they do not reflect an increased bleeding tendency. Therefore, it is clear that the more complex cross talk between circulating hemostatic factors, platelets, and endothelial surfaces comprising the cell-based model must be considered as the basis of pediatric hemostasis.

Development

Although the introduction and characterization of the cell-based model has expanded understanding and treatment options for hemostatic derangement in the mature adult hemostatic system, differences in pro- and anticoagulant factor levels in infants and children relative to adults illustrate the need for better understanding of developmental hemostasis. In initial seminal research, Andrew et al detailed hemostatic factor level differences in healthy full-term⁵ and healthy premature⁶ infants relative to adults and described the maturation of the coagulation system through childhood.⁴ They noted that infants maintain an effective hemostatic system despite differences relative to adults.

Changes in Coagulation Factors

As noted, plasma pro- and anticoagulant factor levels in neonates, infants, and children differ from adults as show in Figures 2 and 3.⁴⁻⁶ Average levels of most procoagulant factors remain 20%

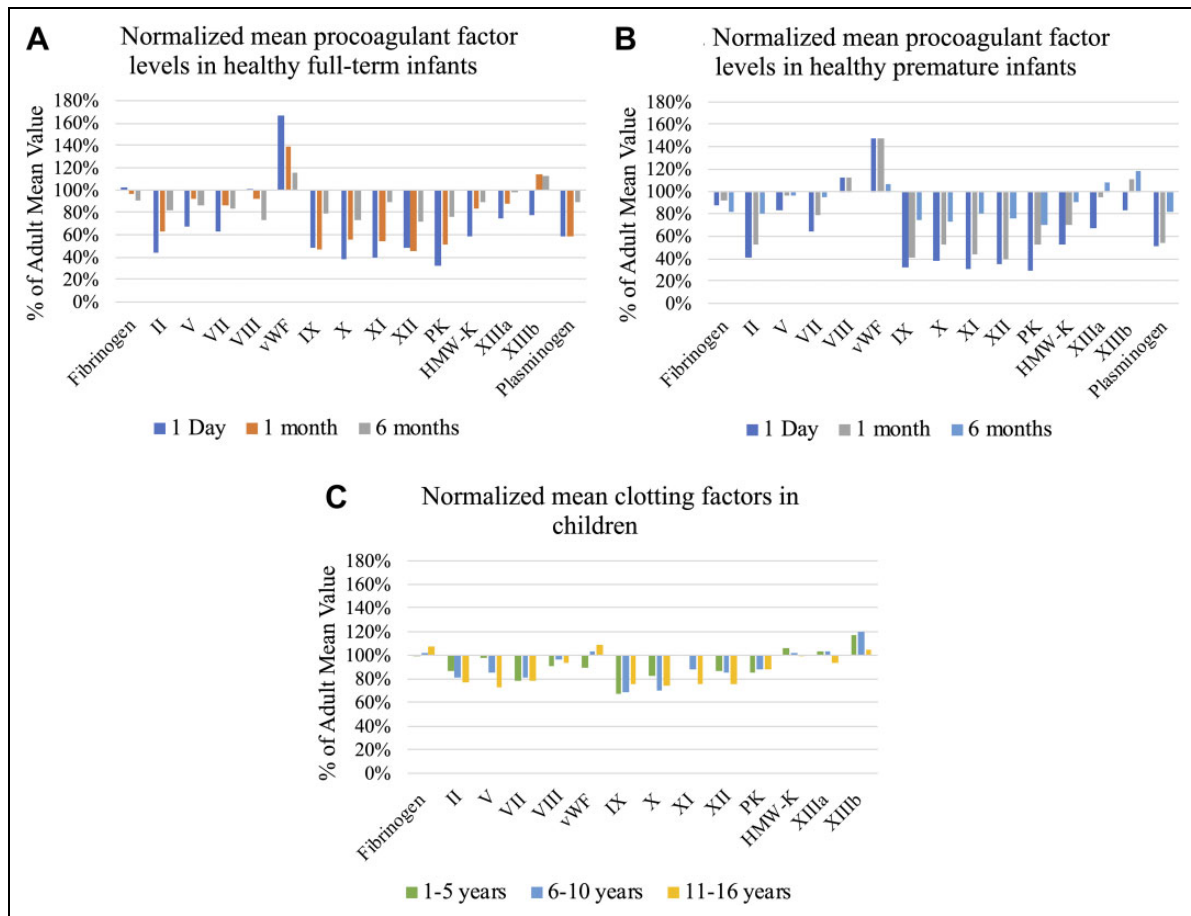


Figure 2. (A) Changes in procoagulant factor levels in healthy full-term infants in the first 6 months of life, relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁵ (B) Changes in procoagulant factor levels in healthy premature infants in the first 6 months of life relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁶ (C) Changes in procoagulant factor levels in children 1 to 16 years of age relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁴

lower than adult levels throughout childhood.¹⁴ Both tissue factor pathway inhibitor and antithrombin (AT) are present in reduced quantities,^{5,6,15} while in contrast, neonatal tissue factor levels are high compared to adult levels. There are also reduced concentrations of anticoagulants⁴⁻⁶ such as heparin cofactor II and protein C/S (Figure 3).^{4-6,16} Antithrombin and heparin cofactor II are at 50% of adult levels at birth and increase to adult levels by 3 months of age.^{4,5} Perhaps to compensate for the low level of AT, another anticoagulant, α_2 -macroglobulin (α_2 -m), is present in larger amounts at birth than in adults and increases to twice adult values at 6 months of age.^{4,5} Protein C and S levels are also low relative to adults; protein C starts out in fetal form; and remains at low levels until 6 months of age.^{4,5} Despite these differences, neonatal thrombin generation is equivalent to approximately 90% of that in adults, which is ample for hemostatic clot formation.¹⁷ Although the differences are most pronounced in the neonatal period, the school aged children and adolescent hemostatic systems still differ significantly from adults, further complicating the diagnosis and management of hemostatic disorders in the pediatric population.¹⁴

Changes in Platelet Function and Fibrinolysis

Platelet and fibrin function and physiology are also age dependent. After birth, platelet adhesion is augmented by increased plasma concentrations of vWF and higher functional high-molecular-weight vWF forms.¹⁸ At the same time, the platelet response to physiologic agonists is diminished: Studies of cord and peripheral blood have shown transient platelet hyporeactivity to thrombin, collagen, adenosine diphosphate, and U46619 (a thromboxane A₂ analog) in the first days after birth.¹⁹⁻²¹ Reactivity reaches normal adult levels between days 5 and 9 of life. In contrast to (and perhaps in order to counteract) this diminished platelet activity, children demonstrate increased plasma vWF concentrations until about 3 months of age (Figure 2).^{5,6,16}

Levels of both pro- and antifibrinolytic components differ in children as well. Plasminogen and α_2 -antiplasmin are similar to adults throughout childhood (Figures 2 and 3).⁴⁻⁶ However, tissue plasminogen activator (tPA) is less abundant in children, and plasminogen activator inhibitor is found at higher levels than in adults.^{4,6} Additionally, there are unique fetal glycoforms of fibrinogen and plasminogen, which are less active overall and have

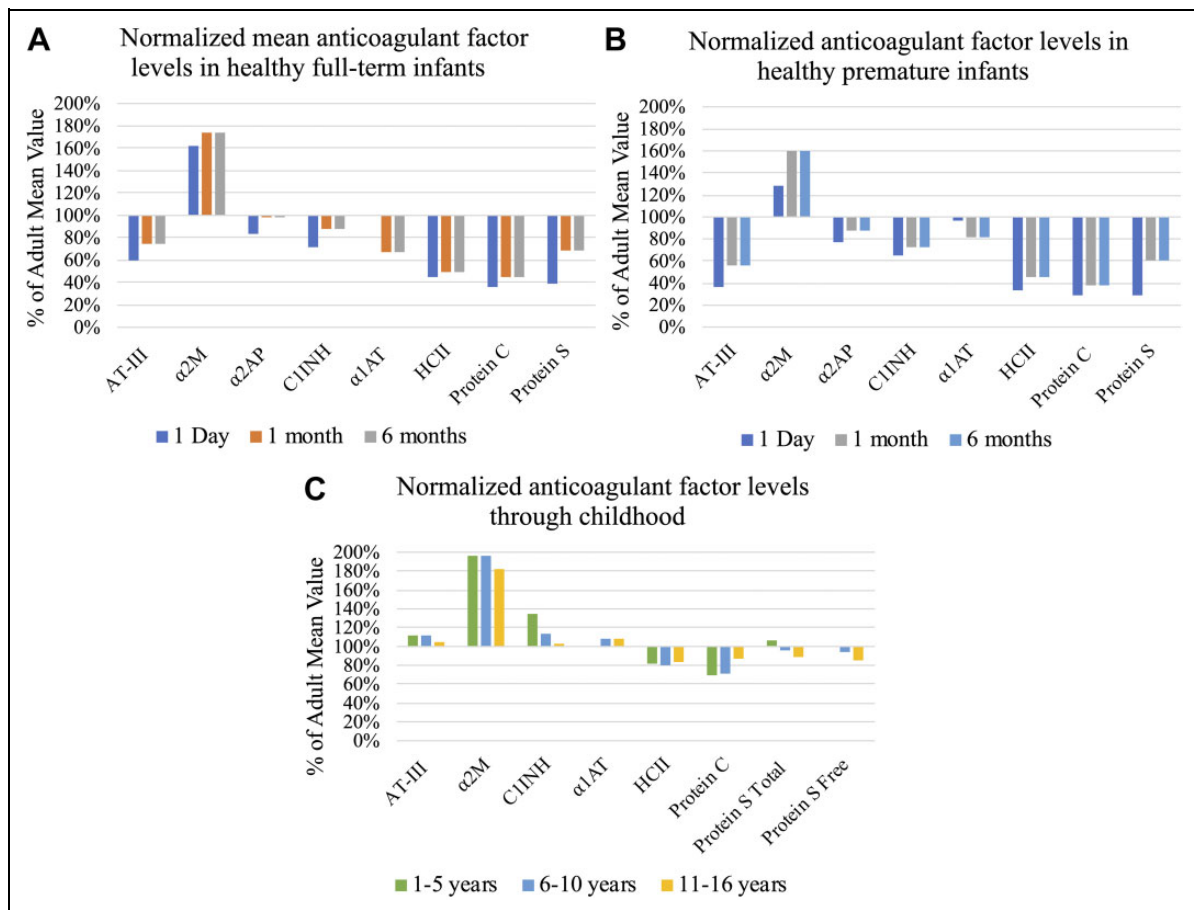


Figure 3. (A) Changes in anticoagulant factor levels in healthy full-term infants in the first 6 months of life relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁵ (B) Changes in anticoagulant factor levels in healthy premature infants in the first 6 months of life relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁶ (C) Changes in anticoagulant factor levels in children 1 to 16 years of age relative to adult levels. Normalized values calculated as (mean for age)/(mean for adult) using data from Andrew et al.⁴

less effective receptor binding compared to adult forms.²² The fetal glycoform of plasminogen is less efficiently converted to plasmin by tPA, but the kinetics of activation by urokinase plasminogen activator do not differ.¹⁶ Recent work has demonstrated that fetal fibrinogen forms different clot structures than adult fibrinogen. Furthermore, the addition of adult fibrinogen to fetal fibrinogen results in disorganized clot microstructure in vitro.²³ These structural differences may help to explain the reduced fibrinolysis observed in infants.²² A recent study in neonatal piglets demonstrates that these animals recapitulate the observed differences in clot structure between human neonates and adults, suggesting that piglets may serve as a useful laboratory model.²⁴

Immune Contribution

In adult populations, the interplay between immune activation and regulation of the coagulation cascade within the inflammatory response has been established. In particular, there is extensive cross talk between the complement system (another evolutionarily conserved enzymatic cascade) and coagulation pathways. There are multiple sites of interaction between

complement and coagulation that include endothelium, cells, and platelets as well as nonendothelial surfaces such as plastic tubing. A few aspects of these complex interactions are worth highlighting. First, activated coagulation cascade components can initiate and/or cleave complement components in the fluid phase. Second, complement- and coagulation-associated proteins colocalize on vascular and endothelial surfaces, and the contact-associated factors XII, XI, prekallikrein, and high-molecular-weight kininogen communicate with endothelial complement proteins. On circulating cells, including platelets and leukocytes, the cross talk can also modulate the hemostatic response to inflammation. Pathogens can also utilize the cross talk between complement- and coagulation-associated proteins to avoid detection; for example, by binding fibrinogen to coat their surface, they can evade deposition of C3b and the resulting opsonization for phagocytosis by neutrophils.²⁵ This cross talk between adult immune response and coagulation suggests a complex interplay between inflammatory responses and altered coagulation.²⁶ In infants and children, however, this relationship remains incompletely characterized.

The pediatric innate and adaptive immune responses are immature at birth and emerge over time as has been reported

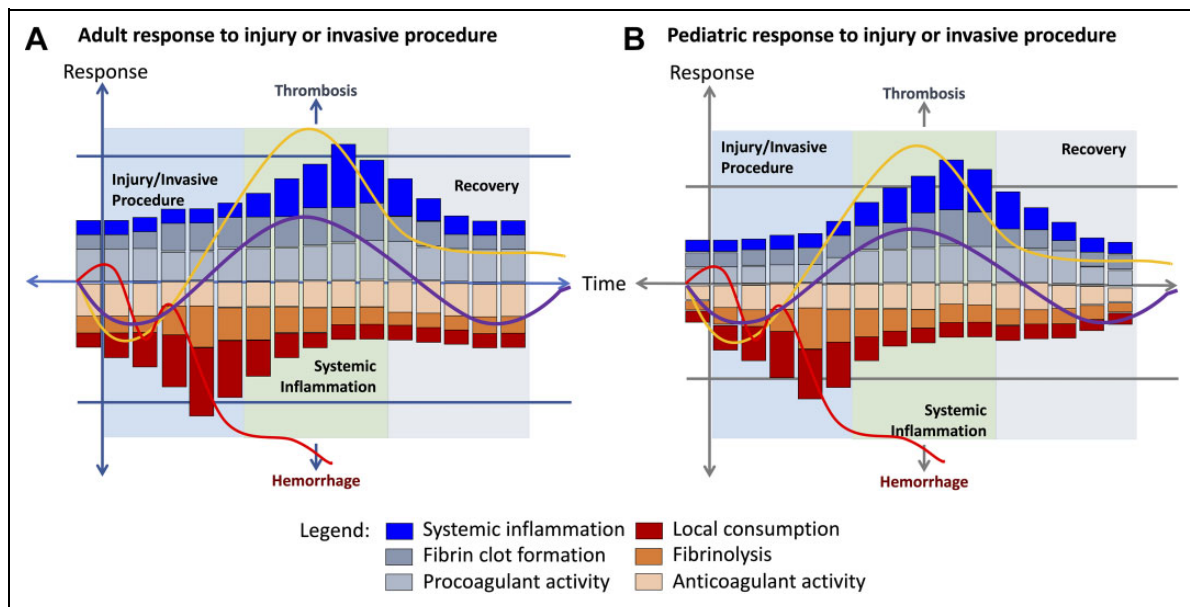


Figure 4. The normal hemostatic response to injury or invasive procedures involves an initial bleeding phase (which if left unchecked could result in hemorrhage) followed by a pro-thrombotic, pro-inflammatory phase (which if left unchecked could lead to pathologic thrombosis) then recovery (A). In adults, qualitative and quantitative age-related changes in various components of hemostasis result in a wider range “safe” range in which normal hemostasis can be restored without deviating into life-threatening hemorrhage or thrombosis than in children (B).

previously.²⁷ However, a few points merit particular attention. First, cross talk between complement and coagulation factors in adults has been well established²⁵ but is yet to be fully characterized in pediatric populations. However, reports suggest that there is an important association between activation of the complement and coagulation systems—particularly contact activation—and increased morbidity in neonates.^{28,29} Preterm infants are known to exhibit reduced levels of both immunoglobulin G and complement, in addition to decreased neutrophil function.³⁰ Monocytes and macrophages demonstrate impaired signaling pathways resulting in weaker cytokine production than adults.^{27,31-34} Adaptive immunity also follows a distinct developmental pathway. Newborns have populations of $\gamma\delta$ T cell receptor (TCR)-positive and innate-like $\alpha\beta$ TCR-positive T cells. While $\gamma\delta$ T cells have limited function in adults, they display a range of receptor chain combinations in neonates. $\gamma\delta$ T cells can produce large amounts of interferon γ that may compensate for the relative immaturity of the classical Th1-type T-cell response.^{27,35,36} The B-cell subpopulations also differ from adult levels. At birth, B1 cells constitute 40% of B-cells in the peripheral blood.³⁷ B2 (“conventional”) B-cells have limited capacity in the neonate due to lower expression of coreceptors such as CD28 and CD40 ligand.³⁸ These differences in both humoral and adaptive immune responses may point to differential contributions of systemic inflammation and immune factors to coagulopathy in neonates, infants, and children relative to adults.

Clinical Problems of Pediatric Bleeding and Thrombosis

Despite these qualitative and quantitative age-related changes in plasma proteins and cell populations, normal hemostatic

mechanisms compensate for most healthy neonates, infants, and children to prevent major bleeding and clotting complications. However, in critically ill children and those undergoing major procedures that induce considerable physiologic stress, maintenance of hemostatic balance is different than in adults (Figure 4). Representative clinical scenarios with increased rates of bleeding and thrombosis include major surgery,³⁹ trauma,⁴⁰ abdominal organ transplantation,⁴¹ and extracorporeal life support systems (ECLS; ie, extracorporeal membrane oxygenation [ECMO] and cardiopulmonary bypass [CPB]).⁴² These procedures generate physiologic derangements that overwhelm the hemostatic system’s compensatory mechanisms, shifting the balance toward life-threatening hemorrhage and/or thrombosis.

Major Surgery

Complex, lengthy, and physiologically stressful surgical procedures are becoming increasingly possible and survivable in children. However, with increased utilization of these procedures, rates of thrombosis and bleeding complications are also increasing.^{3,39} Surgery itself alters coagulation by multiple mechanisms that include hyperadrenergic responses, hyperfibrinolysis, and consumptive coagulopathy.⁴³ In most routine surgical procedures on infants and children, the orchestration of procoagulant, anticoagulant, fibrinolytic, and vascular endothelial responses is sufficiently balanced to prevent pathological bleeding or thrombosis. However, when invasive procedures cause significant vascular endothelial injury, exposure of blood to nonendothelial surfaces, or altered hemodynamics, life-threatening bleeding and/or thrombosis can occur.^{25,44} In an ACS National Surgical Quality Improvement Program—

based study examining rates of venous thromboembolism (VTE) in pediatric surgery patients, Sherrod et al found that the highest VTE risk occurred in patients undergoing cardiothoracic or general surgical procedures, omphalocele repair, CSF-shunt creation, and complete colectomy via abdominal approach.³⁹ In a national study examining rates of femoral arterial thrombosis following cardiac catheterization in pediatric populations, we reported that infants aged 0 to 12 months have the highest rate of arterial thrombosis following cardiac catheterization of any pediatric age-group and that arterial thrombosis is associated with increased morbidity and cost when compared to children aged 1 to 18.^{45,46}

Extracorporeal Life Support

Extracorporeal membrane oxygenation and CPB have made previously high mortality procedures survivable for pediatric and adult patients. However, extracorporeal circuits expose blood to nonendothelial surfaces that initiate prothrombotic and inflammatory responses.⁴⁷ As a result, patients on ECMO or CPB require anticoagulation. Unfractionated heparin is the mainstay of therapy due to its reversibility, rapid onset of action, and ability to titrate effects.^{42,44,47} However, children and neonates exhibit high rates of bleeding and thrombotic complications on ECMO, including high rates of central nervous system bleeding that are associated with increased mortality.^{42,48,49} In infants and children, standard clot-based monitoring tests such as activated clotting time and PTT are unreliable predictors of clotting or bleeding risk, given their lower levels of antithrombin.⁴⁷

The challenges of managing children and infants on ECMO or CPB arise in part from the lack of reliable tests to evaluate their hemostatic state as well as a dearth of well-studied anticoagulation options. Extracorporeal membrane oxygenation induces changes in coagulation that include fibrinolysis in children. In a study of 29 children >30 days of age who bled during ECMO, plasmin–antiplasmin levels were persistently elevated on day 5, while plasmin–antiplasmin decreased in nonbleeders, consistent with fibrinolysis contributing to bleeding. In neonates, all biomarkers were elevated on day 5 when compared with day 1, irrespective of bleeding complications.⁵⁰ Data from experiences with CPB also suggest differential responses to ECMO in children relative to adults. Thrombosis has been demonstrated in up to 20% of children after CPB, with a higher risk in neonates.⁴⁵ Extracorporeal circuits result in a thromboinflammatory response, where tissue factor expression leads to thrombin generation,⁵¹ and contact activation when blood interfaces with the nonendothelial circuit. A recent *in vitro* examination of the effect of flow rate on platelet, leukocyte, and extracellular vesicles within an ECMO circuit using adult blood suggested that both high- and low-flow rates, in contrast to a “clinical” flow rate, increased tissue factor expression on leukocytes and in extracellular vesicles, resulting in increased oxygenator thrombosis, despite infusion of tissue factor pathway inhibitor.⁵² Thrombin levels increase within minutes of CPB initiation.^{53,54} Biomarkers of thrombin formation are also

elevated at the end of CPB in neonates, and this elevation persists for up to 3 days after surgery.⁵⁵ This, in turn, can result in the consumption of clotting factors and the need for allogeneic transfusions. *In vitro*, addition of adult fibrinogen to fetal fibrinogen results in disorganized clot formation and may be detrimental to clot dynamics.²³ Studies in older children have demonstrated decreased levels of the anticoagulant factors AT, protein C, and protein S during CPB. However, in a more recent study, there was no difference in AT, protein C, or protein S levels among neonates who did and did not develop thrombosis after cardiac surgery.⁵⁶ These data suggest that the risk of thrombosis in children cannot easily be predicted on the basis of levels of circulating hemostatic factors alone.

Emerging research in ECMO focuses on the development of novel anticoagulation strategies that specifically target contact activation or other factors to enable effective anticoagulation without increasing the risk of hemorrhage. One successful pre-clinical approach has been to target activated factor XII (FXIIa) using an inhibitory antibody (3F7) in a rabbit model of ECMO, which is predicted to reduce contact activation (Figure 1A).⁹ Another strategy uses a piglet model of CPB to evaluate an RNA aptamer–antidote pair to specifically inhibit activated factor IX (FIXa; Figure 1A and B).¹⁰ While these strategies have shown promise, to our knowledge, they have not yet begun to be evaluated in human patients, let alone in the vulnerable pediatric population. Antisense oligonucleotides, monoclonal antibodies, and small molecules targeting factor XI/factor XIa are currently being evaluated for VTE prophylaxis in adult patients with promising early results in clinical trials (Figure 1A)^{11,57}; to our knowledge, these have not yet been evaluated in pediatric patients. Therefore, improvements in the monitoring and treatment of pediatric patients on ECLS with currently available technology are urgently needed.

Trauma

Pediatric patients with trauma represent another illustrative example of differences in the presentation, course, and management of children at risk for bleeding and thrombosis. Trauma-induced coagulopathy describes the finding of coagulopathy that is inherent to injury. It is present in trauma patients prior to (and then typically is exacerbated by) resuscitation and associated hemodilution,^{58,59} a well-recognized phenomenon in adult populations. Clinically, it is defined as a prolongation of clotting times, prothrombin time (PT)/international normalized ratio (INR), in patients presenting with major trauma. However, the available literature on pediatric ATC suggests that, while this phenomenon occurs in children, this presentation may arise from different derangements in children than in adults and that further research into optimal management is needed.⁴⁰ A recent study from our group focused on the prevalence of ATC in pediatric, adult, and older adult populations at a single site to begin to characterize the differences in presentation and laboratory values that characterize these populations. We found that prolonged activated partial thromboplastin time (aPTT), prolonged INR, and hypofibrinogenemia were associated with mortality in

pediatric and adult populations, suggesting that ATC can be identified in pediatric trauma patients using specific reference values for clotting tests.⁶⁰ However, others have suggested that in both adult and pediatric patients, the use of viscoelastic methods such as thromboelastometry (TEG) or rotational thromboelastometry (ROTEM) may eventually prove more effective in helping to guide management.^{40,61}

In patients requiring massive transfusion, either due to ATC or due to other causes, the need to optimize management protocols specifically for pediatric patients is pressing. A recent systematic review on pediatric massive transfusion protocols by our group demonstrated important deficits in current practice: heterogeneous definitions of massive blood loss, variability in transfusion protocols (or absence of an established pediatric massive transfusion protocol at the institution at all), diverse clinical and laboratory assays guiding transfusion implementation, and persistently high mortality rates in pediatric patients receiving massive transfusion.⁶² Although studies in adults have concluded that a balanced transfusion protocol does reduce exsanguination (despite failing to reduce overall mortality),⁶³ the application of balanced transfusion protocols in pediatric patients has been inconsistent⁶² and requires further study.⁴⁰

Transplant

Abdominal transplant confers a significant risk of both bleeding and thrombosis due to the operation as well as underlying disease. In our institutional series of pediatric liver and multi-visceral transplants, the risk of major bleeding was 20%, and the risk of organ thrombosis was 25% of which 40% required subsequent retransplantation.⁶⁴ Indication for pediatric liver transplantation is cholestatic liver disease in 30% to 50% of cases. These patients have a normal hemostatic profile prior to transplant but a higher incidence of hepatic vessel thrombosis. Whether cholestasis results in a hypercoagulable state in infants and children as it does in their adult counterparts is not known.⁶⁵ Mimuro et al studied the coagulation and fibrinolytic profiles of 63 pediatric patients following liver transplantation by measuring PAI-1, TM, ADAMTS-13, soluble E-selectin, protein C and plasminogen activity, soluble fibrin, and PT.⁶⁶ Results showed a rapid recovery of coagulation activity from day 1 with a full recovery of the coagulation and fibrinolysis system to normal levels by days 21 to 28. Soluble fibrin levels, a marker of thrombin generation, increased significantly on day 1 and then decreased, normalizing by day 14. In a previous study of pediatric liver transplants, AT was reduced in ~70% of patients.⁶⁷ Heparin, which inhibits coagulation by binding to AT, is the most commonly used agent in anticoagulation protocols in pediatric liver transplantation. Anticoagulation strategies to prevent thrombosis of vascular reconstructions may improve with AT repletion or alternative anticoagulants. These changes illustrate that perioperative management of hemostasis in pediatric patients undergoing liver transplantation involves unique considerations.

Challenges of Assessing Hemostasis in Infants and Children

Major challenges complicating clinical assessment and management of bleeding and thrombosis in infants and children include the lack of accurate, rapid, and meaningful assays for assessing hemostasis and the extrapolation of therapeutic targets from adult studies.^{2,14} Standard laboratory tests can provide accurate and reliable information regarding coagulation proteins in plasma and qualitative and quantitative information about plasma coagulation proteins; however, red blood cells and platelets are commonly assessed based on quantitative data only. We lack the ability to evaluate the role of vascular endothelial functions and influence of inflammatory cytokines and other biomarkers. Additionally, interpretation of these tests in children is challenging in practice due to the need for adjustment by laboratory-specific, age-matched standards. Even when standard laboratory tests are appropriately interpreted in light of the normal developmental changes in the coagulation profiles of infants and children, they are limited by capturing only specific aspects of the child's hemostatic picture. For example, healthy newborns exhibit prolonged clotting times on standard coagulation tests,^{5,6} but newborns have no increase in bleeding tendency.⁶⁸

Screening tests for hemostasis include PT/INR, aPTT, and viscoelastic testing methods. Prothrombin time assesses the extrinsic and final common pathways of the coagulation cascade as a measure of the time required for plasma to clot after introduction of calcium, phospholipid, and tissue factor or its analogues (thromboplastin). The INR was introduced to address the variability of PT due to different sensitivities of the thromboplastin reagents made by different manufacturers.⁶⁹ The INR accounts for variances in thromboplastin sensitivity by using the international sensitivity index and the geometric mean of the prothrombin time for at least 20 healthy adults tested at the performing laboratory.⁶⁹ The aPTT measures the contact activation (intrinsic) and final common pathways of the coagulation cascade by reporting the time required for plasma to clot after the addition of calcium, an intrinsic pathway activator (ie, kaolin or ellagic acid), and phospholipid to activate the intrinsic pathway. The aPTT reagent was named "partial thromboplastin" because it lacks tissue factor.^{69,70} More recently, viscoelastic testing—including TEG (Haemostasis Corporation) and ROTEM (Tem GmbH)—has emerged as a potential means to monitor whole blood coagulation in patients by analyzing clot phenotype.⁷¹ These assays evaluate the dynamics of coagulation and clot dissolution by measuring the force transmitted to a pin immersed in the blood during rotation either of the cup (TEG) or of the pin (ROTEM).⁶⁷

These screening tests have limitations. For example, PT and aPTT test results can differ due to variable preanalytical issues (ie, those related to sample acquisition), use of different reagents, or incorrect application of reference ranges. Unfortunately, most aPTT reagents do not have published age-related reference ranges, making the task of accurate interpretation more difficult. If the reference range is not appropriate based on the testing reagent or patient age, children may be incorrectly suspected of

having a bleeding disorder.⁷² Until recently, normative ranges for TEG and ROTEM for children and term neonates had not been described, although recent data among healthy donors have become available to guide the use of these assays.^{68,73,74}

Future Directions

The current deficiencies in evidence-based, practical, robust clinical assessments and guidelines for treating pediatric patients with hemostatic derangements present several opportunities to advance understanding of both normal and pathological hemostasis in children. Further studies are needed to define laboratory tests and parameters that provide rapid, accurate assessments that can be utilized to determine clinically meaningful end points for management, including transfusion or anticoagulation. Studies utilizing viscoelastic measurements in pediatric patients undergoing major surgery, as well as those on ECMO, to better evaluate changes in clot dynamics are underway to suggest future therapeutic targets. Understanding the complex and dynamic coagulation system, its interplay with the immune system, the dynamics of coagulation and inflammatory interactions with foreign surfaces, and the changing human plasma proteome promises more targeted and timely interventions in children at risk for bleeding or thrombosis. Current coagulation tests cannot characterize the role of flow or vascular endothelial interactions, inflammatory influences, or functional activity that are important for hemostasis. Recent advances in “-omics” may enable highly personalized, precise profiling of a patient’s hemostatic system at a given time point, allowing more precise predictions and treatment decisions.⁷⁵ Age-related changes in the plasma proteome of neonates and children, including both quantitative and qualitative changes (such as phosphorylation), have been described.^{75,76} Although many studies of the human proteome have focused on identification of biomarkers for adult disease states,^{77,78} we know little about the clinical relevance of age-related changes in the proteome of infants and children. The plasma proteome may be an accessible way to understand the interactions of multiple hemostatic mechanisms and is an exciting direction of ongoing study.

Conclusion

Bleeding and thrombosis in critically ill pediatric patients and those undergoing major surgery remains a complex clinical problem.³⁹ Increasingly, critically ill children and neonates are undergoing complex surgeries and interventions, but evidence-based, biology-informed guidelines for managing these patients are important.⁷⁹ Risk factors that can guide decision-making and therapeutic interventions in this population are scarce,⁷⁹ often largely extrapolated from adult practice, and do not account for special age-related changes. Prospective, randomized, controlled trials are difficult to conduct in this population, but studies are needed to evaluate the pediatric plasma proteome, understand the cross talk between

coagulation factors and the immune system, and identify novel targets for therapeutics.

Authors’ Note

Ethics board approval and patient consent to share information were not required for this review. M.A.A., U.P.N., V.L.R., C.R.R., and G.M.A. contributed to researching, drafting, and revising the manuscript. J.H.L. contributed to the design and conception of the review, and researching, drafting, and revising the manuscript. E.T.T. contributed to the design and conception of the review, and researching, drafting, and revising the manuscript.


Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: J.H.L. serves on research steering committees, data safety monitoring boards, or advisory boards for CSL Behring, Instrumentation Laboratories, Octapharma, Leading Biosciences, and Merck.

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