HEMOSTATIC CHALLENGES IN PEDIATRIC CRITICAL CARE MEDICINE

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PUBLISHED IN: Frontiers in Pediatrics

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88971-383-7
DOI 10.3389/978-2-88971-383-7

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HEMOSTATIC CHALLENGES IN PEDIATRIC CRITICAL CARE MEDICINE

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Citation: Karam, O., Nellis, M., Crighton, G., Stanworth, S., eds. (2021). Hemostatic Challenges in Pediatric Critical Care Medicine. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-383-7

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frontiers in Pediatrics

EDITORIAL
published: 12 July 2021
doi: 10.3389/fped.2021.697921

Editorial: Hemostatic Challenges in Pediatric Critical Care Medicine

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Keywords: pediatric, critical care, haemostasis, coagulation, bleeding, transfusion, hemorrhage

Editorial on the Research Topic

Hemostasis in Critically Ill Children

"When one thinks about the current state of critical care for children, do we not wonder why and how we got here, how we are doing, and where we are going?" (John J. Downes—US pediatric intensivist).

How we got here?

Hemostasis (derived from Greek *haimostasis*) literally means arrest of bleeding (1). Our knowledge of hemostasis has greatly advanced since ancient Greek times, when Homer described in *The Iliad* and *The Odyssey*, the treatment of war wounds with compression, bandages, cautery and styptics (2). Hemostasis is a complex and highly regulated process, involving the vascular system, platelets, coagulation factors, fibrinolytic systems, serine protease, kinin, and complement systems (3). This finely balanced system functions effectively to protect against both bleeding and thrombotic complications.

Understanding hemostatic dysfunction firstly relies on understanding what is normal. Maureen Andrews was a pioneer in the field of pediatric hemostasis when she reported on the vast differences seen between the hemostatic systems of neonates and adults and reported reference ranges for common coagulation tests (4, 5).

How are we doing?

Blood transfusions are one of the top five overused treatments and in many clinical settings provide no or negligible benefit and potentially expose patients to harm (6). Children are at least twice as likely as adults to have an adverse reaction secondary to transfusion (7, 8). In the Platelets for Neonatal Thrombocytopenia (PlaNeT-2) trial, platelet transfusions to support higher platelet counts in preterm neonates cause more harm than restrictive practice, giving platelets at lower thresholds (9). A key concept of pediatric patient blood management is patient and family-centered care (10). How would children and their families feel about being treated with potentially unnecessary or even harmful interventions?

In this collection of articles, Nair and Parker provide an overview of hemostasis and its regulation, discussing developmental hemostasis, laboratory tests used to evaluate hemostasis in children and their limitations. Gillespie and Doctor discuss how red blood cells (RBC)s contribute to hemostasis, from RBC biomechanics to cell-to-cell signaling and humoral influences and finally RBC's role in thrombosis and bleeding. Davenport and Sola-Visner describe the "*developmentally unique neonatal hemostatic system*," discussing common neonatal bleeding presentations, current and emerging coagulation tests and therapeutic interventions.

In times of health, the hemostatic systems of neonates and children are considered physiologic or balanced, this balance may be greatly disrupted in the context of illness, sepsis, trauma, major

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *20 April 2021*
Accepted: *16 June 2021*
Published: *12 July 2021*

Citation:
Crighton GL, Karam O, Nellis ME and Stanworth SJ (2021) Editorial: Hemostatic Challenges in Pediatric Critical Care Medicine. Front. Pediatr. 9:697921. doi: 10.3389/fped.2021.697921

surgery, liver disease, mechanical circulatory support, [e.g., extracorporeal membrane support (ECMO) or ventricular assist devices (VAD)s] and medications.

In this series, Bulut et al. describe the hemostatic balance in pediatric acute liver failure, limitations of current coagulation testing, the role of viscoelastic haemostatic testing and potential treatment strategies. Haas and Cushing review trauma-induced coagulopathy in children, discussing transfusion strategies in pediatric trauma patients and veno-thromboembolism.

Drop et al. evaluate the risk factors for hemostatic complications in pediatric ECMO patients, alternative anticoagulants and the association between coagulation tests and hemostatic complications. Ghbeis et al. review hemostasis in children with VAD and introduce ACTION (Advanced Cardiac Therapies Improving Outcomes Network), a collaborative international network aiming to improve outcomes for pediatric VAD patients.

Clinically significant bleeding is encountered in approximately one tenth of critically ill children (11, 12) and is a major cause of morbidity and mortality. Transfusions are frequently given to critically ill children to prevent or treat clinical bleeding. Aran et al. provide an overview of bleeding in critically ill children, definitions of bleeding and discuss BASIC (Bleeding Assessment Scale in Critically Ill Children), the first diagnostic criteria for describing bleeding applicable to critically ill children. Kahn et al. evaluate platelet and plasma transfusions in children, highlighting the lack of evidence to support their use and the variability in practice for transfusion indications and thresholds. Crighton and Huisman's two-part article discusses diagnostic tools to measure fibrinogen, fibrinogen replacement products and clinical indications in critically ill children.

What is striking from this collection of articles, is the lack of high-quality evidence to support hemostatic decision making in critically ill children. Only 18 RCTs were discussed amongst the 11 articles, that evaluated either transfusion support, fibrinogen supplementation or anticoagulation in different cohorts of sick neonates or children.

Where we are going?

Several areas recommended for future research include better understanding the coagulopathy of pediatric acute liver failure, trauma-induced coagulopathy including fibrinolysis shutdown in traumatic brain injury (13) and the role of the endothelial glycocalyx and coagulation in pediatric trauma and sepsis (14, 15). New hemostatic methods need evaluation in children specifically, thrombin generation testing and whole-blood hemostatic assays and platelet function assays in neonates. Research is needed to establish the relationship between coagulation parameters and clinical bleeding risk in children and neonates. BASIC bleeding definitions require validation in large patient cohorts and in relation to clinical outcomes.

RCTs are needed to guide recommendations for plasma and platelet transfusions in children, for prophylactic and therapeutic indications and to determine the optimal fibrinogen replacement product and triggers for fibrinogen supplementation in critically ill children in different clinical settings. Studies are also needed to evaluate newer platelet products, such as cold-stored platelets, thrombopoietin mimetics, and artificial platelets. In pediatric ECMO and VAD patients, evidence is needed to reduce the frequency of bleeding and thrombotic events and improve patient outcomes. Given the significant variability in the management of both ECMO and VAD patients it is important to define best practice and reduce treatment-related adverse events. Research priorities in pediatric trauma include determining the optimal resuscitation strategy (including the role of whole blood and fibrinogen), the effect of pre-hospital tranexamic acid and prevention of veno-thromboembolism.

In summary, there remains an urgent need for further research in critically ill neonates and children to guide the evaluation of hemostasis and bleeding risk and its optimal treatment. As seen with recent publications in adults (16, 17), it is possible to conduct high-quality RCTs in critical care settings and we should strive for the same in pediatrics. It is essential to consider the adverse effects of transfusion, limit unnecessary use and ensure effective implementation of any research findings.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to this research topic. GC conceptualized and wrote the first draft of this manuscript. OK, MN, and SS contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

We thank the editors and authors who have contributed to this review.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 11 November 2020
doi: 10.3389/fped.2020.600501

Hemostatic Balance in Severe Trauma

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Acute coagulopathy is prevalent in adult and pediatric trauma patients and is associated with increased morbidity and mortality. While reasonable hypotheses have been created to explain the underlying perturbations of adult trauma coagulopathy (i.e., tissue factor-related increase in thrombin generation, protein C activation, hypoperfusion, and hyperfibrinolysis), only a small number of studies have been performed to prove whether these mechanisms can likewise be detected in pediatric trauma patients. In addition, severe hypofibrinogenemia (<100 mg/dL) is a frequent finding in pediatric trauma patients (>20%). Although the probability of life-threatening coagulopathy is low with minor to moderate injury, it is present in almost all patients with an injury severity score >25, hypotension, hypothermia, and acidosis. As these multifactorial changes in hemostasis cannot be adequately and rapidly measured using standard laboratory testing, the use of viscoelastic measurements has been established in adult trauma management, but prospective studies in children are urgently needed. Apart from diagnostic challenges, several studies have focused on the impact of blood product ratios on the treatment of massively bleeding pediatric trauma patients. The majority of these studies were unable to show improved survival by using higher plasma to red blood cell ratios or higher platelet to red blood cells ratios, but there are no published randomized trials to definitively answer this question. A goal-directed transfusion protocol using viscoelastic tests together with early substitution with an antifibrinolytic and fibrinogen replacement is a promising alternative to traditional ratio-based interventions. Another crucial factor in treating trauma-induced coagulopathy is the early detection of hypofibrinogenemia, a common condition in massively transfused patients. Early treatment of hypofibrinogenemia is associated with improved morbidity and mortality in adults, but needs to be further studied in future pediatric trials. Pediatric trauma patients are not only threatened by coagulopathy-related bleeding but are also at higher risk for venous thromboembolism. Pediatric trauma patients with brain injury, central venous catheters, immobilization, or surgical procedures are at highest risk for developing a deep venous thrombosis. There are no specific pediatric guidelines established to prevent venous thromboembolism in children suffering from traumatic injury.

Keywords: trauma, massive bleeding, thrombosis, viscoelastic testing, transfusion, pediatrics, coagulation factor concentrates

OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *30 August 2020*
Accepted: *15 October 2020*
Published: *11 November 2020*

Citation:
Haas T and Cushing MM (2020) Hemostatic Balance in Severe Trauma. Front. Pediatr. 8:600501. doi: 10.3389/fped.2020.600501

INTRODUCTION

Adult traumatic coagulopathy is a commonly studied, but not fully understood, condition that occurs early and is a major contributor to injury-related mortality (1, 2). Its presence is usually defined by detection of impaired plasmatic coagulation parameters, such as a prolonged prothrombin time (PT) or activated partial thromboplastin time (aPTT) or increased International Normalized Ratio (INR). The occurrence of a prolonged PT or increased INR (specifically INR of $\geq$1.3 at admission) is associated with mortality in trauma patients (3, 4). Although INR seems to predict mortality, prospective data from adult surgical and trauma patients have shown that INR is not a reliable indicator of coagulopathy and should not be used to guide factor replacement (5).

While initial theories to describe trauma-related coagulopathy were based on the effects of hemodilution due to the administration of large amounts of coagulation factor-free solutions, the knowledge about the pathophysiologic changes have evolved substantially. The current understanding of trauma-induced coagulopathy is still evolving, but the main underlying causes are thought to be hemorrhagic shock-related hypoxia/hypoperfusion, and tissue injury. Hypoperfusion and hypoxia promote platelet and endothelial dysfunction that further trigger activation of protein C and impaired thrombin generation, as well as major changes in the fibrinolytic pathway (6). In addition, defects in collagen deposition related to endothelial dysfunction, downregulated platelet glycoprotein receptors, and complement activation are all thought to be contributors to Trauma Induced Coagulopathy (TIC) (7). In a prospective trial in pediatric trauma patients with an age of >15 years, the probability of life-threatening coagulopathy was low with minor to moderate injury, but coagulopathy was present in almost all patients with an injury severity score >25, hypotension, hypothermia, and acidosis (8). It is unknown whether the coagulopathy is similar for children in all age groups suffering from trauma due to a dearth of prospective studies in pediatric populations.

In addition to a brief description of TIC in children and discussion of the major differences between hemostatic changes in adults and children suffering from trauma, this review will focus on the management of coagulopathic bleeding in pediatric trauma patients. Finally, we will review the impact of trauma on the occurrence of venous thromboembolism, as this is an important but frequently overlooked counterpart of trauma-related bleeding complications.

TRAUMA-INDUCED COAGULOPATHY IN CHILDREN

Overview of TIC in Children

The incidence of TIC in severe pediatric trauma patients in the emergency room varies tremendously and is reported to be in the range of 10–77% (9). This remarkable variability may be explained by the fact that to date no specific pattern of tests and no specific thresholds are available to define TIC. There is no universally accepted definition of TIC. In fact, Christiaans et al. (9) described a huge variety of coagulation parameters and thresholds from 18 different studies that were used to define TIC after pediatric trauma (9), thus questioning if the main results of those studies are comparable. In addition, it is known from the adult world that changes in hemostasis following trauma do not uniformly lead to bleeding. There may be transient changes that lead to hypercoagulability as well.

Although the TIC-related changes in hemostasis and fibrinolysis of severely injured adult patients can be likewise observed in children (10), age-dependent differences may occur due to developmental changes. The overall hemostatic balance in an infant prevents an increase in bleeding or hypercoagulable states; however, it is hypothesized that even small changes in this fragile balance may lead to a coagulopathy in one direction or the other depending on the type of injury or time period after injury (10).

The Role of Hypercoagulability and Fibrinolytic Pathway Abnormalities in Pediatric Trauma-Induced Coagulopathy

In adult trauma patients, the presence of hyperfibrinolysis is highly correlated with mortality (11). Fibrinolysis is also a concern in children. In a cohort of 133 pediatric trauma patients, 19.6% of all children presented with hyperfibrinolysis detected by rapid thrombelastography. In addition, 38.3% of the children had fibrinolytic shutdown (12). In that study, hyperfibrinolysis and fibrinolytic shutdown were both associated with mortality and the need for blood transfusion, while fibrinolytic shutdown was additionally associated with disability (e.g., changes in behavior, intellectual functioning, and locomotion) and deep vein thrombosis. A prospective follow up study performed by the same group demonstrated that plasma transfusion and TBI are both independently associated with fibrinolysis shutdown, and that the combination of both is associated with a very poor prognosis, showing a 75% mortality and 100% disability in all survivors (13). Based on those results, the authors concluded that TIC is not a single entity, but a broad group of hemostatic imbalances showing distinct phenotypes.

While the empiric administration of tranexamic acid to address hyperfibrinolysis has shown to reduce mortality in adult trauma patients, data in pediatric trauma are scarce (14). Studies are urgently needed to investigate whether preadmission administration of tranexamic acid in pediatric civilian trauma patients has an impact on bleeding and mortality. There are no studies that address the treatment of fibrinolytic shutdown in children.

Pathophysiology of the Mechanisms of Pediatric Trauma (Including Traumatic Brain Injury)

There is a substantial difference in the underlying mechanisms of trauma in children as compared to adults: a relatively higher rate of blunt trauma and non-accidental trauma, including brain injuries, can be observed in children (15). This is of particular importance as isolated severe brain injury (TBI) is a major contributor to the development of trauma-induced

coagulopathy (TIC), and TIC can be observed in about 42–44% of all severe TBI cases (16, 17). Indeed, pediatric TBI has been identified as a common contributor to TIC and mortality (18). In adult patients suffering from TBI, an early transition from a hyper- to a hypocoagulable state has been observed (19, 20). Zhang et al. (21) have demonstrated in a mouse model that the release of brain-derived cellular microvesicles into the circulation following brain trauma has served as a causal factor for induction of a systemic hypercoagulable state, which was quickly followed by a consumptive coagulopathy. Focusing on the time course of traumatic brain injury-related coagulopathy, D-dimers and fibrinogen degradation products can be detected within minutes of injury, whereas prolonged PT and aPTT are detected late, reaching their peaks ∼3–6 h post-TBI (19, 20, 22). The combination of both, a hyper- and hypocoagulable state have been likewise detected in pediatric TBI (23). Hypercoagulability is suspected to promote ischemic lesions which can further worsen the outcome in this setting (9).

Summary

In summary, when TIC is present in pediatric patients the mortality rate appears to be markedly increased (3, 24). Unfortunately, the definition of TIC is not yet fully understood, especially in children, and may encompass several distinct abnormal hemostatic profiles. Therefore, a timely and reliable diagnostic workup is a basis for a better understanding of the hemostatic balance and optimal bleeding management. This will be discussed in the next section.

DETECTION OF TRAUMA-INDUCED COAGULOPATHY IN CHILDREN

In addition to reviewing global plasmatic coagulation parameters (i.e., PT/INR and aPTT) which provide only a simple assessment of the first phase of clot initiation, it seems worthwhile to review published evidence of other markers to detect TIC, such as platelet count, fibrinogen, signs of fibrinolysis and viscoelastic testing.

Platelet count was significantly associated with mortality in a cohort of 102 pediatric trauma patients, and an abnormal platelet count was observed in 23% of all children (15). However, platelet count does not reflect platelet function, which may also play a role in TIC (25). In adult patients with TBI, platelet ADP receptor inhibition was strongly correlated with the severity of brain injury (6). Unfortunately, sound data in children are lacking to determine the impact of platelet count and function on TIC, and clear thresholds are needed to safely guide transfusion therapy.

Severe hypofibrinogenemia (plasma fibrinogen levels below 100 mg/dL) was detected in 20% of the previously mentioned cohort of pediatric trauma patients (15). It remains unclear if this was due to hemodilution or consumptive coagulopathy on large wound surfaces, or because of hyperfibrinolysis.

When viscoelastic testing such as thrombelastography or thromboelastometry was used to detect TIC in pediatric trauma patients, signs of hyperfibrinolysis (defined as lysis index of 3.0% or greater or maximum lysis >15%, respectively) (12), a prolonged time for initiation of clot building (R-time and k-time, or clotting time and clot formation time, respectively), as well as signs of weakening in clot strength (maximum amplitude or maximum clot firmness, respectively) were observed (26, 27). Signs of TIC using standard coagulation testing and viscoelastic testing are shown in **Table 1**.

TABLE 1 | Signs of trauma-induced coagulopathy using standard laboratory testing (SLT) and viscoelastic testing (VET).

Characteristics	Signs in SLT	Signs in VET
Impaired initiation of coagulation	Prolonged aPTT Prolonged PT/elevated INR	Prolonged R-time (TEG) Prolonged CT (ROTEM)
Weakening of clot strength	*Not measurable*	Reduced MA (TEG) Reduced MCF (ROTEM)
Hypofibrinogenemia	Reduced value in Clauss fibrinogen assay	Reduced MA FF (TEG) Reduced FIBTEM MCF (ROTEM)
Hyperfibrinolysis	*Not measurable*	LY30 ≥ 3% (TEG) ML > 15% (ROTEM)

aPTT, activated partial thromboplastin time; PT, prothrombin time; INR, international normalized ratio; TEG, thrombelastography; ROTEM, thromboelastometry; R-time, reaction time; CT, clotting time; MA, maximum amplitude; MCF, maximum clot firmness; FF, functional fibrinogen test; FIBTEM, fibrin polymerization assay; LY30, Clot lysis at 30 min after maximum clot strength; ML, maximum lysis at 60 min after CT.

PEDIATRIC TRANSFUSION STRATEGIES

There is published evidence that hemorrhagic injuries are less likely in children, and TBI is the main underlying reason for mortality. In a cohort of 776 pediatric trauma patients, clinical bleeding or hemorrhagic injury was only present in 7.0% ($n = 54$) of all children (4). Although in that study an admission INR of ≥1.3 was determined to be an independent predictor of mortality, transfusion of plasma was not effective in changing INR. The authors have therefore questioned whether an INR of 1.3 or greater can be used as a precise term to diagnose coagulopathy in this setting or whether it is just an indicator of disease severity. It is of great importance to understand and to distinguish the interpretation of impaired plasmatic coagulation test results as markers of systemic hemostasis imbalance and outcome predictors vs. our ongoing efforts to find reliable laboratory treatment targets for transfusion therapy. Notably, patients with borderline elevated plasmatic coagulation tests do not necessarily show signs of disturbed clot initiation in viscoelastic tests (28, 29). As mentioned before, transfusion of plasma to treat a moderately elevated INR is not successful and does not have influence on outcome (4). Therefore, the transfusion strategy should be adopted primarily based on the clinical status of a trauma patient, namely by whether clinically relevant bleeding is present or not. A threshold of 37 ml/kg per 4 h or more of transfused blood was determined to be an indicator for the need for hemorrhage control procedures and a predictor of early mortality (30).

If clinically meaningful hemorrhage is present, immediate access to blood and blood products is needed. Institutional protocols should be established. However, there is no evidence to support transfusion protocols using a 1:1:1 approach of red blood cells (RBCs), plasma and platelets in pediatrics. Unlike in the adult setting, several trials have failed to show any survival benefit by using a transfusion protocol with a predefined transfusion ratio (31–37). In a retrospective analysis of a cohort of 364 massively transfused combat-injured children, a high ratio of plasma to RBC transfusion was associated with a longer hospital length of stay and was not associated with a mortality benefit (36). When comparing a cohort of 55 pediatric trauma patients that were transfused with a protocol using a predefined massive transfusion ratio, Chidester et al. (31) were not able to show a significant difference in mortality. There is only one retrospective data analysis that found a benefit. In this study of 465 pediatric trauma patients that excluded severe isolated head injury, a survival benefit was detected for children treated with a high ratio of plasma:RBC, while no differences were observed among the higher platelet:RBC cohort (38). Given that there are major differences in terms of definition of coagulopathy and criteria to activate a massive transfusion protocol in different published studies, it is hardly surprising that there is no clear and easy answer as to whether certain transfusion ratios may have a mortality benefit in civilian pediatric trauma patients. In addition to study differences, differences in age groups, the frequency of severe brain injury, as well as inconsistent adherence to transfusion protocols and laboratory thresholds among published trials also cloud the picture.

Recently, the successful resuscitation using whole blood at a dose of 20 ml/kg was reported in a cohort of 18 pediatric civilian trauma patients (39). However, more evidence is urgently needed before the use of whole blood can be recommended in children.

GOAL-DIRECTED HEMOSTATIC RESUSCITATION

As there is no clear evidence to support ratio driven transfusion protocols in pediatric trauma patients, goal-directed transfusion management using viscoelastic testing offers an alternative approach. Data from an adult pragmatic randomized trial in 111 trauma patients demonstrated that a group of patients whose treatment was guided by thrombelastography had improved survival compared to patients whose treatment was guided by conventional coagulation tests (40). Higher blood product utilization, with more units of plasma and platelets in the first 2 h of resuscitation, occurred in the group guided by conventional coagulation tests. Survival in the thrombelastography group was significantly higher than the conventional coagulation test group. In another adult trauma trial that compared thromboelastometry-guided transfusion management with purified concentrates compared to plasma transfusion vs. a targeted administration, the former group showed superior outcome (41). Not only was the amount of transfused allogeneic blood products smaller in the coagulation factor group, but the time to correct TIC and improve bleeding was significantly shorter in the coagulation factor-group (median of 22.5 min [IQR; 13.5–40.0] vs. 128.0 min [IQR; 48.3–186.3]) vs. the plasma group (estimated time difference of −97 min [−126 to −60], $p < 0.0001$). Another important finding of that study was that almost all patients suffered from severe hypofibrinogenemia after arrival at the emergency room, which was successfully treated by administration of fibrinogen concentrate at a dose of 50 mg/kg, while a dose of 30 ml/kg of plasma failed to normalize plasma fibrinogen levels. The importance of detecting and treating hypofibrinogenemia early was also investigated in a prospective cohort study of 517 adult trauma patients (42). Multiple studies have demonstrated evidence that hypofibrinogenemia is a critical issue in severe bleeding for pediatric intraoperative and trauma patients (43–45). One study in pediatric trauma patients analyzed an institutional transfusion protocol where cryoprecipitate was not included until the third transfusion package. The authors found that 11% of all cases had fibrinogen levels below the detection limit of the assay upon arrival in the emergency department. They concluded that future renditions of their Massive Transfusion Protocol (MTP) would have included cryoprecipitate earlier. Plasma is not adequate to replenish low fibrinogen levels (32). Viscoelastic testing guided bleeding management could guide earlier fibrinogen replacement since acquired hypofibrinogenemia can be detected within 5–10 min. However, the search for a perfect bleeding management approach is hampered by a marked difference in methodologies between published trials. Trials have used different coagulation tests (conventional or viscoelastic testing) and applied different treatment options (ratio-driven transfusion, administration of coagulation factors, or a mix of both), thus leading to a variety of alternative approaches (**Figure 1**).

An example of a goal-directed pediatric bleeding management algorithm is displayed in **Figure 2**. This approach is feasible even in young children. The combination of viscoelastic testing plus resuscitation with factor concentrates is appealing as results are rapidly available to the trauma team and the preparation of coagulation factors can be performed extremely quickly. Furthermore, the lower volume and pathogen-inactivated status of coagulation factors compared to standard blood components are important for safety in pediatric patients. Rapidly available testing and blood components are of particular importance as the management of pediatric trauma patients is further challenged by difficulties and delays in establishing (large bore) venous access for phlebotomy and fluid resuscitation.

VENOUS THROMBOEMBOLISM IN TRAUMA

Children sustaining severe traumatic injuries are not only threatened by the risk of bleeding, but also have an increased risk for developing venous thromboembolism (VTE). Although very young children tend to be protected from VTE by their quantitative and qualitative developmental differences in hemostasis (46), the risk factors for VTE in older pediatric patients are well-established. In addition to individual inherited

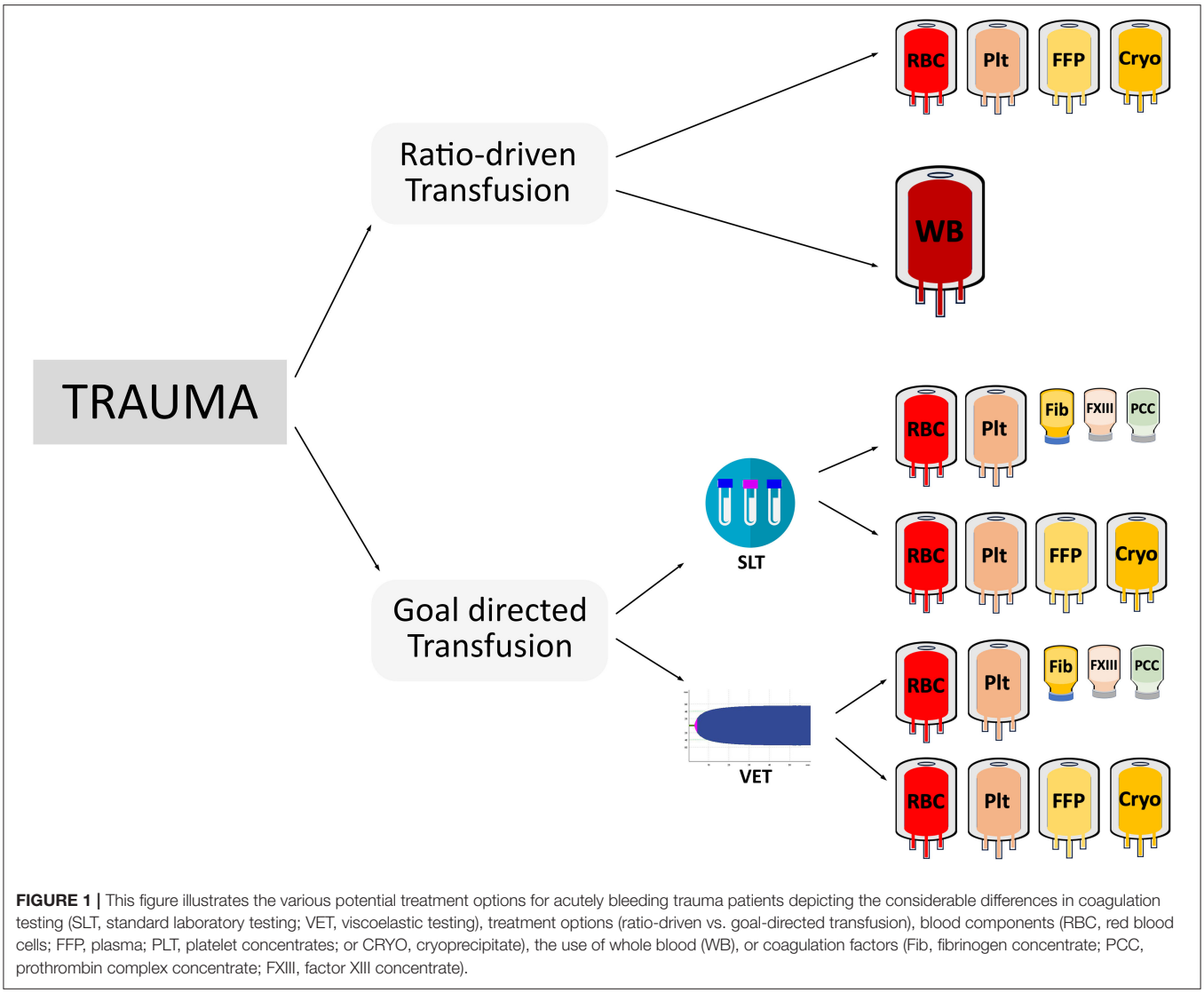

FIGURE 1 | This figure illustrates the various potential treatment options for acutely bleeding trauma patients depicting the considerable differences in coagulation testing (SLT, standard laboratory testing; VET, viscoelastic testing), treatment options (ratio-driven vs. goal-directed transfusion), blood components (RBC, red blood cells; FFP, plasma; PLT, platelet concentrates; or CRYO, cryoprecipitate), the use of whole blood (WB), or coagulation factors (Fib, fibrinogen concentrate; PCC, prothrombin complex concentrate; FXIII, factor XIII concentrate).

or acquired risk factors for thrombosis, multiple disease- or treatment-related risk factors contribute to VTE (47). The incidence of VTE in pediatric trauma patients is < 0.5% (48–50). This is in sharp contrast to the adult trauma patient population where the estimated incidence is as high as 20–58% without appropriate thromboprophylaxis (47). However, there is a paucity of evidence for standardized VTE screening in children and thus children are monitored for VTEs less frequently so the actual incidence may differ. Additional risk factors such as major surgery, placement of a central venous line, injury severity score, age, poor perfusion with necessity for inotrope support, Glasgow coma scale, intubation, and blood transfusion have been identified as contributors to VTE risk.

Although there is currently no consensus for routine administration of thromboembolism prophylaxis in the pediatric trauma setting, a group from Johns Hopkins created their own VTE prediction score based on risk factors identified in their risk assessment model (50). A retrospective review of a pediatric trauma registry encompassing 13,880 patients found no VTE in the age group < 13 years, two VTEs in the age group 13–17 years, and 59 VTEs in the age group > 17 years old (51). A consensus conference regarding the prevention of VTE in pediatric trauma patients judged current scientific data as insufficient for evidence-based decisions. However, they provided the recommendation that injured children < 12 years old do not routinely need VTE prophylaxis, but prophylaxis should be considered in children with a history of VTE, and may be considered in children with a central venous catheter (52). A recently published retrospective cohort of 209 pediatric trauma patients showed that femoral venous catheter-associated thrombosis is significantly more frequent than in non-femoral catheters (53). When thromboembolism prophylaxis with low-molecular-weight

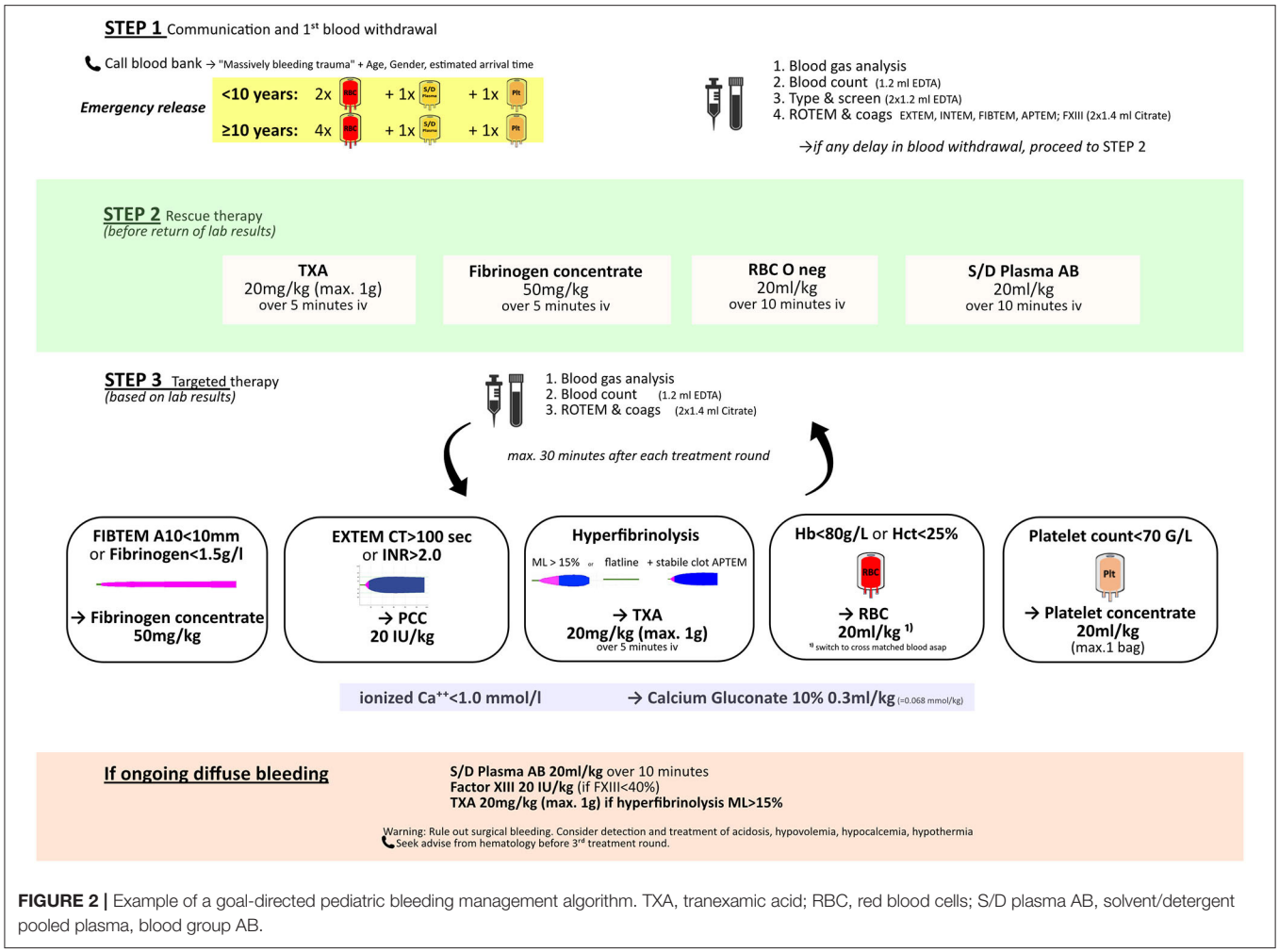

FIGURE 2 | Example of a goal-directed pediatric bleeding management algorithm. TXA, tranexamic acid; RBC, red blood cells; S/D plasma AB, solvent/detergent pooled plasma, blood group AB.

heparin was administered to a cohort of 706 pediatric trauma patients, major bleeding was reported in 0.4% of those children, while thromboembolic events were detected in 2.1% (54).

CONCLUSIONS AND FUTURE DIRECTIONS

At present, the knowledge regarding hemostatic balance in severe pediatric trauma patients is mostly extrapolated from adults. More studies are urgently needed to understand the pathophysiologic changes in this patient population. Trauma-induced coagulopathy is not just one single entity, but a heterogenous group of hemostatic imbalances showing distinct phenotypes depending on timing, mechanism of injury, underlying conditions and other less understood factors. The conditions are even more heterogeneous in children, where the age of the children impacts the mechanism of injury as well the baseline hemostatic factors. As such, a rapid, frequent and reliable laboratory testing should be performed to detect the current and evolving clinical picture. Coagulation tests such as INR or PT may not accurately assess the hemostatic imbalance and may lead to over transfusion of plasma without improving the outcome. The implementation of a viscoelastic testing protocol has been beneficial in adult trauma treatment and should be validated in the pediatric setting. A targeted transfusion approach may offer a feasible alternative in pediatric bleeding management, with focus on the treatment of hyperfibrinolysis and hypofibrinogenemia. Although thromboembolism prophylaxis is not generally recommended in pediatric trauma patients < 12 years old, future studies should investigate whether viscoelastic testing can be used to predict thrombosis, thus giving a more global picture of hemostatic balance in this patient population.

AUTHOR CONTRIBUTIONS

TH and MC conceptualized and wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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Conflict of Interest: TH has received lecturer's fees and travel support from Octapharma and Instrumentation Laboratory, and is a consultant for Octapharma. MC is a consultant for Cerus Corporation and Octapharma.

frontiers in Pediatrics

REVIEW
published: 13 November 2020
doi: 10.3389/fped.2020.601659

Overview of Plasma and Platelet Transfusions in Critically Ill Children

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Critically ill children are a unique population who frequently receive plasma and platelet transfusions for both active bleeding and mitigation of bleeding risk. While these products are frequently administered, transfusion indications in this population remain unclear, and practice varies across institutions and providers. In this manuscript, we will outline the current evidence regarding plasma and platelet transfusions for hemostasis in the pediatric intensive care setting. For both products, we will describe the product composition, epidemiology, and product indications and discuss the potential risks and benefits involved with the transfusion. We will also discuss knowledge gaps and future areas of research.

Keywords: platelet transfusion, plasma, critical ill children, hemostasis, hemorrhage

OPEN ACCESS

Edited by:
Utpal S. Bhalala, Baylor College of Medicine, United States

Reviewed by:
Gaurav K. Gupta, University of Rochester, United States
John Holcomb, University of Alabama at Birmingham, United States
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *01 September 2020*
Accepted: *21 October 2020*
Published: *13 November 2020*

Citation:
Kahn S, Chegondi M, Nellis ME and Karam O (2020) Overview of Plasma and Platelet Transfusions in Critically Ill Children. Front. Pediatr. 8:601659. doi: 10.3389/fped.2020.601659

INTRODUCTION

Plasma and platelet transfusions are frequently administered to critically ill children to prevent bleeding and help treat active bleeding. Transfusion of whole blood began in the early 1900's, and significant advancements have been made since that time. Plasma was first transfused in 1941 (1), followed by platelet-rich plasma in the 1950's (2) and platelet concentrates in 1965 (3). In 2015, the American Association of Blood Banks (AABB) National Blood Collection and Utilization Surveys noted that 48,000 children received 165,000 apheresis platelet units and 29,000 children received 74,000 plasma units (4, 5). While adult transfusions are subdivided by area of administration, inpatient medicine (including oncology), and critical care being the largest location administrations, this breakdown is notably absent for pediatric patients with the only division being neonatal vs. pediatric. Although these therapies have been used in critically ill children for more than 50 years and based on recent data are still frequent interventions, there are still many unanswered questions regarding optimal transfusion practices.

PLASMA

Plasma transfusions remain a common treatment in medicine, specifically for bleeding in the emergency and intensive care settings. In 2017, it was noted that 3,209,000 units of plasma were distributed to hospitals in the United States with 2,374,000 units being transfused. This remains to be a large amount; however, there is a significant decline of 13% in units transfused since 2015 (6). The most recent practice guidelines around transfusion of plasma from the AABB were released in 2010 (7), whereas the British Society of Hematology released newer guidelines in 2018 (8). Of note, pediatric providers were involved in the creation of the AABB guidelines; however, they are

not specific to children. The updated British guidelines also mention children; however, their most recent pediatric specific guidelines were published in 2016 (9). Despite a lack of data in the pediatric population, it is estimated that about 3% of pediatric admissions to the intensive care unit (ICU) received plasma, making this an important area for further research (10).

Product Composition + Processing

Plasma is the acellular portion of blood and is obtained by centrifugation or apheresis of blood post-donation. While it is ∼92% water, the other 8% are vital proteins, such as coagulation factors (factors II, V, VII, VIII, IX, X, XI, and XII; protein C; protein S; antithrombin), albumin, and immunoglobulins (11). Plasma can also be further fractionated and used for its derivatives. Via fractionation, blood banks are able to isolate specific factors, antithrombin III, albumin, immunoglobulins, etc. to be given individually for a variety of indications.

The mean volume of a plasma unit is ∼200–250 ml with a small portion of this volume being anticoagulant for storage. Units are stored at −18°C (−0.4°F) or lower as some coagulation factors, specifically factors V and VIII, have shortened half-lives at room temperature (approximately 22°C). The term fresh-frozen plasma (FFP) is used for plasma frozen within 8 h of collection, whereas frozen plasma, also known as FP24 or PF24, is used to refer to plasma frozen after the 8 h mark but within 24 h of collection. Both can be safely stored for up to 1 year post-collection if kept frozen. FFP can then be thawed and kept in the refrigerator for up to 24 h, and some countries allow for FFP or FP to be kept as thawed plasma for up to 5 days (12). The clinical efficacy is similar between FFP and FP, and they are often used interchangeably; however, cryoprecipitate can only be manufactured from FFP (7). For the purposes of this review when we discuss plasma, we will use them interchangeably unless otherwise noted.

Coagulation factor concentrations may vary between single donor units (13). Units can also be pooled from multiple (300–5,000) donors to allow for a more constant concentration of factors (13). In some European countries, plasma is frequently pathogen reduced by one of three methods, the use of methylene blue, amotosalen, or riboflavin, to further decrease the risk of infection. While beneficial for its impact on infectious risk, this process can also significantly impact the amount of coagulation factors present in the unit. The extent to which coagulation factors are impacted depends on the specific pathogen reduction process employed (8, 13). This is not currently standard of practice in the United States. Solvent detergent units, pooled units pathogen reduced by exposure to a solvent and detergent, are the only ones licensed by the Food and Drug Administration (FDA) (7, 13).

Epidemiology

In addition to a decrease in units transfused over the past several years, there was also an overall decline in plasma collection in the United States by 14%. Approximately 61% of the units collected in 2017 were distributed to centers throughout the country as frozen plasma with 91% manufactured from whole blood collection, and the remaining percentage was collected via apheresis (14). In the United Kingdom, while there was an increase in the use of FFP from 2008 to 2012, there is a notable decline from 2012 onward, with the data extending to 2016 (8).

As noted above, in the US, the number of plasma units transfused decreased by 13% from 2015 to 2017. However, it is notable that the breakdown of product varies from the collection data. FFP and frozen plasma are more evenly split. There was also an increase in the utilization of AB plasma (the universal donor) by 19.8% (14).

Indications and Dosing

Plasma is given to critically ill children for a variety of reasons with the most common being coagulopathy, bleeding, and prevention of bleeding in those preparing for an invasive procedure. Initiation of extracorporeal membrane oxygenation (ECMO) in critically ill children causes depletion of coagulation factors, fibrinogen depletion, hemolysis, and thrombocytopenia. This results in a need for blood product transfusions, including FFP. Plasma is also used for exchange transfusions for a variety of disorders, which we will not cover in this review. There is a paucity of data in critically ill children regarding the efficacy of plasma transfusions and the risk–benefit ratio; therefore, practices vary widely.

Therapeutic Transfusion (Treatment of Bleeding)

Plasma is frequently given to correct coagulopathies in patients with active bleeding; however, there are limited data to support its benefit. In a systematic review, Levy et al. did not find any randomized controlled trials (RCTs) that evaluated the benefit or safety of plasma to treat coagulopathic bleeding in children and adults (15). We looked at critically ill children in 101 pediatric intensive care units (PICUs) in 21 countries and found that 22% of plasma transfusions were given for an indication of critical bleeding. The international normalized ratio (INR) was only significantly improved in patients with a baseline INR of >2.5, supporting that only significant coagulopathy can be improved with plasma transfusion (16). In children and adults with active bleeding who are on vitamin K antagonists for anticoagulation, such as warfarin, FFP transfusions are a common intervention. However, recent adult studies have shown increased incidence of volume overload and ICU admission as a result (17, 18). Refaai et al. compared the use of prothrombin complex concentrates (PCC) with the use of FFP for anticoagulant reversal in adults and found that plasma was independently associated with volume overload (18). In addition, a small single center retrospective study by Sisti et al. found that the use of PCC in pediatric patients with ventricular assist devices, for anticoagulation reversal prior to heart transplant, limited their exposure to exogenous blood products (19). Additional studies are needed to determine the best situation for each product in the pediatric population.

The role of plasma transfusions in pediatric trauma patients with bleeding has been explored. Coagulopathy following trauma has been associated with worse outcomes in the pediatric population (20–22). However, no specific ratio of plasma to other products has been particularly effective in controlling coagulopathy. A recent small retrospective study found no significant difference in the transfusion ratios of surviving and

deceased pediatric patients at the 3- or 24-h timepoints (22). In addition, a recent study by Sehdev et al. found no difference in mortality rate of pediatric trauma patients when comparing >2:1 packed red blood cell (pRBC) to plasma ratios and <2:1 pRBC to plasma ratios (23). Therefore, the optimal dose of plasma in children with massive bleeding is still unknown.

Prophylactic Transfusion (Prevention of Bleeding)

The use of plasma transfusions in non-bleeding patients is controversial with minimal evidence to support its utility. A study by Deitcher found that in adults with liver disease, an INR of 1.3–1.9 correlated with mean factor levels of 31–65% of factor II, 40–70% of factor V, and 22–60% of factor VII, all adequate concentrations to support hemostasis—further supporting that transfusion would not be indicated for mild coagulopathies (13, 24). Plasma transfusions given prior to invasive procedures have also not been shown to change bleeding rates or outcomes in both children and adults (25–27).

We noted that one-third of patients received plasma transfusions prophylactically and had no bleeding and no planned procedure (16). A meta-analysis including 80 RCTs concluded that there was no evidence of significant benefit for prophylactic plasma transfusion across a range of indications evaluated in both children and adults (28). Likewise, a systematic review in 2013 examining restrictive vs. liberal transfusion of plasma found no RCTs that met their criteria, further highlighting the lack of data surrounding this practice (29). The AABB guidelines for plasma transfusion in children provide the following indications: in massive bleeding, support during treatment of disseminated intravascular coagulation (DIC), as replacement therapy (when specific factor concentrates are unavailable; if indicated during therapeutic plasma exchange), or for emergency reversal of warfarin (7, 30).

Dosing

FP transfusions should be ABO compatible but are not required to be identical, and unlike RBC transfusions, AB positive plasma is the universal donor and can be given in emergency situations. The average dose of plasma given is 10–20 ml/kg as this increase factor levels by 15–25% that should allow for hemostasis (31).

Adverse Effects

There is conflicting evidence regarding the benefits of plasma transfusions for many indications, but the risks of administering plasma are clear. As with all blood products, plasma carries the risk of transfusion reactions, such as transfusion-related acute lung injury, transfusion-associated circulatory overload, and allergic reactions (30). In addition, plasma in the United States is not routinely pathogen reduced, as noted above, and therefore carries the risk of infection. However, transmission of viral infections, such as HIV and hepatitis, from plasma are rare. Children with IgA deficiency are at specific risk for anaphylaxis given the presence of anti-IgA antibodies. Studies in adults have shown associations between plasma transfusions and acute respiratory distress syndrome, nosocomial infections, and septic shock (26, 32). One recent study demonstrated that patients who received FFP developed acute lung injury in 12% of cases, in contrast to 3% in the non-transfused group (24). We performed an observational, prospective single center study in critically ill children and found that 42% of patients who received at least one plasma transfusion in comparison with 8% of patients did not develop new or progressive multiorgan dysfunction syndrome (MODS) ($p < 0.001$), with an adjusted odds ratio of 3.19 (95% CI 1.55–6.58, $p = 0.002$). A significant dose relationship was noted—the more plasma received, the higher the proportion of new or progressive MODS. This study also demonstrated an increased incidence of nosocomial infections, longer PICU length of stay, and a higher 28 day non-adjusted mortality rate in those who received plasma (33).

Plasma transfusions have been shown to have significant risks and impact on clinical outcomes; therefore, given the lack of current evidence to support liberal usage, plasma should not be given prophylactically or used solely as volume expander (7, 9, 30).

Knowledge Gap and Future Directions

The usage of plasma in both the United States and Europe has decreased in recent years—possibly due to a lack of data regarding indications and appropriate utilization. While there are notably some benefits in patients with major bleeding and severe coagulopathy, the use of plasma in patients with minor coagulation derangements and no bleeding appears to not outweigh the many risks seen in observational studies. RCTs are needed in both the adult and pediatric populations to guide recommendations for plasma transfusion.

PLATELETS

Platelets are essential for normal hemostasis. Critically ill children often present with quantitative and/or qualitative platelet abnormalities. The goal of platelet transfusion is to prevent or to limit bleeding. In the 1950's, whole blood and then concentrated platelet transfusions were shown to reduce the mortality associated with bleeding in patients with leukemia. Over the years, there were significant advances in terms of platelet collection, storage, and transfusion practices. However, in children, the indications of platelet transfusions and optimal platelet count thresholds remain unclear.

Standard Platelet Products

Platelets are commonly prescribed for critically ill children in the PICU (4). Platelet products are pooled from the whole blood derived via a single donor (34).

The Whole Blood-Derived Platelet Concentrate (WB-PC)

Also known as pooled platelets or random donor platelets (RDP) are extracted before cooling the blood to <20°C using the platelet-rich plasma or buffy coat method either manually or semiautomated systems. In the US, the platelet-rich plasma is used to extract PC, whereas in Europe, buffy coat is preferred (34). Typically, four to six whole blood units are pooled for a single RDP transfusion. One unit of RDP contains about 55×10^9 platelets suspended in 50–70 ml plasma.

The Single Donor PC Using Apheresis Technique (SDA-PC)

Often considered as the gold standard method. Compared with WB-PC, SDA-PC takes a longer time to collect, exposes the donor to an extracorporeal circuit, and involves higher production cost. It contains a higher platelet count, 300×10^9 platelets suspended in 150–300 ml plasma. In addition, apheresis allows the production of specific platelet products, such as human leukocyte antigen (HLA) or human platelet antigen (HPA) compatible (35). One unit of apheresis platelets is equivalent to four to six units of WB-PC. The plasma proteins, including all the coagulation factors, are lower due to platelet added solution.

Warm vs. Cold Platelets

Platelets are typically stored at a warmer temperature, 20–24°C under gentle agitation. The advantage of warm storage platelets (WSP) is the longer shelf life of 5–7 days with increased circulation time and beneficial to use prophylactic platelet transfusion in patients at risk for bleeding (36). However, the risk of bacterial contamination is higher with WSP (37). Ever since the renunciation of cold storage platelets (CSP) in the early 1980's, there is an increasing evidence of using CSP in recent years. The CSP are stored at a colder temperature, 1–6°C without agitation. The CSP exhibit better hemostasis, especially useful in patients with active bleeding and major trauma with less risk of bacterial contamination. Though CSP shelf life is shorter than WSP (3 vs. 5 days), the *in vitro* studies suggest extended shelf life for CSP (38). Currently, CSP for the most part are considered experimental, and they are not routinely stocked in blood banks.

Special Platelet Concentrates

Leukocyte-Depleted Platelets

Pre-storage white blood cell (WBC) count reduction is a standard procedure. Leukocytes from the platelet units are depleted using centrifugation and filtration techniques. Leukocytes are depleted following an average of 6 h of contact time to neutralize bacterial contamination risk through the leukocyte mediated phagocytosis (39). The WBC count is $<8.3 \times 10^5$ in the platelet concentrate. The result of leukocyte depletion is a significant reduction in transmission of bacterial and viral diseases, such as cytomegalovirus (CMV) and Epstein–Bar virus (EBV), and reduced frequency of febrile reactions and transfusion-related immunomodulation (TRIM) (40). Bedside leukocyte depletion is not recommended as it decreases the platelet number in the concentrate.

CMV Negative Platelets

Platelets can transmit CMV infection. CMV negative platelets are indicated for CMV negative patients with solid organ or bone marrow transplant, immunodeficiency, and intrauterine transfusion (41).

Irradiated Platelets

For all HLA-matched platelet concentrates, pre-transfusion irradiation is a must. Irradiation is also recommended in infants at risk of transfusion-associated graft-vs.-host disease (TA-GVHD) and intrauterine transfusions (42).

Pathogen-Reduced Platelets

Multiple techniques are available worldwide to reduce the potential infection risk with platelet transfusion. Pathogen reduction protects against bacterial contamination, and it potentially increases the platelet shelf life up to 7 days (43). In addition, pathogen reduction obviates the need for irradiation. However, these platelets hemostatic efficacy may be lower. Other efficacy and safety issues of pathogen-reduced platelets are currently under investigation (43).

Cryopreserved Platelets

These platelets are considered an experimental product, and due to storage at −80°C, they require standardized thawing protocols, which may not be possible at all centers. The advantage of cryopreserved platelets is a significant increase in shelf life, up to 2 years. However, the efficacy and safety issues are not well-described (44).

Epidemiology of Platelet Transfusion

Over 2.2 million platelet units are transfused annually in the US (45), and 275,000 units in UK (46). The current trend shows a decline in the surplus inventory (45, 46). In 2015, the AABB reported 165,000 apheresis platelet units among 48,000 children in the US (5). The rate of platelet transfusion in critically ill children is 3.4% during their PICU stay (4). Over two-thirds of platelet transfusions are prescribed prophylactically and remaining for active bleeding (4). The existing literature is limited in children. The platelet transfusion practice follows adult transfusion protocols, and there is a wide variation in platelet transfusion practice across US health care centers and globally (4, 47).

Thrombocytopenia is defined as a platelet count of $<150 \times 10^9$/L. In critically ill children at the time of PICU admission, the reported incidence of thrombocytopenia was 17%, and during the PICU stay, 25% of children were thrombocytopenic (48). The underlying mechanisms of thrombocytopenia are decreased production, increased destruction of platelets, and dilutional. Among the critically ill children, common etiologies for thrombocytopenia may include sepsis, chemotherapy, DIC, MODS, and hemolytic uremic syndrome. Heparin-induced thrombocytopenia (HIT), hemophagocytic lymphohistiocytosis (HLH), and massive transfusion are less common etiologies (49, 50). Qualitative platelet defects are more common due to sepsis, exposure to hypothermia, cardiopulmonary bypass, chemotherapy, antiplatelet drugs, and rarely due to hereditary disorders (47). This platelet dysfunction might also be another factor in prescribing transfusions at higher platelet count.

Indications for Platelet Transfusion

In critically ill children, platelets are transfused as prophylactic or as therapeutic for the ongoing bleeding. Since the transient rise of platelet count in these children does not address the underlying etiology, the main aim of transfusion is to prevent major bleeding. Thrombocytopenia has been identified as an independent risk factor for major bleeding and mortality in critically ill children at admission or during their stay in the PICU (48, 51, 52). In children with a higher risk of mortality, platelet

transfusion may be beneficial, whereas in children with low risk of mortality, the transfusion risks may outweigh the benefits (52). This relationship suggests that besides thrombocytopenia, disease severity should be considered while prescribing platelet transfusion. Platelet transfusions are also indicated for reversal of antiplatelet drugs, such as aspirin and clopidogrel; however, clear guidelines are lacking.

Therapeutic Platelet Transfusion

The therapeutic transfusion of platelets is indicated for clinically significant bleeding associated with thrombocytopenia or due to dysfunctional platelets. With major bleeding, often, platelets are transfused to keep platelet count $>50 \times 10^9$/L and, while on ECMO support, above 100×10^9/L (53). However, without a clear definition of major bleeding and a paucity of data to guide therapeutic platelet transfusions in critically ill children, indications for transfusion are unclear. One cannot apply the existing adult platelet transfusion guidelines in children, which are based on expertise opinions (54).

The current practice in children with massive bleeding is to give empiric transfusion of WB-derived platelets and RBCs transfused between 1:1 and 1:2 ratio until the bleeding resolves. When using apheresis platelets, the ratio is between 1:5 and 1:10, as each unit is concentrated. However, this data is based on RCTs in adult patients (55).

While therapeutic transfusion strategy may result in a lower rate of platelet transfusions, reduced cost, and resource utilization, the patient safety and risk of major bleeding are of concern. There is conflicting evidence as to the efficacy of platelet transfusions preventing bleeding. Though adult studies reported no relationship between the lowest platelet count and the risk of bleeding in adult oncology patients (56), in a prospective observational study, Moorehead et al. reported an increased risk of major bleeding with low platelet count in critically ill children (51). Most recently, a large RCT in pre-mature neonates showed increased bleeding and/or mortality in those transfused at a liberal threshold (50×10^9 cells/L) as compared with a restrictive threshold (25×10^9 cells/L) (57). Animal models have suggested that it is not thrombocytopenia alone that leads to bleeding, but rather thrombocytopenia in the setting of inflammation (58).

Platelet transfusions have been associated with increased morbidity and mortality in children (49, 52). In an international point prevalence study, we showed an independent association between dose of platelets and mortality (4). A recent study by Cashen et al. suggested that platelet transfusions are associated with increased risk of mortality, bleeding, and thrombosis in children receiving ECMO support (59).

Prophylactic Platelet Transfusion

The majority of platelet transfusions in children are given prophylactically to prevent bleeding (4). Currently, there is a lack of evidence to suggest a platelet count threshold that can be generalizable to all children. Previous studies report that between 50 and 67% of platelet transfusions are prescribed to prevent bleeding in children, and that the median pre-transfusion platelet count was between 39 and 50×10^9/L (4, 52). In a recent point prevalence study, we reported that 34% of children received platelet transfusion even when the platelet count was above 50×10^9/L to meet the arbitrary platelet count threshold (4). The current transfusion threshold recommended by the AABB with hypoproliferative thrombocytopenia is 10×10^9/L. This threshold platelet count is based on the Platelet Transfusion Trigger Trial, which reported no significant difference in the occurrence of bleeding comparing the platelet count 10×10^9/L vs. 20×10^9/L in adolescents and adults with acute myeloid leukemia (AML) undergoing induction therapy (60). A secondary analysis of the prophylactic platelet dose (PLADO) trial in adults and children with hematological malignancies reported a poor association with the thrombocytopenia severity and risk of bleeding (61). Studies in neonates also suggest that the risk of clinically significant bleeding including intraventricular hemorrhage is likely not related to the degree of thrombocytopenia (62, 63). The poor correlation between thrombocytopenia and risk of bleeding suggests considering other factors, such as underlying etiology, to assess the bleeding risk.

There is a wide variation in the practice of prophylactic platelet transfusion in children, and most of the clinical practice guidelines are based on expert opinion. Often the platelet count threshold for transfusion varied depending on the geographic location of clinical practice (29), with underlying clinical indication and diagnosis (4, 5, 64, 65). The clinical practice of platelet transfusion for hypoproliferative thrombocytopenia cannot be applicable in thrombocytopenia due to immune-mediated platelet destruction. The American Society of Hematology recommends platelet transfusion only with life-threatening bleeding or during the preparation for surgical procedures (66). In critically ill children with qualitative platelet dysfunction, often platelets were transfused even with normal platelet count. Currently, there is no consensus for transfusion threshold platelet count (47). The AABB, in adults, recommend prophylactic platelet transfusion at the following threshold platelet counts: for hospitalized patients with hypoproliferative thrombocytopenia at a threshold platelet count of 10×10^9/L, for patients requiring elective central venous catheter placement at 20×10^9/L, patients with a lumbar puncture at 50×10^9/L, and patients with major non-neuraxial surgery at 50×10^9/L (67).

Dosing

Unlike adults, the platelet doses are smaller in children. However, the platelet type and preparation methods are similar. The standard dose for platelet transfusion in adults is typically 3×10^{11}–6×10^{11} platelets. The PLADO trial compared the prophylactic platelet transfusion at low (1.1×10^{11}), medium (2.2×10^{11}), and high (4.4×10^{11}) doses of platelets per square meter (m^2) of body surface area (BSA) among adults and children with hematologic cancers and solid tumors undergoing hematopoietic transplant and chemotherapy. This study reported no difference with the bleeding incidence among patients who received low or high dose platelet transfusion (68). The current clinical practice suggests 1 unit

of pooled platelets for every 10 kg body weight and 5–10 ml/kg of apheresis platelets. Expert guidelines recommend not to transfuse more than six pooled or a single apheresis unit at a time.

How to Transfuse Platelets

The volume of the WB-PC is 50 ml, whereas the apheresis unit volume ranges from 200 to 300 ml. Up to 95%, this volume is plasma or plasma and platelet additive solution (PAS). Volume reduced platelet units are only recommended when circulatory overload risk is significant. Besides delaying the platelet unit release from the blood bank, the volume reduction process decreases platelet count by 20% and activates the platelets (42). Platelets must be used within 4 h once they are released from the blood bank and complete transfusion within 30 min. While transfusing platelets, a filter with 80–170 micropores must be used to remove the aggregates. The benefit of ABO matching platelet transfusion is inconclusive, and currently, there is no consensus guideline that exists (69). However, Rh compatibility is highly desirable in childbearing age women to prevent Rh alloimmunization as platelet units invariably contain few RBCs. If the platelet transfusion is Rh incompatible, an anti-D immunoglobulin should be administered within 48 h (70). Withholding immunoprophylaxis for Rh incompatible platelet transfusion is safe, especially in males and women past the reproductive age as the rate of anti-D alloimmunization is very low with increased SDA-PC utilization (71).

Platelet Dose Response

Response to the platelet count is measured by the count increment (CI), which is defined as the platelet count increase within 1 h post-transfusion. With adequate platelet dose transfusion, the platelet CI should be above 20% of the pre-transfusion platelet count if measured within 10–60 min and higher than 10% if measured with 24 h post-transfusion. By body weight, one unit of platelet transfusion per 10 kg should increase platelet count by 35,000–50,000/mm^3 and by 7,000–11,000/mm^3/m^2 of BSA. In neonates and infants, a dose of platelet volume 5–10 ml/kg should increase the platelet count by 50,000–100,000/mm^3 (70). Though the CI method is simple, it has been shown not to be very accurate. Another accurate method to assess post-transfusion response is corrected count increment (CCI), which includes BSA and the number of platelets transfused. The CCI is usually measured within 10–60 min post-transfusion, and its value is expected to be $>7{,}500/\mu l \times 10^{11}$ platelets transfused/m^2 BSA. Lack of adequate platelet CI response suggests platelet refractoriness, and it is defined as CCI $<7{,}500$ with two subsequent platelet transfusions. The platelet refractoriness is often seen with increased platelet consumption due to DIC, acquired HLH, splenomegaly, and medications, such as amphotericin-B therapy. Managing the underlying clinical condition improves platelet refractoriness. Failure to have the CCI response within 1 h of post-transfusion in the absence of hypoproliferative and consumptive pathology suggests immune-mediated causes, such as presence of HLA or HPA antibodies (70). Immune-mediated platelet refractoriness conditions require HLA matching, cross-matching, and antibody specificity prediction methods to identify compatible platelet units. If antibody-matched platelets are not available, in the absence of bleeding, rituximab, recombinant factor VIIa, plasma exchange, intravenous immunoglobulins, and platelet continuous infusion can be used (68).

Platelet Transfusion Reactions

Common transfusion reactions associated with platelet transfusion are also similar to other blood component transfusions. Since the platelet storage temperature is around 24°C, almost all bacteria can grow, and therefore platelet concentrates have the highest risk of bacterial contamination as compared with other blood components. Compared with WB-PC, the risk of bacterial infection is five times with the SDA-PC (34). Febrile non-hemolytic transfusion reactions (FNHTR) and transfusion-related acute lung injury (TRALI) are common with platelets compared with other blood product transfusions (34). Other risks include alloimmunization with HLA, HPA, and Rh antigens and graft-vs.-host disease (34). A recent study reported an increased risk of organ dysfunction, sepsis, nosocomial infections, prolonged ICU stay, and mortality with platelet transfusions (49). The use of PAS reduces the plasma content of the platelets by two-thirds and decreases the incidence of adverse reactions related to plasma (34).

Knowledge Gap and Future Directions

There had been a significant advancement in terms of platelet preparation, storage, and transfusion safety. There remain opportunities to improve platelet transfusion practice in children. Using the WB-PC and SDA-PC is standard practice. However, the superiority of these products in terms of dose response and overall safety is still under investigation. A recent interest in using cold platelet transfusion in children needs further evaluation. The relevance of ABO compatibility in platelet transfusion is still controversial. The current prophylactic transfusion practice in the PICU is based on expert opinion, and there is a lack of objective evidence to favor prophylactic vs. therapeutic platelet transfusion. The optimum platelet count threshold for transfusion is significantly variable depending on the underlying etiology, clinical practice setting, and geographic location, which needs further evaluation (72). At present, there is a lack of evidence to replace platelet transfusions with the newer platelet substitutes, such as artificial platelets, recombinant interleukin-6 or-11, and thrombopoietin mimetics (73).

CONCLUSION

Over the last century, significant advancements have been made in the field of transfusion medicine. Platelet and plasma transfusions have become safer in many ways with the changes in processing methods and advances in storage

techniques. While these products are commonly used in critically ill children, there is still much to be learned regarding the optimal usage of platelets and plasma in this group.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

SYSTEMATIC REVIEW
published: 16 December 2020
doi: 10.3389/fped.2020.612467

Challenges in Maintaining the Hemostatic Balance in Children Undergoing Extracorporeal Membrane Oxygenation: A Systematic Literature Review

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OPEN ACCESS

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: 30 September 2020
Accepted: 26 November 2020
Published: 16 December 2020

Citation:
Drop JGF, Wildschut ED, Gunput STG, de Hoog M and van Ommen CH (2020) Challenges in Maintaining the Hemostatic Balance in Children Undergoing Extracorporeal Membrane Oxygenation: A Systematic Literature Review. Front. Pediatr. 8:612467. doi: 10.3389/fped.2020.612467

Background: Despite advances in technology and clinical experience, the incidence of hemostatic complications, including bleeding and thrombosis, remains high in children supported with extracorporeal membrane oxygenation (ECMO). These hemostatic complications are important to prevent, since they are associated with increased morbidity and mortality. This systematic literature review aims to outline the most important risk factors for hemostatic complications in children undergoing ECMO treatment, to summarize the reported alternative anticoagulant drugs used in pediatric ECMO and to describe studied associations between coagulation tests and hemostatic complications.

Methods: A literature search was performed in Embase, Medline, Web of Science Core Collection, Cochrane Central Register of Controlled Trials, and Google Scholar in February 2020. Included studies were studies evaluating children (<18 years old) treated with ECMO, and studies evaluating risk factors for hemostatic complications, alternative anticoagulants, or the association between coagulation tests and hemostatic complications.

Results: Out of 1,152 articles, 35 studies were included. Thirteen out of 49 risk factors were investigated in three or more studies. Most consistent results were found regarding ECMO duration and pH. However, evidence for risk factors was equivocal in the majority of studies, which is explained by the variability of populations studied, definitions of hemostatic complications, ECMO circuits, anticoagulation protocols, transfusion triggers and monitoring of anticoagulation. Five studies described alternative anticoagulants, including bivalirudin ($n = 3$), argatroban ($n = 1$) and FUT ($n = 1$). Higher anti-factor Xa levels were associated with less clotting events in one of nine studies, investigating the association between tests and hemostatic complications. Two studies revealed an association between anti-factor Xa assay-based protocols and a decreased number of transfusions, bleedings and need for circuit change.

Conclusion: Studies regarding risk factors showed conflicting results and a few retrospective studies reported the use of new anticoagulants and data on coagulation tests in relation to hemostatic complications. To decrease hemostatic complications in ECMO children, prospective multicenter studies are needed with clear bleeding and thrombotic definitions, and the best possible standardization of ECMO circuits used, anticoagulation protocols, and transfusion triggers.

Keywords: pediatrics, risk factor, bleeding, thrombosis, bivalirudin, unfractionated heparin (UFH), extra corporeal membrane oxygenation (ECMO)

WHAT THIS STUDY ADDS

The incidence of hemostatic complications, including bleeding and thrombosis, in children undergoing extracorporeal membrane oxygenation (ECMO) is high. It is important to prevent hemostatic complications since these complications are associated with decreased survival. Unfractionated heparin is most frequently used to remain patency of the circuit and to prevent patient thrombosis. However, alternative anticoagulants including direct thrombin inhibitors have been described. To evaluate the hemostatic balance, several coagulation tests are used. Knowledge about risk groups for hemostatic complications, alternative anticoagulants, and the association between coagulation tests and bleeding and thrombotic complications in ECMO children may help decrease the number of hemostatic complications and improve outcome. Hence, this systematic review aims to assemble existing evidence of (1) risk factors for hemostatic complications, (2) suitable alternative anticoagulants, and (3) associations between coagulation tests and hemostatic complications in children undergoing ECMO support. Studies regarding risk factors showed conflicting results and a few retrospective studies reported the use of new anticoagulants and data on coagulation tests in relation to hemostatic complications. To decrease hemostatic complications in ECMO children, prospective multicenter studies are needed with clear bleeding and thrombotic definitions and the best possible standardization of ECMO circuits used, anticoagulation protocols, and transfusion triggers.

INTRODUCTION

Extra Corporeal Membrane Oxygenation (ECMO) is the last treatment option for children with refractory cardiac and/or pulmonary failure. The methodology was introduced by Bartlett when he performed the first successful ECMO treatment in an infant in 1976 (1). Over time, through improvements in materials, components and techniques, the role of ECMO as a supportive therapy has expanded in pediatric patients (2). In 2019, the registry of the extracorporeal life support organization (ELSO) contained 12,850 ECMO runs from 430 participating centers worldwide (2). Neonatal and pediatric ECMO runs accounted for 33.5 and 21.6% of the total number of ECMO runs, respectively. Despite improvements in technology and increasing clinical expertise, hemostatic complications, including bleeding and thrombosis, remain an important cause of mortality and morbidity in ECMO treated children worldwide. Hemorrhagic complications, including intracranial hemorrhage, were reported in up to 29.1% of neonatal and 28.5% of pediatric ECMO patients. Thrombotic complications, including circuit thrombosis and cerebral infarction, arose in up to 16.7% of neonatal and 12.4% of pediatric patients (3). These hemostatic complications may be life threatening, especially when they occur in the central nervous system. In a multicenter, prospective study in 514 children with ECMO support, bleeding appeared to be independently associated with a higher risk of mortality (4). One of the policies to improve outcome in pediatric ECMO patients should, therefore, focus on decreasing the frequency of bleeding and thrombotic complications.

The hemostatic complications in children undergoing ECMO support are related to the continuous contact between circulating blood and the foreign surface of the extracorporeal circuit, shifting the hemostatic balance to a hypercoagulable state with risk of thrombosis. To restore the hemostatic balance and prevent thrombosis, the use of systemic anticoagulation is necessary, but also increases the risk of bleeding. Many other factors may be associated with the development of hemostatic complications, including circuit factors, such as mode of cannulation (venoarterial [VA] or venovenous [VV]), high shear stress causing acquired von Willebrand disease, and patient factors, such as developmental hemostasis, sepsis, and cardiopulmonary resuscitation (CPR) (5). Knowledge of important risk factors for hemostatic complications may help to define high risk groups for which the anticoagulation protocol can be adjusted. The type of anticoagulant drug may also have an effect on the frequency of hemostatic complications. Most centers use unfractionated heparin (UFH) owing to the short half-life and effective reversal ability by protamine sulfate (6, 7). Besides UFH, alternative anticoagulants like direct thrombin inhibitors (DTIs), including bilivarudin or argatroban, are increasingly used (8, 9). These DTIs get their name from their direct binding to thrombin to exert anticoagulant effects. They may induce a more stable anticoagulant effect leading to less hemostatic complications. In addition, the choice of coagulation test may affect the risk of hemostatic complications. To monitor the UFH effect, several coagulation tests are used, including activated clotting time (ACT), activated partial thromboplastin time (APTT), anti-Xa assays (aXa), thromboelastography (TEG), rotational thromboelastometry (ROTEM), or a combination of them in a variability of coagulation protocols (10). The best coagulation test

in ECMO patients would be one which correlates well with the occurrence of hemostatic complications.

Knowledge about risk factors for hemostatic complications, the efficacy and safety of alternative anticoagulants, and the association between coagulation tests and bleeding and thrombotic complications in ECMO children may help decrease the number of hemostatic complications and improve outcome. Hence, this systematic review aims to assemble existing evidence of (1) risk factors for hemostatic complications, (2) suitable alternative anticoagulants, and (3) correlations between coagulation tests and hemostatic complications in children undergoing ECMO support.

METHODS

This systematic review was performed in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (11). This review is registered in the PROSPERO database (CRD42019133803). Embase.com, Medline ALL via Ovid, Cochrane Central Register of Controlled Trials, Web of Science Core Collection and Google scholar (top 100) were systematically searched from inception until February 27th, 2020. The search strategy is attached in **Supplementary Material 1**.

Study Selection

In this systematic review the following studies were included: all studies evaluating pediatric patients (<18 years old) treated with ECMO, and studies evaluating risk factors for hemostatic complications, alternative anticoagulants, or the association between coagulation tests and hemostatic complications in children treated with ECMO. Outcome was defined as any hemostatic complication and comprised of all hemorrhagic and thrombotic complications. Furthermore, only English language and human studies were included. Case-reports, editorials, conference abstracts and letters, and unavailable full-text articles were excluded.

Two independent review authors (JD, HO) screened titles and abstracts to select eligible studies. Disagreements about study selection were resolved by discussion. One reviewer (JD) examined the full text records to determine which studies met the in- and exclusion criteria. Any doubt was resolved via discussion and consensus with the second author (HO).

After study selection based on the in- and exclusion criteria, the Newcastle Ottawa Score (NOS) was used to assess the methodological quality of studies describing risk factors (12). This scale assesses patient selection bias, comparability of cases and controls and determination of outcome. The NOS values are depicted in **Supplementary Material 2**. Studies with a NOS above five were considered high quality and were included in this manuscript.

Data Extraction

Extracted data from eligible studies investigating risk factors for hemostatic complications included number of patients, authors, year of publication, study design, years of patient inclusion, outcome, odds ratios (ORs) or mean values and corresponding *P*-values. A list of all risk factors for hemostatic complications was drafted and risk factors examined in three or more studies were included in this manuscript. Outcome measures were OR or relative risk (RR) estimates with corresponding 95% confidence intervals (CIs). If a risk factor was described in multiple types of analyses (i.e., multivariate, univariate, comparing means or incidence) for the same outcome, only the highest level of evidence was considered eligible for this review. Continuous variables were appraised higher than categorical variables. Extracted data from eligible studies investigating alternative anticoagulants involved type of anticoagulant, used dosages and target ranges, reason for use, patient characteristics, and bleeding and thrombotic complications. Obtained data from studies examining the association between coagulation tests and hemostatic complications included type of tests and hemostatic complications, patient characteristics and the associations found.

RESULTS

Study Identification

The systematic search strategy yielded 1,152 articles (**Figure 1**). The majority of studies was excluded ($n = 1{,}097$), as they did not fulfill the eligibility criteria after screening of titles and abstracts. A total of 35 out of 55 studies were included after full text screening: 20 studies investigated risk factors for hemostatic complications, five studies described alternative anticoagulants, six studies examined the association between coagulation tests and hemostatic complications, and four studies described both risk factors and the association between coagulation tests and hemostatic complications. Twelve studies were considered low quality and excluded. A substantial part of studies had a retrospective design. The years of publication ranged from 1986 to 2020 and 2013 was the median year of publication. Eleven studies retrospectively investigated the ELSO registry.

Risk Factors for Hemostatic Complications

Qualitative assessment was performed in 24 studies concerning risk factors for hemostatic complications. After application of NOS, twelve studies were considered high quality, including 11 retrospective studies and one prospective observational study. In these 12 studies, a total of 49 potential risk factors was described. (**Supplementary Material 3**) 13 risk factors were investigated in three or more studies and included in this manuscript. Eight studies performed multivariate analyses, which are described in **Tables 1**, **2**. The average NOS score of included studies was 5.66.

Age at ECMO Initiation

Age at ECMO initiation as risk factor for hemostatic complications was investigated in eight studies (4, 13–15, 17, 18, 20, 21). Dalton and colleagues performed a prospective, multicenter cohort study, investigating 514 children undergoing ECMO support. It was concluded that neonatal age was associated with a significantly increased risk of daily bleeding events (RR 1.04, 95%CI 1.02–1.05, $p < 0.01$), defined as an intracranial hemorrhage or blood loss that required transfusion (4). In addition, in the ELSO study of Rollins et al. examining 2617 ECMO patients between 1 month and 18 years

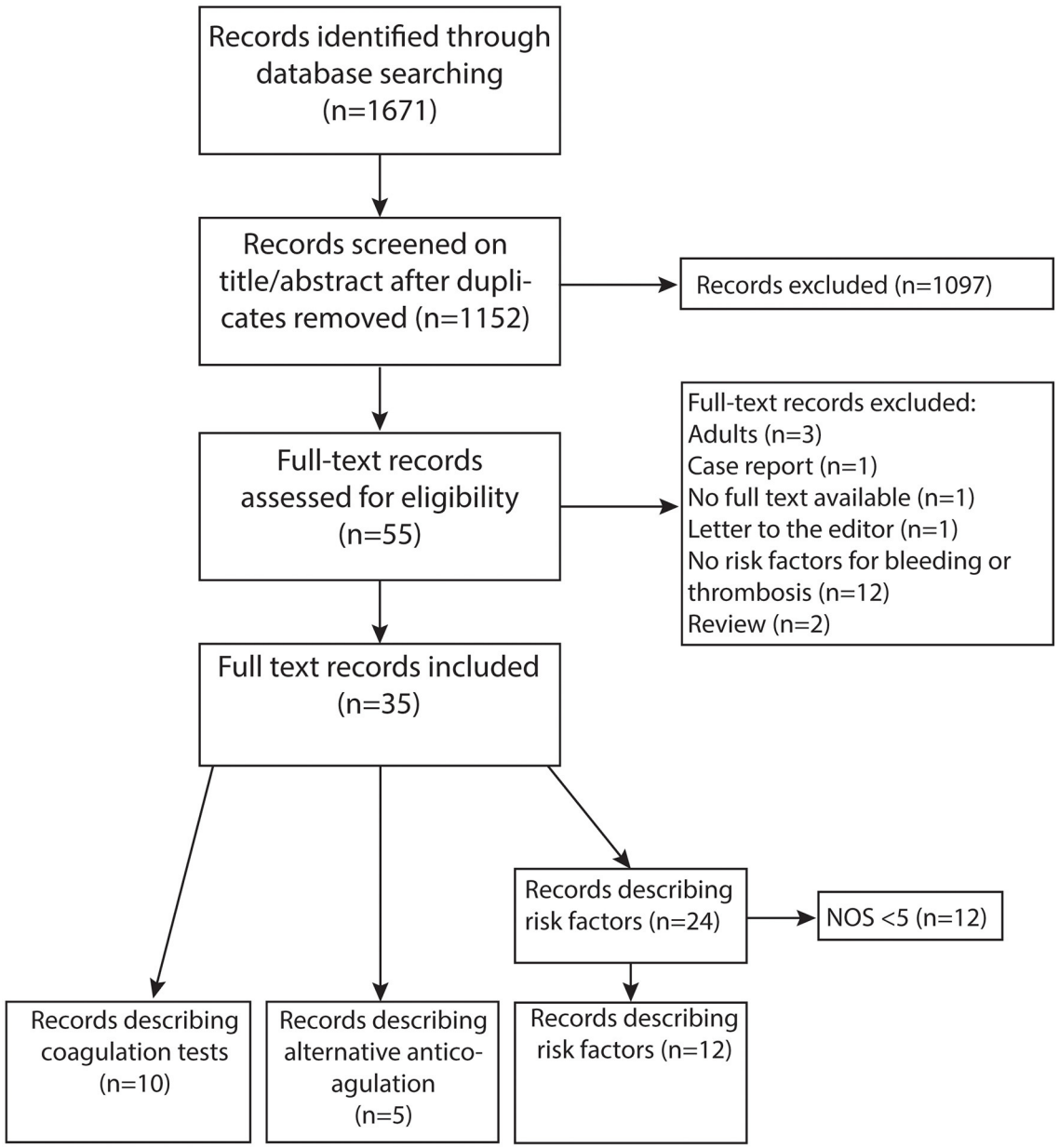

FIGURE 1 | PRISMA flow diagram of literature search (11).

old with respiratory failure, increasing age was associated with decreased odds of CNS infarction or hemorrhage (17). However, no significant association could be found in two retrospective ELSO reviews of Hardart et al. and in the case-control study by Mol et al. (13–15) Contradictory results were also found for thrombotic complications. In the retrospective study of Muensterer et al., age at cannulation was 83 ± 108 h in 112 patients without clotted circuits and 70 ± 111 h in 62 patients with clotted circuits ($p = 0.45$) (20). In addition, no association was found between age and change of circuit secondary to thrombus formation in 62 pediatric patients in the retrospective study of Irby et al. (21). However, Werho et al. concluded that neonatal age was associated with significantly increased odds of stroke from a retrospective ELSO study with 3,517 cardiac surgical patients requiring ECMO postoperatively (18).

Gestational Age

Gestational age was investigated in four studies (13–16). Increasing gestational age between 29 and 34 weeks was found to be significantly associated with decreasing odds of cerebral infarction (OR 0.79, 95%CI 0.64–0.98, $p = 0.03$), but not with ICH in a retrospective ELSO study of 752 neonates by Church

TABLE 1 | Risk factors for bleeding complications in pediatric ECMO studies with multivariate analyses.

Author	Year	Study population	Study type	Outcome	Risk factors	Univariate OR	*p*	Multivariate OR	*p*
Hardart and Fackler (13)	1999	3,777 neonates	Retrospective ELSO review (1992–1995)	ICH	Age	0.71	<0.001–0.01	ns	
					Gestational age				0.0001
					<34 weeks	11.6	<0.001	12.1 (6.6–22.0)	0.0001
					34–<36 weeks	5.0	<0.001	4.1 (2.9–5.8)	0.0001
					36–<38 weeks	2.5	<0.001	2.1 (1.6–2.8)	
					CPR/cardiac arrest pre-ECMO	1.9	<0.001	ns	
					Gender (male)	1.3	0.01–0.05	ns	
					$PaCO_2$ >50 mmHg	1.8	<0.001	ns	
					Birthweight (kg)				
					<2.5	3.3	<0.001	ns	
					2.5–<3.0	1.9	<0.001	ns	
					Coagulopathy/DIC	2.5	<0.001	1.6 (1.1–2.2)	0.0009
					Primary diagnosis sepsis	2.6	<0.001	1.8 (1.4–2.4)	0.0001
					pH				
					<7.0	3.9	<0.001	2.5 (1.6–3.9)	0.0001
					7.0–<7.2	2.6	<0.001	1.8 (1.3–2.5)	0.0001
					7.2–<7.35	1.5	<0.001	1.6 (1.1–2.2)	0.0001
					Mode: VV ECMO vs. VA ECMO	0.7 (0.53-0.92)	<0.05	0.97 (0.73–1.30)	
Hardart et al. (14)	2004	1,398 neonates < 37 weeks gestation	Retrospective ELSO review (1992–2000)	ICH	Age		0.01	ns	
					Gestational age		0.09	–	
					CPR/cardiac arrest pre-ECMO		<0.001	ns	
					Gender (male)		ns	–	
					Increasing $PaCO_2$		0.03	ns	
					Birthweight		ns	–	
					ECMO mode		ns	–	
					Primary diagnosis sepsis			1.78 (1.24–2.56)	0.004
					pH				
					<7.0			2.41 (1.27–4.56)	0.0004
					7.0–<7.2			1.65 (1.10–2.45)	
De Mol et al. (15)	2008	25 newborns with ICH vs. 40 control patients matched for diagnosis, gestational age, birth weight	Retrospective case control study	ICH	Age	1.0 (0.9–1.0)		–	
					Gestational age	0.4 (0.1–1.4)		–	
					CPR pre-ECMO	2.0 (0.4–9.0)		–	
					PH ≤7.3 6 h pre- ECMO	4.5 (1.4–14.0)		ns	
					$PaCO_2 \geq$ 45 mmHg 6 h pre-ECMO	3.3 (1.0–10.9)		ns	
					ECMO duration	ns			
					Birth weight	ns			
Church et al. (16)	2017	752 neonates between 29 and 34 weeks of gestation	Retrospective ELSO review (1976–2008)	ICH	Gestational age	ns			
					ECMO mode	ns			
					Birthweight			0.59 (0.37–0.93)	0.02
					CPR/cardiac arrest pre-ECMO			1.42 (0.83–2.43)	0.20
Dalton et al. (4)	2017	514 children < 19 years	Prospective cohort study	Daily bleeding event	Neonatal age			1.04 (1.02–1.05)	<0.001
					CPR/cardiac arrest pre-ECMO			1.76 (1.45–2.13)	<0.001
					ECMO indication				
					Cardiac vs. Resp.			1.34 (1.11–1.63)	0.002
					CPR vs. Resp.			1.52 (1.16–1.98)	
					ECMO duration			Significant	

ECMO, extracorporeal membrane oxygenation; ELSO, extracorporeal life support organization; CPR, cardiopulmonary resuscitation; ns, not significant; ICH, intracranial hemorrhage; VV, venovenous; VA, venoarterial; OR, odds ratio; DIC, disseminated intravascular coagulation.

et al. (16). In both the study of de Mol et al., a retrospective case control study, and in the retrospective ELSO review of Hardart in 2004, gestational age was also not significantly associated with ICH (14, 15). However, in 1999 a positive association was found by Hardart et al.: newborns with a gestational age below 34 weeks (OR 12.1, 95%CI 6.6–22.0, $p = 0.0001$), 34–36 weeks (OR 4.1, 95%CI 2.9–5,8, $p = 0.0001$) and 36–38 weeks (OR 2.1, 95%CI 1.6–2.8, $p = 0.0001$) had a significantly increased risk of ICH (13).

CPR/Cardiac Arrest Pre-ECMO

Four studies investigated the association between CPR and/or pre-ECMO cardiac arrest and hemostatic complications (13, 15–17). In three retrospective ELSO studies in various patient groups, no association could be found in the multivariate analyses (13, 16, 17). In addition, the incidence of children with CPR before ECMO did not significantly differ between cases and controls in the study of de Mol et al. (15).

TABLE 2 | Risk factors for thrombotic events in studies with multivariate analyses.

Author	Year	Study population	Study type	Outcome	Risk factors	Univariate OR (95%CI)	*p*	Multivariate OR (95%CI)	*p*
Church et al. (16)	2017	752 neonates between 29 and 34 weeks of gestation	Retrospective ELSO review (1976–2008)	Cerebral infarct	Birthweight		ns		
					ECMO mode		ns		
					Gestational age			0.79 (0.64–0.98)	0.03
					DIC			3.01 (1.39–1.75)	0.04
Rollins et al. (17)	2012	2,617 children 1 mo-< 18 years respiratory failure	Retrospective ELSO review (1993–2007)	CNS infarct or hemorrhage	Age			0.96 (0.93–0.98)	
					Male gender		ns	–	
					Weight (kg)		<0.05	ns	
					Cardiac arrest pre ECMO		<0.05	ns	
					$paCO_2$ pre-ECMO		<0.05	ns	
					DIC		<0.05	ns	
					Hours on ECMO		<0.05	ns	
					pH pre ECMO				
					pH 6.4–7.18		<0.05	2.1 (1.5–2.8)	
					pH 7.19–7.29		<0.05	1.5 (1.1–2.1)	
					pH >7.29 ref group		<0.05	ref group	
					ECMO mode (VA vs. VV)			1.6 (1.1–2.3)	
Werho et al. (18)	2015	3,517 cardiac surgical patients ECMO postop. < 18 years	Retrospective ELSO review (2002–2013)	Stroke	Neonatal age	1.73 (1.41–2.14)	<0.0001	1.77 (1.32–2.36)	0.0001
					Weight for age z score	1.06 (0.98–1.15)	0.13	1.14 (1.04–1.25)	0.004
					Duration of ECMO ≥ 167 h	1.45 (1.16–1.80)	0.001	1.38 (1.06–1.78)	0.02
					Cannulation site: neck	1.38 (1.03–1.83)	0.03	1.29 (0.91–1.81)	0.15
Dalton et al. (4)	2017	514 children < 19 years	Prospective cohort study	Daily thrombotic event	ECMO mode (VV vs. VA)			0.62 (0.40–0.95)	0.024
					ECMO duration			Significant	
Maul et al. (19)	2020	274 patients with 299 ECMO runs	Retrospective cohort study	Circuit change	Weight (mean)		ns	–	<0.05
					ECMO duration (mean)		<0.05	1.007 (1.004–1.010)	<0.05
					ECMO mode (VV vs. VA)		<0.1	–	
					Respiratory indication		<0.05	2.8 (1.4–5.8)	

ECMO, extracorporeal membrane oxygenation; ELSO, extracorporeal life support organization; CPR, cardiopulmonary resuscitation; ns, not significant; VV, venovenous; VA, venoarterial; OR, odds ratio; DIC, disseminated intravascular coagulation; h, hour; postop, postoperatively; kg, kilogram; CNS, central nervous system.

Gender

In five studies, gender was not correlated with a hemostatic complication in a univariate analysis (14, 15, 17, 20, 21). In the retrospective ELSO review of Hardart in 1999, 240 of 2,210 male patients (10.1%) had ICH (RR 1.3, $p < 0.05$). However, after adjustment for other significant predictors for ICH by multivariate analysis, no correlation was found (13).

$PaCO_2$

Three studies examined the association between ICH and higher pre-ECMO $PaCO_2$ in ECMO children (13, 15, 17). In the ELSO study of Rollins et al. the mean $PaCO_2$ before ECMO was significantly higher in 233 children with CNS injury (64 mmHg; IQR 87 mmHg) than in 2,384 children without CNS injury (55 mmHg, IQR 75 mmHg, $p < 0.05$). However, after multivariate analysis no correlation was present (17). Similarly, no significant association was found in the multivariate analyses of another ELSO review of Hardart et al. in 2004 and in the case control study of de Mol et al. (14, 15).

(Birth)Weight

Eight studies investigated the association between bleeding or thrombotic events and (birth)weight, showing conflicting results (13–20). In the ELSO review of Church et al. lower birthweight increased the odds of ICH, but not for infarction in neonates between 29 and 34 weeks of gestation (OR 0.59, 95% CI 0.37–0.93, $p = 0.02$) (16). Three other studies with neonates did not find an association between birthweight and ICH after multivariate analyses (13–15). However, average cannulation weight of 62 neonates requiring a circuit change (2,988 g ± 602) was significantly lower than 112 neonates without a circuit change (3,212 g ± 580; $p = 0.02$), as concluded from the study of Muensterer (20).

In older children, lower weight-for-age z score was independently associated with stroke in cardiac surgical patients requiring ECMO postoperatively (OR 1.14, 95%CI 1.04–1.25, $p = 0.004$) (18). However, weight was not a risk factor for CNS injury in ECMO children with respiratory failure (17, 19). Furthermore, weight was not significantly different in children with circuit change (11 ± 14 kg) compared to children without circuit change (14 ± 21 kg, $p > 0.05$) (19).

Coagulopathy/Disseminated Intravascular Coagulation

The association between hemostatic complications and coagulopathy has been investigated in three studies (13, 16, 17). In the ELSO review of Hardart et al. in 1999, coagulopathy increased the odds for ICH in neonates significantly (OR 1.6, 95%CI 1.1–2.2, $p = 0.0009$) (13). In addition, the presence of DIC was associated with cerebral infarction in neonates between

29 and 34 weeks (OR 3.01, 95%CI 1.39–1.75, $p = 0.04$) in the ELSO review of Church et al. (16). In older children with ECMO due to respiratory failure, no association between DIC and CNS infarct or hemorrhage was found by Rollins et al. (17).

Platelet Count

Three studies have investigated platelet count and hemostatic complications (21–23). In a retrospective review of 25 children undergoing ECMO support by Sell et al., the incidence of ICH was significantly lower in patients with platelet count >100.000/uL and significantly higher in patients with platelet count <100.000/uL (23). Stallion et al. showed that in 42 children, the incidence of bleeding complications in patients managed with a platelet count >200.000/uL was not significantly different compared to patients with a goal platelet count >100.000/uL (22). In addition, platelet count was similar in 62 pediatric patients with or without circuit change due to thrombus formation in the retrospective study of Irby et al. (21).

Duration of ECMO Support

The association between duration of ECMO support and hemostatic complications has been described in six studies (4, 15, 17–20). In 2017, Dalton reported a strong association of ECMO duration in neonates and older children with the development of bleeding and thrombotic complications during the total ECMO period (4). In the study of Werho et al., ECMO duration >167 h increased the odds of stroke in cardiac surgical patients requiring ECMO postoperatively (OR 1.38, 95%CI 1.06–1.78, $p = 0.02$) (18). In addition, in the single center study of Maul et al., the risk for a circuit change increased with longer ECMO duration (OR 1.007, 95%CI 1.004–1.010, $p < 0.05$) (19). Moreover, Muensterer et al. reported an increased ECMO duration in patients with circuit clots (195 ± 108 h in patients without clots vs. 504 ± 224 h in patients with clotted circuits, $p = 0.02$) (20). In addition, in the ELSO review of Rollins et al., ECMO duration significantly increased the risk for CNS injury in children with respiratory failure (CNS injury 176 IQR 326, no CNS injury 201 IQR 356, $p < 0.05$) (17). However, ECMO duration was not associated with ICH in the study of de Mol et al.: ECMO duration was 155 ± 76 h in neonates with ICH and 171 ± 81 h ($p = 0.43$) in control patients (15).

ECMO Indication

ECMO indication is investigated in three studies (4, 19, 21). Both cardiac (OR 1.34, 95%CI 1.11–1.63, $p < 0.002$) and ECPR (OR 1.52, 95%CI 1.16–1.98, $p < 0.002$) indications in the study of Dalton et al. increased the risk for daily bleeding events compared to children with a respiratory ECMO indication (4). In contrast, in the study of Maul et al. the risk for circuit clots was increased in patients with respiratory indications (OR 2.8, 95%CI 1.4–5.8, $p < 0.05$) (19). However, no association between ECMO indications and circuit change was revealed in 62 pediatric patients from the single center of Irby et al. ($p = 0.327$) (21).

Mode of Cannulation

Mode of cannulation in relation to hemostatic complications was described in five studies (4, 13, 17–19) In the 2,617 children requiring ECMO support for respiratory failure, VA ECMO increased the odds of CNS infarction or hemorrhage compared to VV ECMO (OR 1.6, 95%CI 1.1–2.3) (17). Moreover, VV ECMO decreased the odds for daily thrombotic events, including patient and circuit related thrombosis, in the study of Dalton et al. (4). Maul et al., however, showed a trend toward a higher number of VV ECMO patients requiring circuit changes (19). In neonates, VV ECMO was not associated with significantly increased odds of ICH and cerebral infarction in three retrospective ELSO studies (13, 17, 18). In 3,517 cardiac surgical patients requiring ECMO postoperatively, cannulation in the neck did not increase the risk of hemostatic complications (18).

Sepsis as Primary Diagnosis

Sepsis as primary diagnosis has been described in four studies (13, 14, 16, 20). In the two retrospective ELSO studies of Hardert et al. comprising 3,777 and 1,398 neonates, sepsis as primary diagnosis increased the odds for ICH significantly (13, 14). In the retrospective review of Church et al., none of the primary diagnoses, including sepsis, were associated with ICH in neonates between 29 and 34 weeks of gestational age (16). In the study of Muensterer et al. sepsis as primary diagnosis was significantly less present in patients with clotted circuits (3%) than without clotted circuits (31%, $p < 0.01$) (20).

pH

Four studies described the association between pH and hemostatic complications (13–16). Additionally, the case control study of de Mol et al. showed an increased risk for ICH in neonates with pH <7.3 in children (15). In three ELSO registry studies, low pre-ECMO pH was associated with increased risk for ICH in neonates and for CNS infarction and hemorrhage in children (13, 14, 16).

Summary of Risk Factors

Thirteen risk factors were described in at least three studies in various patient groups on ECMO. In the majority of these 13 risk factors, contradictory results were found. Most constant results were found between hemostatic complications and ECMO duration and pH: longer ECMO duration was associated with increased risk of bleeding and thrombotic complications in all age categories, and a low pre-ECMO pH was associated with an increased risk of intracranial injury in neonates.

Alternative Anticoagulants

Five studies reported the use of alternative anticoagulants in pediatric ECMO patients (8, 9, 24–26). Three studies described bivalirudin, one case series covered the use of argatroban, and one case series outlined FUT. Nagle et al. reported the use of bivalirudin in pediatric patients on ECMO in a case series of 12 children with a median age of 8 days. All patients were managed with heparin initially, but anticoagulation was changed to bivalirudin due to heparin induced thrombocytopenia, heparin resistance, thrombus formation or unstable ACTs. The initial infusion ranged from 0.05 to 0.3 mg/kg/h. The maintenance dose that corresponded with an initial target APTT ranged from 0.045–0.48 mg/kg/h with a median rate of 0.16 mg/kg/h. Two patients suffered from bleeding from chest tubes requiring

re-exploration and 8 patients had a circuit change, while on bivalirudin (9). Two groups of 21 post cardiotomy ECMO patients, including four neonates and six children, using UFH or bivalirudin were retrospectively compared in the study of Ranucci et al. Bivalirudin infusion was started at an initial dose of 0.03 to 0.05 mg/kg/h depending on the bleeding rate and adjusted according to ACT (target range 160–180 s), APTT (50–80 s) and r time values (12–30 min) of TEG. Blood loss and transfusion with platelets and fresh frozen plasma was significantly higher in the heparin group. The number of thrombotic events and mortality did not differ (25). Teruya et al. described six pediatric patients with bivalirudin on ECMO. The initial infusion rate was 0.05–0.15 mg/kg/h and was thereafter adjusted to maintain APTTs within a specific target range. The target range of 60–80 s was adjusted by the treating physician based on the patient condition (26).

Another direct thrombin inhibitor, argatroban, was described by Potter et al. in a case series of three children on ECMO. The circuit was primed with 30–50 ug argatroban on the basis of patient's ACT. Initial infusion ranged from 0.5 to 1 ug/kg/min and was titrated to individually set ACT target ranges between 250 and 300 s. None of the patients suffered from any significant hemorrhagic complications. However, all patients suffered from thromboembolic disease in varying severity during argatroban therapy (8).

FUT is a serine protease inhibitor with anticoagulant activity due to the inhibition of the coagulation and fibrinolytic systems (factor II, Xa, and XIIa). Due to its short half-life of 8 min it has been used in continuous renal replacement therapy (27, 28). Nagaya et al. presented evidence for the clinical use of FUT in 12 neonatal ECMO patients in whom hemorrhagic complications had occurred. They attempted to decrease only the patient's ACT levels, while keeping the ACT levels in the ECMO circuit at normal high levels. After administration of FUT in the drainage route, the heparin dose was decreased. FUT and heparin dosages were regulated to maintain the ACT at the reinfusion route at 190–220 s. The average dose of heparin was 21 ± 7.5 IU/kg/h and FUT 0.48 ± 0.22 mg/kg/h. In eight cases, the bleeding could be controlled by FUT administration. No difference was described in thrombotic formation in ECMO circuits between patients managed with FUT and heparin or heparin alone (24).

Summary of Alternative Anticoagulants

The use of bivalirudin, argatroban and FUT in pediatric ECMO patients has been described in a total of 55 children, but pharmacokinetic data, clear dosing and monitoring guidelines are lacking.

Association Between Coagulation Tests and Hemostatic Complications

Nine studies investigated the association between coagulation tests or coagulation protocol and hemostatic complications (21, 29–36). In an autopsy study of 29 pediatric ECMO non-survivors (median age 2 months), thrombosis and bleeding were diagnosed in 69 and 52% of the patients, respectively. ACT, APTT, and PT results of the last ECMO support day were not significantly associated with these hemostatic events (ACT: no hemorrhage group 214, hemorrhage group 209, $p = 0.005$, data APTT and PT not shown) (34). Additionally, ACT, APTT, and aXa levels did not show any differences during 24 and 72 h before a cerebrovascular event between 36 cases and controls in the study of Anton-Martin (29). Furthermore, in the study of O'Meara mean aXa levels, APTT and INR were not statistically different between the failed oxygenator ($n = 7$, PTT 137 ± 27;INR 1.5 ± 0.2) and no failed oxygenator group ($n = 15$, PTT 137 ± 45; INR 1.7 ± 0.5) (33). In the retrospective chart review of Grayck et al., the minimum and maximum ACT values did not significantly differ between 82 neonates with or without ICH (minimum: ICH 13 ± 7, no ICH 157 ± 4, $p = 0.65$; maximum ICH 271 ± 10, no ICH 254 ± 4, $p = 0.90$) (37). Irby et al. studied 62 mostly neonates and young infants with ECMO therapy of whom 17 patients required change of circuit or membrane oxygenator due to thrombosis. No difference in mean daily ACT measurements between patients with and without circuit or membrane oxygenator change was found (no circuit change: 195.26 ± 5.74; circuit change: 202.62 ± 3.96, $p = 0.192$). However, the mean aXa factor was significantly higher in the patients without thrombus formation (21). In the retrospective study of McMichael et al. of 69 pediatric ECMO patients, aXa levels and APTT were not significantly associated with bleeding or thrombotic complications (any bleed aXa 0.44 95%CI 0.23–0.44, no bleed aXa 0.43 95%CI 0.36–0.48, $p = 0.56$; any bleed APTT 79 95%CI 63–100, no bleed APTT 78 95%CI 67–97, $p = 0.96$; circuit clot formation aXa 0.44 95%CI 0.4–0.5, no circuit clot formation aXa 0.42 95%CI 0.36–0.47, $p = 0.41$; circuit clot formation APTT 78 95%CI 64–91, no circuit clot formation APTT 83 95%CI 67–101, $p = 0.50$) (30). Saini et al. investigated the value of a viscoelastic test, TEG-platelet mapping, in the prediction of bleeding complications in 24 children on ECMO. No difference in kaolin-activated heparinase TEG parameters were found between the bleeding and the non-bleeding group. Prediction of bleeding based on ROC revealed that the AUC for ADP-mediated platelet aggregation, AA-mediated platelet aggregation and ACT was 0.64 (95% CI: 0.48–0.79, $p = 0.11$), 0.68 (95%CI 0.53–0.83, $p = 0.03$), and 0.56 (95%CI 0.39–0.75, $p = 0.42$), respectively (36).

Northrop et al. showed that after initiation of a revised anticoagulation protocol, which included the incorporation of aXa, TEG and antithrombin measurements in addition to the standard laboratory tests ACT, PT/INR, and APTT, the median blood product usage, and the frequency of cannula and surgical site bleedings decreased. Moreover, the median circuit life increased from 3.6 to 4.3 days ($p = 0.02$) (32). Niebler et al. also showed a statistically significant association between a change from an ACT based anticoagulation protocol to an aXa based protocol and a decreased incidence of ICH and need for circuit change (31). Yu et al. prospectively compared a simple anticoagulation protocol of one center (ACT every 2 h and daily APTT and aXa levels) with an intensive anticoagulation protocol (ACT every 2 h, APTT, PT, and aXa every 12 h, daily antithrombin) in another center. No significant differences in outcomes including major bleeding [5 [15%] vs. 14 [23], $p = 0.4$], patient thrombosis (6 [18%] vs. 10 [16%], $p = 0.8$) were found, but the center with the extensive monitoring protocol performed

significantly more circuit changes (19 [31%] vs. 3 [9%], $p = 0.02$) (35).

Summary of Coagulation Tests

No clear association has been described between coagulation tests, such as APTT, AXA, ACT, INR, and TEG, and bleeding or thrombotic complications in pediatric ECMO patients. However, in one study higher anti-factor Xa levels were associated with less clotting events (21). Two studies revealed an association between anti-factor Xa assay-based protocols and a decreased number of transfusions, bleedings and need for circuit change (31, 32).

DISCUSSION

Hemostatic complications remain an important cause of morbidity and mortality during ECMO support in children (38). Over the last 6 years, the frequency of bleeding complications and circuit clotting has not changed significantly (39). Decreasing the number of hemostatic complications will improve outcome of pediatric ECMO patients. Unfortunately, this systematic literature review revealed conflicting results regarding most risk factors for hemostatic complications in pediatric ECMO patients and only a few studies reported the use of new methods of anticoagulation. In addition, data on coagulation tests in relation to hemostatic complications were rare.

This literature review shows that about 50 risk factors for hemostatic complications have been investigated in various neonatal and pediatric patient groups with ECMO support. The large number of risk factors studied reflects the multifactorial etiology and the complex and dynamic mechanisms of bleeding and thrombosis in ECMO patients. Some of these risk factors may contribute through similar pathways to a disrupted hemostatic balance, for example sepsis and DIC. In addition, the severity of the patient's condition changes during ECMO support contributing to an alternating risk of bleeding and thrombotic complications. The majority of papers had a retrospective design, resulting in an unclear detection and timing of hemostatic complications. However, performing prospective studies in ECMO patients is challenging due to difficulties with obtaining informed consent and gathering enough patients to provide sufficient statistical power. As result of the retrospective design, timing of thrombotic or bleeding events may have been unclear or these events may have been missed because they were not described in the patient file. Identifying risk factors for ICH has predominantly been performed in neonates with ECMO support. In the most recent ELSO registry report, 11% of neonates with ECMO support for respiratory or cardiac indications developed an ICH (40). In the first ELSO review, gestational age was significantly associated with ICH (13). However, in the subsequent ELSO reports, gestational age did not increase risk for ICH (14–16). This might be the result of improved technology over time. Sepsis as primary diagnosis was the most consistent risk factor for ICH in neonates. However, this risk factor was investigated in two ELSO registries with overlapping patient populations (13, 14). Duration of ECMO support and the last pH before ECMO initiation in neonates were consistent risk factors in this review. However, it is difficult to draw conclusions about the other potential risk factors as mostly contradictory results were found. This is partly explained by the various populations studied, and differences in definitions of bleeding and thrombotic complications among studies. In addition, ECMO circuits, anticoagulation protocols, transfusion triggers and monitoring of anticoagulation varied among centers participating in multicenter studies influencing the risk for bleeding and thrombotic complications. One clear definition of bleeding and thrombotic complications, which can be used in all future studies, is needed. Moreover, description of the circuit variables, anticoagulation and monitoring practices, and transfusion triggers in the publications may be helpful in comparing studies and interpret the risk for hemostatic complications.

The incidence of hemostatic complications may decrease by using an alternative anticoagulant drug for UFH. UFH is still the anticoagulant of choice in pediatric ECMO patients, due to its availability, reversibility by protamine, familiarity among physicians, and rapid onset of action. The use of anticoagulants other than UFH is rarely investigated in children undergoing ECMO support. This literature review revealed five studies, four of them concerning DTIs. DTIs have advantages over UFH, including direct binding of both circulating and clot-bound thrombin, resulting in increased efficacy compared to UFH, antithrombin independence and no risk of heparin-induced thrombocytopenia (41). Bivalirudin is the most commonly used alternative for UFH in pediatric patients. However, the dose range of bivalirudin as described in the available reports is rather large and no direct antidote exists. Furthermore, none of the easily available tests, such as ACT and APTT, are validated for DTI monitoring. The relationship between APTT and DTI concentration is non-linear, especially with high dosages of DTIs (42). Dedicated assays, based on a dilated thrombin time, are not available in all centers. Moreover, as concluded by this literature review, few data are available about the safety and efficacy of DTIs in pediatric ECMO patients. Large prospective, observational or controlled clinical trials with uniform thrombotic and bleeding definitions are needed to compare UFH with a DTI.

Optimal monitoring of anticoagulation may decrease the incidence of hemostatic complications as well. Unfortunately, the best monitoring strategy is still unknown. Several reports have investigated the relationship between ACT, APTT and aXa factor and heparin dose on ECMO (43). These data show that the correlation between aXa levels and heparin dose is better than that of APTT or ACT. Only few studies investigated the association between coagulation tests and hemostatic complications. Only one of them showed in 62 neonates that the mean aXa factor was significantly higher in neonates without thrombus formation. All studies were performed retrospectively, which may have hampered the identification of hemostatic complications, particularly the exact timing of them. Interestingly, two studies revealed an association between protocols incorporating aXa measurements and a decreased number of transfusions, bleedings and the need for a circuit change (31, 32). Since aXa has a good correlation with heparin dose, it is the most valuable test to monitor heparin dosing in neonatal and pediatric ECMO patients (39). However, aXa targets are variable between studies and aXa fails to monitor

hypercoagulability or other derailments of the coagulation such as DIC. A multi test approach to monitor overall coagulation as well as an effective test for the anticoagulant administered seems necessary in managing pediatric ECMO patients. Future prospective studies investigating the association between hemostatic complications and separate or combinations of coagulation tests, including viscoelastic tests, will help to find the optimal strategy for anticoagulation monitoring in pediatric ECMO patients.

CONCLUSION

To improve outcome of pediatric ECMO patients, a reduction of the frequency of hemostatic complications is required. To effectuate such a reduction, we should be aware of the risk factors for hemostatic complications, the safety and efficacy of alternative anticoagulants and the association between coagulation tests and hemostatic complications to improve monitoring of anticoagulant therapy. This review showed a lack of prospective studies, uniform definitions of outcome parameters and therefore inconsistent and conflicting data on risk factors, coagulation monitoring and alternative anticoagulant drugs. The large variation in studied risk factors, patient groups, used ECMO circuits, anticoagulation protocols, monitoring methods and transfusion triggers hinder further development in this field. To appropriately study risk factors, new anticoagulants and coagulation monitoring, prospective multicenter trials are needed with clear bleeding and thrombotic definitions, and the best possible standardization of abovementioned variables. This should be a joint effort of all disciplines who take care of pediatric patients on ECMO support.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

JD and SG: performance of search. JD and CO: selection of studies. JD: drafting of tables. JD, EW, MdH, and CO: writing of manuscript. All authors: contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.612467/full#supplementary-material

Supplementary Material 1 | Search strategy.

Supplementary Material 2 | Table with all risk factors for bleeding or thrombosis in pediatric ECMO patients.

Supplementary Material 3 | Newcastle Ottawa Scores.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 23 December 2020
doi: 10.3389/fped.2020.618119

Hemostatic Balance in Pediatric Acute Liver Failure: Epidemiology of Bleeding and Thrombosis, Physiology, and Current Strategies

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Pediatric Acute Liver Failure (PALF) is a rapidly progressive clinical syndrome encountered in the pediatric ICU which may rapidly progress to multi-organ dysfunction, and on occasion to life threatening cerebral edema and hemorrhage. Pediatric Acute Liver Failure is defined as severe acute hepatic dysfunction accompanied by encephalopathy and liver-based coagulopathy defined as prolongation of International Normalized Ratio (INR) >1.5. However, coagulopathy in PALF is complex and warrants a deeper understanding of the hemostatic balance in acute liver failure. Although an INR value of >1.5 is accepted as the evidence of coagulopathy and has historically been viewed as a prognostic factor of PALF, it may not accurately reflect the bleeding risk in PALF since it only measures procoagulant factors. Paradoxically, despite the prolongation of INR, bleeding risk is lower than expected (around 5%). This is due to "rebalanced hemostasis" due to concurrent changes in procoagulant, anticoagulant and fibrinolytic systems. Since the liver is involved in both procoagulant (Factors II, V, IX, XI, and fibrinogen) and anticoagulant (Protein C, Protein S, and antithrombin) protein synthesis, PALF results in "rebalanced hemostasis" or even may shift toward a hypercoagulable state. In addition to rebalanced coagulation there is altered platelet production due to decreased thrombopoietin production by liver, increased von Willebrand factor from low grade endothelial cell activation, and hyperfibrinolysis and dysfibrinogenemia from altered synthetic liver dysfunction. All these alterations contribute to the multifactorial nature of coagulopathy in PALF. Over exuberant use of prophylactic blood products in patients with PALF may contribute to morbidities such as fluid overload, transfusion-associated lung injury, and increased thrombosis risk. It is essential to use caution when using INR values for plasma and factor administration. In this review we will summarize the complexity of coagulation in PALF, explore "rebalanced hemostasis," and discuss the limitations of current coagulation tests. We will also review strategies to accurately diagnose the coagulopathy of PALF and targeted therapies.

Keywords: PALF, hemostasis, coagulopathy, children, critical care, transfusion, thrombosis, liver failure

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *16 October 2020*
Accepted: *08 December 2020*
Published: *23 December 2020*

Citation:
Bulut Y, Sapru A and Roach GD (2020) Hemostatic Balance in Pediatric Acute Liver Failure: Epidemiology of Bleeding and Thrombosis, Physiology, and Current Strategies. Front. Pediatr. 8:618119. doi: 10.3389/fped.2020.618119

INTRODUCTION

Pediatric acute liver failure (PALF) is a life-threatening illness, characterized by the sudden onset of coagulopathy, thrombocytopenia, and systemic inflammation that may ultimately result in dreaded complications such as cerebral edema and hemorrhage. The liver is responsible for the synthesis of a majority of blood coagulation factors and plays a central role in the hemostatic system. As a result of decreased coagulation factor synthesis, acute liver failure patients were historically believed to be "autoanticoagulated" and protected from thrombosis. However, this belief has been challenged as the understanding of the coagulopathy in liver failure has evolved. In acute liver failure, there is a parallel reduction of anti-coagulant factors along with pro-coagulant factors and this leaves the patient in a rebalanced coagulation state that may sometimes favor thrombosis (1–7). Consequently, thrombotic events are more common than bleeding complications in patients with acute liver failure (2–4, 8–11). Clinically significant bleeding is rare, in part due to the absence of esophageal varices and portal hypertension, which are the major risk factors for bleeding in chronic liver failure. Recent data shows that in acute liver failure the spontaneous significant bleeding rate is <5%, the spontaneous intracranial bleeding rate is <1% and the risk does not correlate with INR values (12).

Since PALF is defined as severe acute hepatic dysfunction with prolongation of International Normalized Ratio (INR) >1.5 with encephalopathy, close monitoring of INR and correction of elevated levels occur frequently (13). Although the INR value is followed as a prognostic tool it is important to recognize the limitation of this classic test for evaluating the coagulopathy of liver disease. The prothrombin time (PT) and INR are measures of pro-coagulant factors (or lack thereof) and do not take into consideration changes in anti-coagulant factors, platelets, and other hemostatic changes. As such, the PT and INR are poor predictors of bleeding risk. It is essential to be cognizant of the risk of over-transfusion and tipping this delicate balance toward hemorrhage or thrombosis for an arbitrary INR number (14). Transfusion of any blood component has been associated with increased mortality, increased need for liver transplantation and reduction in graft survival (15). Transfusion-related immunomodulation (TRIM), transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), hemolytic reactions, and infection risk are some of the potential reasons for transfusion-related poor outcomes (16). This review will discuss the pathophysiology of "rebalanced hemostasis" in pediatric acute liver failure, diagnostic challenges and therapeutic considerations.

REBALANCED HEMOSTASIS

The hemostatic system consists of three major arms. Primary hemostasis involves formation of the primary platelet plug on the injured vascular endothelium. Secondary hemostasis involves the cascade of coagulation factors leading to formation of the fibrin clot. Fibrinolysis follows primary and secondary hemostasis; and involves a process that removes the fibrin clot and prevents uncontrolled thrombosis (17, 18).

In acute liver failure, all three of these arms of hemostasis are affected. The delicate balance between prevention of bleeding and excessive clot formation is impacted by the concomitant changes in pro- and anti-hemostatic factors in PALF. These changes are a result of decreased hepatic synthesis of pro- and anti-coagulation factors, thrombocytopenia, elevated von Willebrand factor (vWF), reduced a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13), reduced vitamin-K dependent carboxylation of pro- and anti-coagulant factors, and consumptive coagulopathy (10, 19).

Primary Hemostasis

Platelets are responsible for the initial primary hemostatic response by adhering to the damaged blood vessels and promoting aggregation and clot formation with the aid of the endothelial-derived protein vWF. They are also responsible for facilitating the Tissue Factor (TF) and factor VII complex formation, which catalyzes the activation of factor X and formation of "priming thrombin" (19) (**Figure 1**).

Both platelet number and function are reduced in PALF, although the changes are less pronounced compared to chronic liver failure (2, 9). Thrombopoietin (TPO) is a glycoprotein hormone produced primarily in the liver that regulates the production of platelets in bone marrow by stimulating megakaryocytes. In children with acute liver failure there are reductions in TPO levels as evidenced by reduced serum TPO and hepatic TPO mRNA levels (20). Moreover, increased consumption of platelets further contributes to thrombocytopenia. Increased production of endothelial-derived platelet inhibitors prostacyclin and nitric oxide likely also contributes to impaired function of platelets (21).

This thrombocytopenia is compensated, however, by elevated levels of adhesive protein vWF and decreased ADAMTS-13 (**Table 1**). While vWF promotes platelet adhesion and aggregation at sites of vascular injury, it is negatively modulated by ADAMTS-13. ADAMTS-13 is a metalloproteinase that is primarily synthetized in the liver as well as in endothelial cells and platelets. It cleaves large vWF multimers into smaller less procoagulant forms and decreases clot formation. In acute liver failure vWF levels are elevated and ADAMTS-13 levels are reduced. These opposing effects promote platelet adhesion with the net result of rebalanced hemostasis (1, 22).

Thrombocytopenia is also compensated by the presence of platelet-derived extracellular vesicles (EV); microparticles (0.1–1 μm) which are highly pro-coagulant and contribute to ongoing activation of coagulation (23). Recently a study by Stravitz et al. showed that patients suffering from acute liver failure had an increased amount of these circulating microparticles (23, 24).

Overall, thrombocytopenia in ALF is compensated by elevated levels of vWF, reduced ADAMTS-13 levels and presence of platelet-derived extracellular vesicles that partially offset the impact of low platelet count.

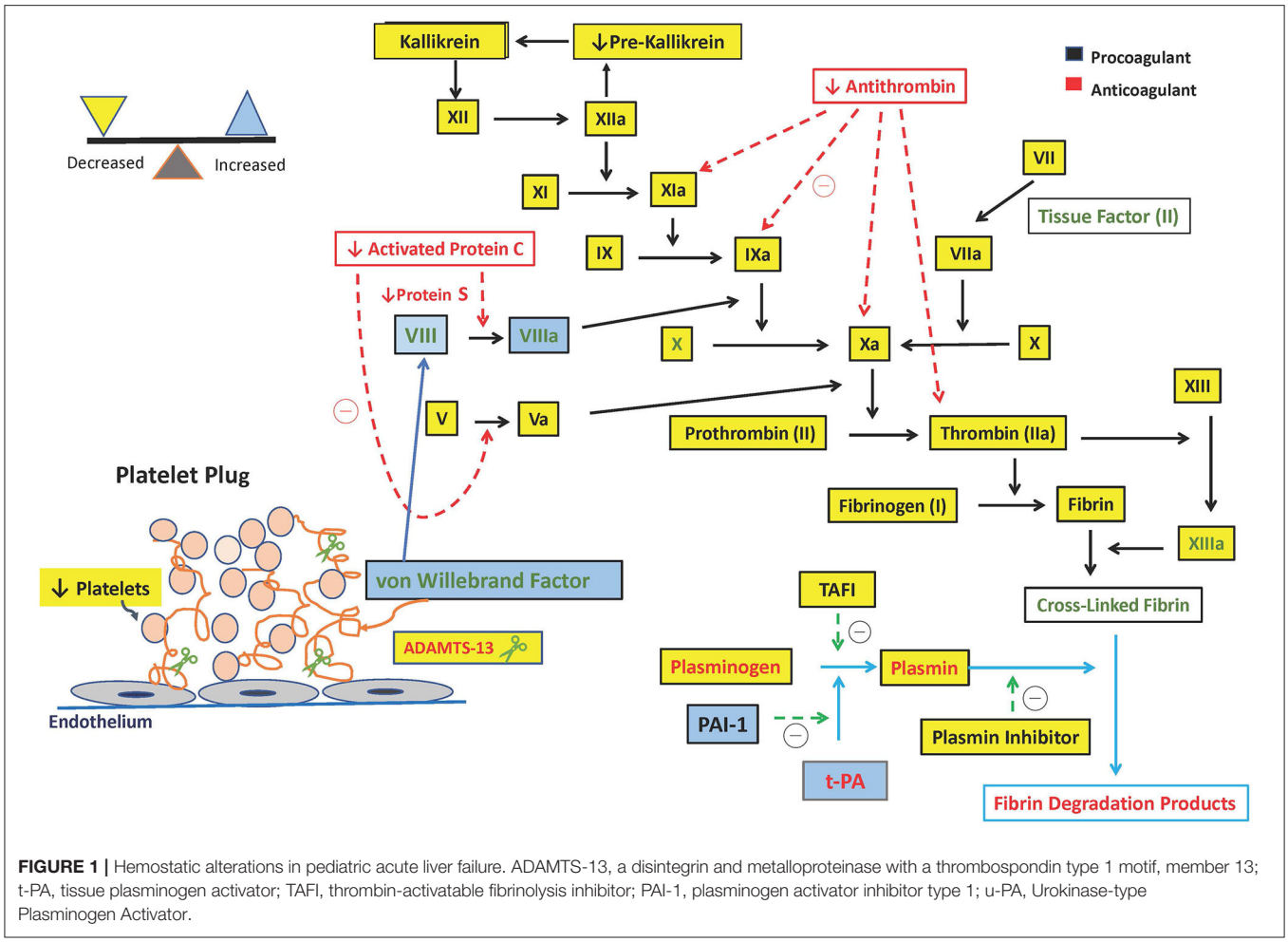


FIGURE 1 | Hemostatic alterations in pediatric acute liver failure. ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; t-PA, tissue plasminogen activator; TAFI, thrombin-activatable fibrinolysis inhibitor; PAI-1, plasminogen activator inhibitor type 1; u-PA, Urokinase-type Plasminogen Activator.

Secondary Hemostasis

Secondary hemostasis is characterized by the formation of an insoluble fibrin clot by activated coagulation factors and thrombin. Fibrin in turn stabilizes the primary platelet plug to stop the hemorrhage.

Reduction of coagulation factors is more extensive in acute liver failure compared to chronic liver failure, and factor levels can be as low as 1–10% of normal values (11, 21). Due to synthetic dysfunction of liver, there is reduced production of both pro-coagulant factors: V, VII, IX, X, XI, XIII, and prothrombin (II), and anti-coagulant factors: antithrombin, protein C, and protein S. Factor VIII is the only coagulation factor that is increased in PALF since Factor VIII is synthesized both in liver and in endothelial cells and is an acute phase reactant. In addition, activity of factor VIII is enhanced as a result of elevated levels of vWF, a protein that stabilizes Factor VIII in the circulation (25). These simultaneous and opposing pro- and anti-coagulant protein deficiencies results in a net rebalanced hemostasis.

Fibrinogen, an essential coagulation factor produced by the liver, it is the precursor of fibrin and is necessary for clot formation. Pediatric acute liver failure is accompanied by hypofibrinoginemia, though the reduction in fibrinogen levels is modest compared to the other pro-coagulant factors (2). Dysfibrinogenemia, or abnormal functioning of fibrinogen, is a result of increased content of sialic acid residues that leads to abnormalities of fibrin monomer polymerization (26, 27).

Vitamin K is essential for the synthesis of both pro-coagulant factors and anti-coagulant factors by the liver. Unlike in chronic liver failure, vitamin K deficiency is uncommon in PALF unless there is coexisting biliary tract disease or prolonged use of gut sterilizing broad-spectrum antibiotics. Deficiency has the potential to worsen the associated coagulopathy, and so differentiation between vitamin K-dependent and vitamin K non-dependent coagulopathy should be part of initial evaluation in PALF (28).

Fibrinolysis

Fibrinolysis is the process by which the body breaks down clots. Fibrinolysis limits the extent of thrombosis, begins clot degradation, and maintains vascular patency. It starts with conversion of plasminogen to plasmin by the pro-fibrinolytic drivers tissue Plasminogen Activator (t-PA), urokinase-type Plasminogen Activator (u-PA), and activated factor XII. At the same time these pro-fibrinolytic drivers are opposed by anti-fibrinolytic drivers such as Plasminogen Activator Inhibitor

TABLE 1 | Opposing effects of hemostasis in acute liver failure.

Hemostasis	Anti-hemostatic	Pro-hemostatic
Primary hemostasis: activated platelets and vessel wall interaction	↓Platelet Count ↓Platelet Function ↓Thrombopoietin ↑Nitric Oxide and Prostacyclin	↑ vWF ↓ ADAMTS-13
Secondary hemostasis/ coagulation: thrombin generation and inhibition	Low pro-coagulants factors ↓Factor II, V, VII, IX, X, XI, XIII ↓Fibrinogen Dysfibrinogenemia	Low anti-coagulant factors ↓Protein S and C ↓Antithrombin ↓Heparin Co factor ↑Factor VIII
Fibrinolysis: Fibrin removal	↓Plasmin Inhibitor ↓TAFI ↑t-PA	↓Plasminogen ↑PAI-1
Outcome	**BLEEDING**	**THROMBOSIS**
Treatment	Vit K, PCC, Factor VII, FFP, platelets, cryoprecipitate	Anticoagulation

vWF, von Willebrand factor; ADAMTS-13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; t-PA, tissue plasminogen activator; TAFI, thrombin-activatable fibrinolysis inhibitor; PAI-1, plasminogen activator inhibitor type 1; PCC, Prothrombin complex concentrate.

type 1 (PAI-1), Plasmin inhibitor, and Thrombin-Activatable Fibrinolysis Inhibitor (TAFI) (1).

In liver disease, increased levels of t-PA and reduced levels of plasmin inhibitor and TAFI favors hyper-fibrinolysis and bleeding. On the other hand, reduced levels of plasminogen and increased levels of PAI-1 favors hypo-fibrinolysis and clot formation. These competing factors result in rebalanced pro- and anti-fibrinolytic activity in liver failure. In acute liver failure, high PAI-1 levels and low plasminogen levels may be substantial enough to tip the balance toward a hypofibrinolytic state whereas in cirrhosis, fibrinolysis is usually normal or hyper-fibrinolysis is present (2, 9, 21, 25).

Hyperfibrinolysis can also be seen during the anhepatic phase of liver transplantation due to increased tPA derived from endothelial cells; this is secondary to failure of tPA clearance in the absence of the liver (29).

Differentiating DIC and liver mediated coagulopathy can be quite challenging due to overlapping laboratory abnormalities and possibility of both coexisting at the same time. Patient with ALF may develop DIC if they become septic and hypotensive. Fibrinolysis of DIC is accompanied by fibrinogenolysis and by thrombin generation. Presence of fibrin-related markers such as D-dimer, Fibrin Degradation Products (FDPs) which is an important index of fibrinogenolysis may help differentiate true DIC from liver associated coagulation dysfunction (30–32). Decreased Factor VIII levels can also be used to distinguish DIC from hepatic disease in acquired hypofibrinogenemic states (33).

In summary, contrary to the common belief that acute liver failure patients are "autoanticoagulated," they may be more prone to thrombosis rather than bleeding. There is a combination of (a) thrombocytopenia compensated with elevated levels of vWF, factor VIII and reduced ADAMTS-13, (b) a hypofibrinolytic state due to elevated PAI-1 and low plasminogen, and (c) elevated levels of pro-coagulant microparticles that may all lead to an increased risk of thrombosis and worsen outcome (23, 24).

LIMITATION OF STANDARD HEMOSTATIC TESTS

Current conventional laboratory tests such as PT/INR and partial thromboplastin time (PTT) have limitations for evaluating the coagulopathy of liver disease. Specifically, the PT/INR measures only procoagulant factors and omits the contribution of the platelets, *in vivo* inhibitors, fibrinolytic enzymes, and other cellular components. These tests do not provide information on actual fibrin formation or clot lysis. Lack of predictive power can be explained by the deficiency of anti-coagulant proteins which are reduced in parallel with pro-coagulant factors (34, 35).

The international normalization ratio (INR) is commonly used as a prognostic and decision-making tool when calculating the Pediatric End Stage Liver Disease Model (PELD) score which includes INR, bilirubin, albumin, and growth velocity of the child. International Normalized Ratio is a valuable biomarker for prognostication and determination of organ allocation; however it is inadequate for the measurement of bleeding risk. In children undergoing liver biopsy, hemorrhagic complications can occur in 0.91–4.2% of cases and coagulopathy markers do not predict bleeding complications (36, 37).

International Normalized Ratio calculation is derived from the Prothrombin Time (PT) and is standardized across laboratories among patients receiving vitamin K antagonists. Thrombomodulin is essential for PT measurement. Commercially available Prothrombin time tests do not contain a sufficient amount of thrombomodulin, and although the PT measures the function of pro-coagulants, it fails to measure the thrombin inhibited by the anticoagulants (1). Thrombin generation is downregulated by thrombomodulin, a transmembrane protein on endothelial cells which activates Protein C. Protein C is activated by thrombin and activated protein C forms a complex with its plasma co-factor, protein S and inhibits activated factor VIII and factor V that leads to less thrombin generation (**Figure 1**).

Furthermore, there is a significant laboratory to laboratory variation in the INR tests from patients with liver disease (10). One study demonstrated 26% variability in INR results among three laboratories, which could be problematic for treatment and organ allocation (3).

While standard assays of hemostasis are limited and misleading to evaluate hemostatic status in PALF, viscoelastic hemostatic assays (VHA) can accurately evaluate the current state of rebalanced hemostasis by measuring clot formation, ultimate clot strength, and the stability of the clot by integrating the contribution of platelets (8).

Viscoelastic hemostatic assays (VHA) such as thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are whole blood tests that are a functional measure of clot formation and degradation in real time. Viscoelastic hemostatic assays measure the time to initial fibrin formation, the rate of clot formation, the strength of the clot, clot lysis and the contribution of sepsis-induced heparinoids to coagulation abnormalities. Since clot formation is the endpoint, VHAs have an obvious advantage over thrombin generation tests which have

thrombin generation as the endpoint rather than formation of the fibrin clot (10, 14).

Both TEG and ROTEM are commercially available point-of-care tests of whole blood coagulation. For TEG, blood is placed into an oscillating cup and treated with calcium and a kaolin-cephalin reagent. As the blood starts to clot, the viscoelasticity changes, and the blood starts to exert force on a pin that is suspended in the blood. The torque on the pin is converted into an electrical signal that results in a graphical representation of the clot formation and strength over time. As fibrinolysis begins and the clot starts to degrade, the force on the pin decreases and the resulting graph shows the clot dissolving. ROTEM is performed similarly, but with blood placed into a stationary cup, and an oscillating pin suspended into the blood sample. ROTEM also offers the possibility to use different activator reagents to assess different components of coagulation independently (such as the intrinsic pathway, extrinsic pathway, heparinization, fibrinogen contribution, and fibrinolysis. For an example of a ROTEM graph, see **Figure 2**.

Similar to patients with cirrhosis, patients with ALF generally have TEG parameters within normal limits. It has been shown that despite the average INR values of 3.4 (1.5–9.6) TEG parameters are within normal limits for adult ALF patients (8). Data on the use of VHAs specifically in pediatric patients with acute liver failure are lacking, and so one must extrapolate from the adult literature.

Hemostasis in children is an evolving process; there are age-dependent differences in the quantity and quality of hemostatic proteins that may further complicate the diagnosis of coagulopathy in children (29). A recent review summarized the published reference ranges for VHA testing in pediatrics and found that clotting times and clot formation times were shorter in healthy infants <6 months of age, suggesting more rapid initiation of clotting in this youngest pediatric age group (38). Generally, however, there were no significant differences found between children older than 6 months and adults in the parameters assessed by the assays, suggesting that assessment of clotting by VHAs is age-independent for children >6 months and adults. Utilization of VHAs in PALF patients may therefore provide a more accurate and reliable measure guiding transfusion practices and minimize unnecessary blood product administration.

THERAPEUTIC IMPLICATIONS

The coagulopathy and bleeding risks in acute and chronic liver failure are significantly different. Since portal hypertension is not significant in PALF and the thrombocytopenia is mild, bleeding risk is usually less compared to chronic liver disease (12).

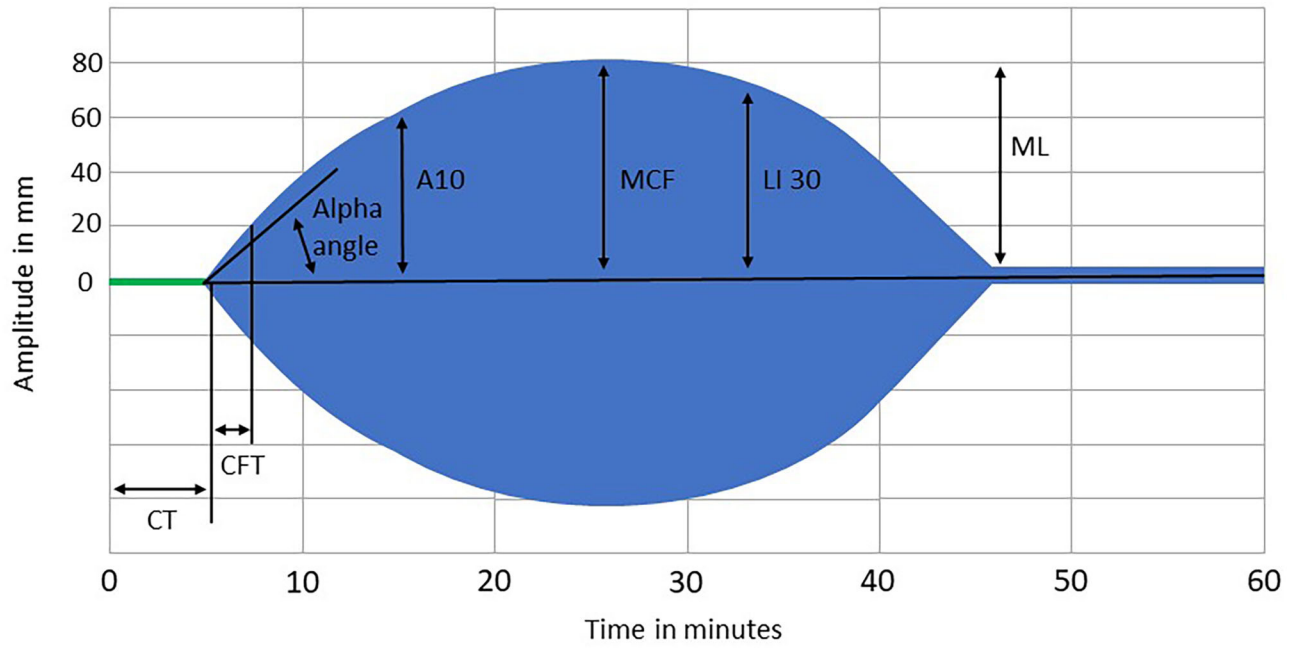
There are no transfusion guidelines available for PALF and this leads to the substantial use of prophylactic blood products. There are no current pediatric cutoff values for the use of prophylactic transfusions of plasma, yet in adults an INR value of >7 with TEG confirming marked prolongation of clot formation, FFP transfusion is advised to maintain an INR between 5 and 7 (8). In PALF prophylactic correction of coagulopathy with fresh frozen plasma or thrombocytopenia with platelet administration is not recommended and should be avoided since the spontaneous bleeding complications are relatively uncommon (9, 12). In addition, INR trend, which is a commonly followed prognostic indicator in PALF, will be obscured with plasma administration. The currently available coagulation tests, other than VHA tests, do not measure the risk of bleeding accurately. Relying solely on those tests to guide transfusions will lead to patients being exposed to unnecessary blood products, leading to risk of fluid overload, transfusion-related acute lung injury (TRALI), and thrombosis risk (8).

Viscoelastic hemostatic assay tests, in contrast, may be able to provide the clinician with a more accurate assessment of coagulopathy. TEG and ROTEM have been used to develop resuscitation guidelines in adult trauma patients (39–41) and their use has been shown to decrease transfusions and mortality (41). Unfortunately there are currently no such guidelines published for either adult or pediatric acute liver failure patients. The use of VHAs in PALF would therefore require as astute clinician, trained in the interpretation of either TEG or ROTEM, in order to appropriately determine when interventions such as plasma, cryoprecipitate, platelets, or antifibrinolytics would be indicated for the pediatric patient with acute liver failure who is either at risk for, or currently bleeding.

Although it is generally mild, thrombocytopenia is the most significant contributor to the coagulopathic state in acute liver failure (8). Correction of platelet count and coagulopathy for high-risk procedures, such as liver biopsy and intracranial pressure (ICP) monitor placement, should be considered (18, 42). There are no cut-off values for preprocedural correction of coagulopathy in PALF, however in a recent survey among the participants of the Coagulation in Liver Disease Symposium, 50% of respondents suggested a cut-off value of INR > 1.5 and platelets of >30,000/mm^3 for liver biopsy, and INR > 1.5 and platelet count of >50,000 mm^3 for intracranial pressure monitor placement (18, 19, 42).

Vitamin K and fibrinogen deficiencies may prolong the INR/PT values and should be corrected as well. Oral or parenteral vitamin K should be considered if deficiency is suspected (34, 35). Cryoprecipitate may be used to keep fibrinogen in the low-normal range (150 mg/dl) and can be adjusted according to TEG values. Cryoprecipitate is a plasma-derived, concentrated product containing fibrinogen, Factor VIII, Factor XIII, vWF, and fibronectin. One unit of cryoprecipitate per 5 kg of body weight will increase fibrinogen by about 100 mg/dL. There are also plasma-derived fibrinogen concentrates available for the repletion of fibrinogen, but they are currently only approved for use in congenital fibrinogen deficiency and may not be available at all centers.

Pre-procedure correction of coagulopathy can be accomplished with low volume products such as Prothrombin Complex Concentrate (PCC) and recombinant activated factor VII (rFVIIa) (43). Prothrombin Complex Concentrate is a plasma-derived product containing clotting factors II, VII, IX, and X that is currently approved for reversing vitamin K antagonists. It has a low volume of administration and higher concentration of factors compared to plasma, which makes it



CT	Clotting Time (time from start to amp. 2mm)
CFT	Clot Formation Time (time from amp. 2mm to amp. 20mm)
Alpha Angle	Alpha Angle (slope of the line at amp. 2mm)
A10	Amplitude at 10 Minutes after CT
MCF	Maximum Clot Firmness
LI 30	Lysis Index at 30 Minutes after CT (% drop in amp. from MCF)
ML	Maximum Lysis (% lysis at end of run)

FIGURE 2 | Rotational thromboelastometry (ROTEM).

an attractive alternative to FFP for factor repletion. There are several reports using PCC for patients with liver disease, some using a standard dose such as 25 units/kg, and others using an INR-based dosing regimen, similar to what would be used in vitamin K antagonist reversal (44). Caution is advised with administration of PCC given the lack of natural anticoagulants in this product compared to plasma, and the possibility of provoking thrombosis.

rFVIIa given at a dose of 20–40 mcg/kg, administered 30 min before the procedure has a rapid onset of action and a low volume of infusion making it another option for use prior to invasive procedures (42). However, administration of rFVIIa requires caution due to high cost and the risk of potentially serious thrombotic events (8, 45, 46).

Treatment goals in PALF should focus on acute bleeding management and prevention of infection, uremia, and GI bleeding, rather than prophylactic correction of laboratory values.

Antifibrinolytic agents such as aminocaproic acid and tranexamic acid (TXA) inhibit plasmin and prevent fibrin clot degradation. Although it has been shown that TXA may reduce blood loss during liver trauma or liver transplantation, there is no proven benefit for acute bleeding in PALF (46). These agents may be useful, however, in bleeding patients with documented hyperfibrinolysis seen on VHAs. These agents are frequently used in pediatric liver transplantation surgery at variable doses (29). A starting dose of TXA is typically 10 mg/kg given intravenously for bleeding. Similar to PCC and rFVIIa, caution should be taken with antifibrinolytics in patient with a history of thrombosis, and also any history of DIC or renal impairment.

Bacterial infections in ALF may potentiate the risk of bleeding, although the mechanism is speculative. Endotoxins and cytokines produced during infection may induce disseminated intravascular coagulation (DIC), inhibit platelet function, and enhance the effects of nitric oxide. Therefore, appropriate antibacterial prophylaxis and treatment of known infection is recommended (13).

Renal failure is also a common complication of acute liver failure. Uremia may further hinder primary hemostasis

due to platelet dysfunction and impaired platelet-vessel wall interaction (47). Prevention and treatment of superimposed infections and renal failure are essential in order to prevent bleeding complications.

Clinically significant gastrointestinal bleeding is rare in PALF, however the American Association of Study of Liver Disease (AASLD) guidelines recommend histamine (H2) blockers or proton pump inhibitors for prophylaxis of gastric bleeding in ICU setting (8, 34, 48).

There are no guidelines for the resuscitation of acute bleeding in acute liver failure and transfusion of blood products have been associated with increased morbidity (48). Restrictive transfusion strategy with a threshold hemoglobin of >7 g/dL with a post-transfusion target of 7–9 g/dL are frequently utilized in a hemodynamically stable patient (15, 25, 42).

A catastrophic bleeding in PALF is very rare, most bleeding complications are clinically insignificant. Spontaneous bleeding usually due to self-limited upper gastrointestinal bleeding or post-procedural bleeding due to ICP placement (35). In case of catastrophic bleeding utilization of massive transfusion protocol may be warranted. Depending on patient laboratory values, a decisions can be made to give platelets, FFP, cryoprecipitate accordingly. If there is continues bleeding despite appropriate replacement of blood products, then PCC, of rFVIIa and TXA can be utilized.

SUMMARY AND FUTURE DIRECTIONS

Hemostasis in pediatric acute liver failure exists in a rebalanced state due to concomitant changes in pro- and anti-coagulant mechanisms. This rebalanced hemostasis and lack of additional risk factors for bleeding (such as esophageal varices and portal hypertension that are commonly seen in chronic liver failure), leads to a low rate of spontaneous significant bleeding in PALF.

Current screening coagulation tests, such as the INR and PT are not predictive of acute bleeding and have certain limitations. International Normalized Ratio is useful for assessing severity of disease, however its use to evaluate one's bleeding risk is limited. Prophylactic FFP transfusion has not been shown reduce the risk of spontaneous bleeding and is not recommended in PALF. Arbitrary FFP and blood product transfusions may harm the patient with increased risks for fluid overload, cerebral edema, and elevated intracranial pressure. In addition, they will obscure the INR trend as a prognostic marker. Coagulopathy should be carefully evaluated and treated prior to high-risk procedures. Utilization of viscoelastic tests in PALF patients may provide an accurate assessment of bleeding risk, prevent unnecessary administration of blood products, lessen complications, and improve outcome. Further studies are necessary to characterize the coagulopathy in children with acute liver failure and develop treatment guidelines.

Understanding the pathophysiology of coagulopathy in PALF is important when evaluating pros and cons of transfusion and maintaining the delicate hemostatic balance. Etiology of PALF is different from adults, and there are age specific differences in pro-coagulant and anti-coagulant factors. It is important to maintain the fragile balance of hemostasis with restrictive transfusion strategies, and prevention of infection and uremia.

AUTHOR CONTRIBUTIONS

YB prepared the initial draft. GR and AS revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 08 January 2021
doi: 10.3389/fped.2020.606643

Hemostatic Testing in Critically Ill Infants and Children

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Children with critical illness frequently manifest imbalances in hemostasis with risk of consequent bleeding or pathologic thrombosis. Traditionally, plasma-based tests measuring clot formation by time to fibrin clot generation have been the "gold standard" in hemostasis testing. However, these tests are not sensitive to abnormalities in fibrinolysis or in conditions of enhanced clot formation that may lead to thrombosis. Additionally, they do not measure the critical roles played by platelets and endothelial cells. An added factor in the evaluation of these plasma-based tests is that in infants and young children plasma levels of many procoagulant and anticoagulant proteins are lower than in older children and adults resulting in prolonged clot generation times in spite of maintaining a normal hemostatic "balance." Consequently, newer assays directly measuring thrombin generation in plasma and others assessing the stages hemostasis including clot initiation, propagation, and fibrinolysis in whole blood by viscoelastic methods are now available and may allow for a global measurement of the hemostatic system. In this manuscript, we will review the processes by which clots are formed and by which hemostasis is regulated, and the rationale and limitations for the more commonly utilized tests. We will also discuss selected newer tests available for the assessment of hemostasis, their "pros" and "cons," and how they compare to the traditional tests of coagulation in the assessment and management of critically ill children.

Keywords: pediatric, developmental hemostasis, diagnosis, coagulopathy, viscoelastic

OPEN ACCESS

Edited by:
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Royal Children's Hospital, Australia

Reviewed by:
Genny Raffaeli,
IRCCS Ca 'Granda Foundation Maggiore Policlinico Hospital, Italy
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *15 September 2020*
Accepted: *10 December 2020*
Published: *08 January 2021*

Citation:
Nair AB and Parker RI (2021) Hemostatic Testing in Critically Ill Infants and Children. Front. Pediatr. 8:606643. doi: 10.3389/fped.2020.606643

INTRODUCTION

Hemostatic dysfunction and resultant pathologic bleeding and/or thrombosis is a common complication of critical illness in children. There are several important considerations of this dysfunction that are imperative for accurate diagnosis and clinical management in the pediatric population. First, the hemostatic system changes and develops from birth to infancy, childhood, adolescence, and ultimately adulthood. Understanding this evolution is important in the interpretation of physiologic and pathologic hemostasis. Second, traditional tests of hemostasis have focused on platelet count and plasma-based measurements of clot formation. There are limitations in this traditional approach including lack of consideration for (1) platelet function and activity, (2) the imperfect sensitivity of plasma-based tests, particularly to abnormalities in fibrinolysis and conditions of enhanced clot formation, (3) the critical role of the endothelium in hemostasis and crosstalk between the endothelial, inflammatory, and coagulation systems, and (4) the stages of clot formation and lysis over time. Newer studies address some of these challenges by directly measuring thrombin generation in plasma and assessing the stages of hemostasis in whole blood. Successful diagnosis and management of hemostatic dysfunction in critically ill children

requires both an age-dependent understanding of clot formation and regulation of hemostasis as well as of the strengths and weaknesses of the tests employed.

HEMOSTASIS AND THE REGULATION OF CLOT FORMATION

Hemostasis is the normal physiologic process by which blood is maintained in fluid state while also allowing for blood clot formation at the site of injury maintaining the integrity of the closed circulatory system after vascular damage (1). It is a highly complex series of interwoven processes that result in a rapid, localized, and highly regulated response. Key elements of hemostasis include (1) formation of the platelet plug (referred to as primary hemostasis), (2) soluble phase coagulation with propagation of clotting through the clotting cascade, (3) termination of clot formation, and (4) clot dissolution (fibrinolysis). Under physiologic conditions, a clot is formed at the site of injury to stop bleeding locally with clot lysis and tissue remodeling to follow (1). Abnormalities in any part of these processes can result in dysfunctional hemostasis manifest as abnormal bleeding or thrombosis.

The hemostatic process starts with injury to the vascular endothelium (2). The undisturbed endothelium has multiple guards against undesired coagulation by functioning to counteract platelet activation and aggregation, and to maintain blood fluidity (2). When the integrity of the endothelium is compromised, exposure of subendothelial elements trigger thrombus formation (3). The endothelium also responds to injury through vasoconstriction which results from impairment of the vasodilatory action of damaged endothelial cells as well as direct access of smooth muscle cells to locally generated vasoconstrictive agents (3).

Platelet activation and adhesion upon exposure to vascular injury occurs in a highly coordinated method involving tethering, rolling, activation, and firm adhesion (4, 5). The initial interactions between platelets and the extracellular matrix are highly dictated by local rheological conditions (4). Platelet activation is triggered by several highly adhesive macromolecules including collagen and von Willebrand factor (vWf) along with the weaker agonists adenosine diphosphate (ADP) and epinephrine (4–6). This activation is also triggered by thrombin (4, 5). As part of the platelet activation process, platelets undergo shape change and develop elongated pseudopods that aid in the subsequent adhesion process (7). Platelet adhesion is primarily mediated by attachment of the platelet surface receptor GPIb/IX/V complex to vWf in plasma and the subendothelial matrix (7). Further, platelet activation results in exposure and conformational change of the GPIIb/IIIa receptor on the platelet surface. The GPIIb/IIIa receptor then binds vWf and fibrinogen ultimately resulting in platelet-platelet cohesion (8, 9).

After platelet binding to subendothelial structures and subsequent signaling to the platelet cytoplasm, platelet granules fuse with the open canalicular system of the platelet membrane and empty their contents into the local environment. The paracrine and autocrine nature of these bioactive contents causes increased activation of nearby platelets in both number and degree (4). This results in secondary secretion and significant amplification of the platelet activation and adhesion processes. Alpha (α)granules contain a heterogeneous complement of proteins that effect multiple biologic systems beyond primary hemostasis and coagulation including inflammation, angiogenesis, wound healing, and others (4). Important to formation of the platelet plug is the α-granule release of vWf which further increases formation of the platelet scaffold via GPIIb/IIIa and GPIb/IX/V as well as release of fibrinogen which crosslinks with GPIIb/IIIa and provides an additional source of fibrinogen to what is present in the plasma (4, 10). Dense (δ)-granules secrete ADP and serotonin which stimulate and recruit additional platelets (11). Finally, platelet procoagulant activity is an important facet of platelet activation. This procoagulant activity includes both exposure of procoagulant phospholipids such as phosphatidylserine and the ensuing formation of enzyme complexes on the platelet surface essential to the clotting cascade (12).

The soluble phase of clotting primarily involves the clotting cascade (**Figures 1**, **2**) (13). In general, the clotting cascade involves successive activation of a series of proenzymes or zymogens into active enzymes resulting in an incremental but amplified clotting response. The clotting cascade is classically described by the intrinsic, extrinsic, and common pathway, though further investigation has revealed these pathways to be much more complex and interrelated (**Figure 1**) (13).

The clotting cascade is initiated through the extrinsic pathway and starts with exposure of tissue factor (TF) at the site of injury. Blood is exposed to TF either directly by the subendothelial matrix or by cytokine-induced expression on endothelial cells or activated monocytes (14, 15). Platelets may also generate their own TF in proximity to the injured vessel (16). TF operates as a cofactor in the activation of factor VII and together these two factors form the extrinsic tenase multimeric complex which goes on to activate factors X and IX to factors Xa and IXa, respectively (17, 18).

The intrinsic or contact activation pathway is initiated through the interaction of negatively charged surfaces resulting in the activation of factor XII, high-molecular-weight kininogen, and plasma kallikrein among others (13). Activated factor XII in conjunction with high-molecular-weight kininogen activate factor XI which then activates factor IX (19). Activated factor IX forms a multimeric complex with activated factor VIII, which has been activated by factor X and thrombin produced in the extrinsic pathway. This multimeric complex, referred to as intrinsic tenase, subsequently activates sufficient factor X for clot formation (13, 19). This process is amplified because (1) factor VIII is activated by both activated factor X and thrombin and (2) activated factor IX is further activated by the thrombin-induced activation of factor XI (19, 20). As a result, there is a progressive increase in factor VIII and factor IX activation as factor Xa and thrombin are formed. Through these mechanisms, sustained and amplified generation of thrombin is achieved through the intrinsic pathway. Of note, while more activated factor X is generated through the intrinsic pathway due to amplifying steps

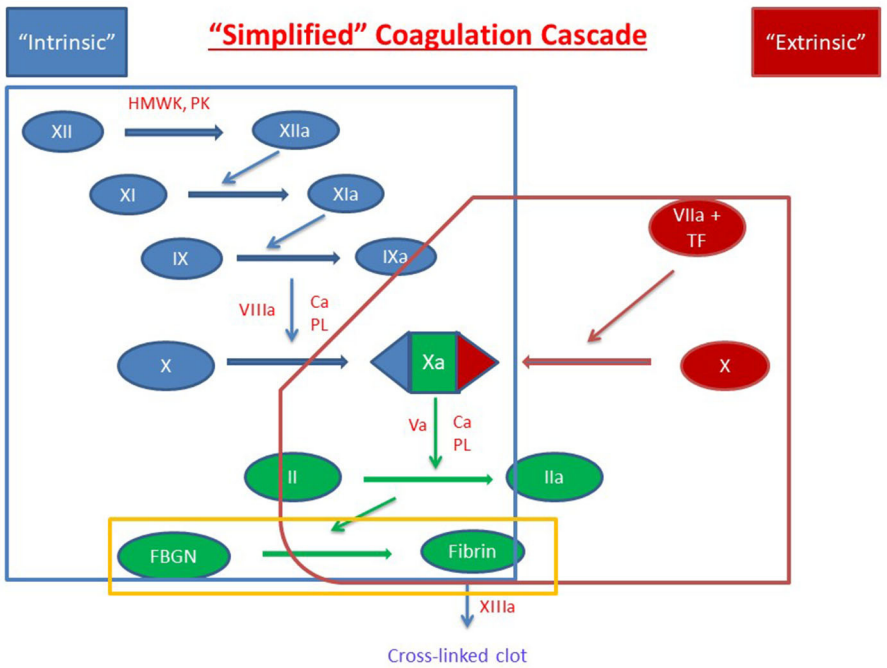

FIGURE 1 | Simplified Coagulation Cascade. Clot formation as activated through the extrinsic pathway (red), the intrinsic pathway (blue) and the common pathway (green). Components of these pathways are measured by prothrombin time, activated partial thromboplastin time, and thrombin time are circled in red, blue, and yellow, respectively. Factors are indicated with a roman numeral and an "a" if activated. Abbreviations: Ca^{2+}, calcium ions; FBGN, fibrinogen; HMWK, high-molecular-weight kininogen; PL, platelet membrane phospholipid; PK, prekallikrein; TF, tissue factor.

in the cascade, the extrinsic pathway is physiologically more important clinically (21).

From there, both the extrinsic and intrinsic pathway proceed to the common pathway. Factor V, which is released from platelet α-granules, is cleaved by thrombin to form activated factor V (22, 23). Activated factors X and V bind on the platelet phospholipid surface to form the prothrombinase complex which converts prothrombin (factor II) to thrombin (activated factor II, factor IIa) (24). Thrombin then converts fibrinogen to fibrin and activated factor XIII crosslinks overlapping fibrin stands which leads to stabilization of the clot (25).

Intrinsic to the built-in amplification systems of formation of the platelet plug and the coagulation cascade, hemostasis also requires highly regulated mechanisms of control of clot extension and termination of clot formation (**Figure 2**). Systemic control of this localized response is modulated through several mechanisms including (1) dilution of procoagulants in the bloodstream, (2) elimination of activated factors through the reticuloendothelial system, and (3) control through antithrombotic pathways anchored on vascular endothelial cells (1). Physiologic inhibitors of coagulation include tissue factor pathway inhibitor (TFPI) which inhibits TF-mediated and factor VIIa-mediated factor X activation, and C1 esterase inhibitor which inhibits activated factor XII, plasma kallikrein, and several complement proteases (26, 27).

Cessation of clot formation is critical in maintaining a targeted response and mediating the extent of the clot. Clot termination involves two key components, antithrombin (antithrombin-III, AT) and the protein C pathway. AT is a serine protease inhibitor that neutralizes many enzymes in the clotting cascade by irreversibly binding to them (28). Its function is enhanced by endogenous heparins and heparan sulfate, which induce a conformational change and increase its affinity for thrombin approximately 300-fold (28–30). Vascular endothelial cells are coated with activated AT and therefore equipped to rapidly inactivate any excess generated thrombin (29, 30). The protein C pathway is initiated through the binding of thrombin to the endothelial membrane-expressed thrombomodulin which induces the ability of the thrombomodulin-thrombin complex to activate protein C (31, 32). In association with protein S, activated protein C then proteolytically inactivates activated factors V and VIII (33, 34).

Also important to the limitation of clot formation is the regulation of vascular and platelet reactivity which is primarily modulated through prostacyclin, thromboxane, and nitric oxide. Specifically, undisturbed endothelial cells adjacent to endothelial injury release arachidonic acid which is subsequently converted to thromboxane A2 by cyclooxygenase-1 in platelets and prostacyclin via cyclooxygenase-1 on the endothelium (35). Prostacyclin blocks platelet aggregation and counteracts thromboxane A2-mediated vasoconstriction (36). In addition to its vasodilatory effects, nitric oxide inhibits platelet adhesion and aggregation (37). In fact, platelets may enhance their synthesis of nitric oxide in the setting of platelet adhesion to collagen

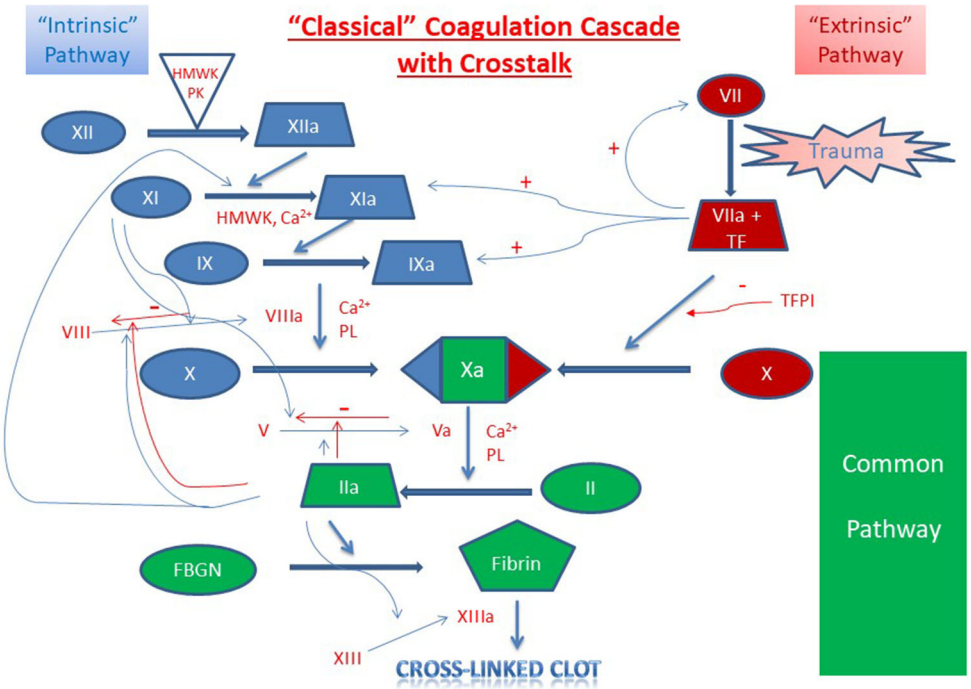

FIGURE 2 | Classical Coagulation Cascade with Crosstalk. Clot formation as activated through the extrinsic pathway (red), intrinsic pathway (blue) and the common pathway (green). Crosstalk and feedback between pathways is shown in blue if positive and red if negative. Factors are indicated with a roman numeral and an "a" if activated. Abbreviations: Ca^{2+}, calcium ions; FBGN, fibrinogen; HMWK, high-molecular-weight kininogen; PL, platelet membrane phospholipid; PK, prekallikrein; TF, tissue factor; TFPI, tissue factor pathway inhibitor.

providing negative feedback to limit excessive platelet adhesion and vasoconstriction at the site of injury (38).

Clot removal following hemostasis is achieved through fibrinolysis. Central to this process, plasmin is formed from the cleavage of plasminogen by bound fibrin and tissue-type plasminogen activator (38, 39). Urokinase is a secondary plasminogen activator, primarily acting in the extravascular compartment (39). Once formed, plasmin cleaves fibrinogen, fibrin, activated factor XIII, as well as a variety of plasma proteins and other clotting factors (39–41). Fibrinolytic activity can be generated either on the surface of the fibrin-containing thrombus or on cells that express profibrinolytic receptors (38). Plasmin activity is regulated by vascular endothelial cells that secrete both serine protease plasminogen activators and plasminogen activator inhibitors (42). Fibrinolysis works in conjunction with wound healing and tissue remodeling to restore vessel patency following injury.

The above discussion focuses primarily on plasma proteins with only limited mention of the role cellular components play in hemostasis. Indeed, a major limitation of plasma-based coagulation tests is that they do not take into consideration the myriad roles platelets, endothelial and leukocytes play in this process (43). Platelets are recruited to areas of vascular injury and adhere to endothelial cells and subendothelial structures (e.g., collagen) via specific receptors on these structures, a process augmented through binding to von Willebrand factor (vWf), thereby forming a platelet plug (**Figure 3A**). As noted, endothelial cells are critical in modulating the balance between activation of coagulation and fibrinolysis mediated through the binding of thrombin to thrombomodulin (**Figure 3B**). Additionally, platelet-neutrophil interactions are important in the formation of Neutrophil Extracellular Traps (NETs) and the process of NETosis which can induce a process referred to as immunothrombosis (**Figure 4**) (44, 45).

DEVELOPMENTAL HEMOSTASIS

Developmental hemostasis describes the physiologic changes that occur with an increase in age. These changes are particularly relevant in the pediatric population from birth through infancy when plasma levels of several important procoagulant and anticoagulant factors rapidly increase or decrease to normal adult levels. Because of the dynamic development of the hemostatic system, an understanding of age-specific physiology and quantification of normal reference ranges for coagulation parameters is of particular importance in pediatric medicine.

Early study of developmental hemostasis included the establishment of reference ranges for basic global measures of coagulation in infants using cord blood (46, 47). Andrew et al. were the first to define the field of developmental hemostasis in a series of articles describing reference values for coagulation parameters in (1) healthy term infants from birth to 6 months of age, (2) healthy preterm infants from birth to 6 months of age, and (3) healthy children and adolescents between 1 and 16

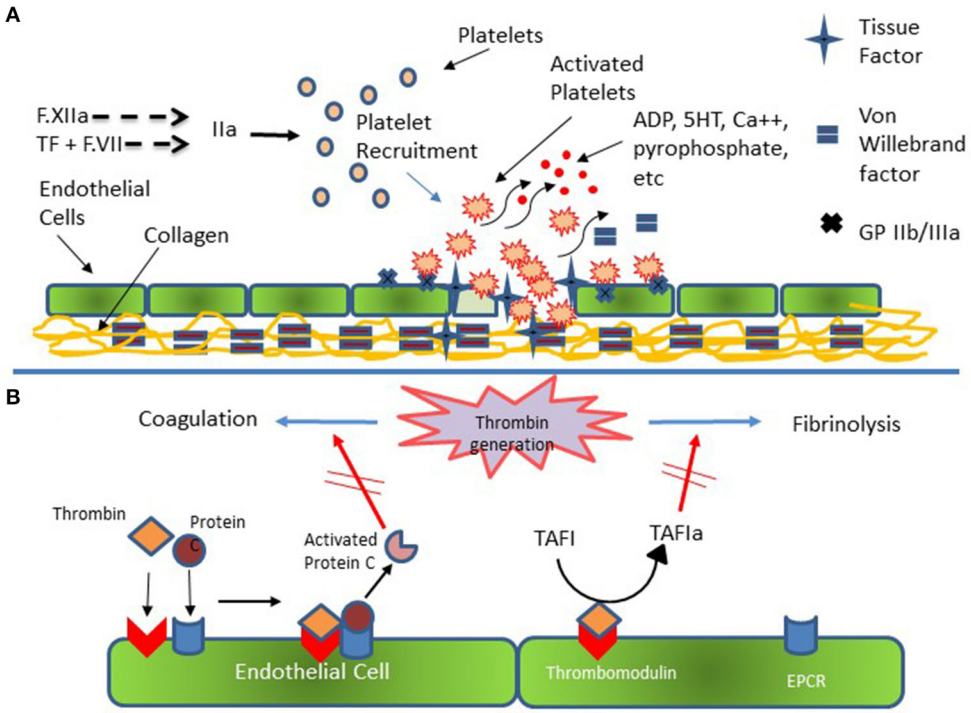

FIGURE 3 | Platelet and Endothelial Cell Interactions in Hemostasis. **(A)** Injured endothelial cells elaborate tissue factor (TF) with consequent activation of the coagulation cascade and generation of thrombin (F.IIa). Thrombin activates platelets which subsequently adhere to injured endothelial cells and to subendothelial collagen via specific receptors on endothelial and platelet membrane and via von Willebrand [vWf]. Additional platelets are recruited to the area of injury with subsequent formation of platelet plug. **(B)** Thrombin generation results in activation of coagulation and fibrinolysis. Thrombin bound to endothelial cell surface thrombomodulin catalyzes the conversion of Protein C bound to endothelial cell protein C receptor (EPCR). Activated Protein C then down regulates further thrombin formation by inactivating activated F.VIII and activated F.V. Additionally, Thrombomodulin bound thrombin catalyzes Thrombin Activatable Fibrinolysis Inhibitor (TAFI) to its active form (TAFIa) which damps the activation of fibrinolysis thereby promoting clot formation and stability.

years of age (**Table 1**) (48–50). This work defined normal values of global measures of coagulation, individual factors that facilitate clot formation, and inhibitors of coagulation (**Table 2**).

Overall, Andrew et al. found that coagulation tests varied with age and different parameters showed different patterns of maturation (**Tables 1**, **2**) (48–50). At birth, plasma levels of many important coagulation factors were found to be around half of that found in adults. Full maturation to adult levels of these factors varied between a few months to 16 years of age (48–50). While there were continued increases in some factors through later childhood, most of these factors reached near normal adult levels by 6 months of age. Specifically, in both term and preterm infants the mean values for the vitamin K-dependent factors, contact factors, and selected inhibitors including antithrombin (AT), protein C and protein S were well-below adult values. In contrast, fibrinogen, factors V, VIII, and XIII, vWf and the inhibitors α_1-antitrypsin, α_2-antiplasmin, α_2-macroglobulin, and C_1 esterase inhibitor were noted to be at normal or somewhat supra-normal adult levels at birth. Globally, both prothrombin time (PT) and activated partial thromboplastin time (aPTT) were prolonged in neonates. While there was wide variation in normal PT in infancy, mean PT normalized to adult levels by 1 month of age whereas aPTT normalized by 6 months of age (48, 49). Differences between preterm and term infants tend to be present but relatively small (48, 49). By 6 months of age both preterm and full term infants exhibit equivalent levels of all but four components of the coagulation system, namely factor VIII (mean 0.99 in preterm compared to 0.73 in term), plasminogen (mean 2.75 in preterm compared to 3.01 in term), antithrombin (mean 0.9 in preterm compared to 1.04 in term), and heparin cofactor II (mean 0.89 in preterm compared to 1.2 in term) (49).

Subsequent studies went on to confirm the highly selective pattern of maturation of the coagulation system described by Andrew et al. using different analyzer and reagent combinations (**Table 3**) (51–55). Compared to Andrew et al. these studies report the same trends by age and value relative to adult values but establish different reference intervals due to use of different equipment and reagents instead of the manual methods employed by Andrew (51–55). In addition to updated analysis techniques, several studies have expanded the panel hemostatic markers (**Table 3**) (51–53). Flanders et al. described differences by sex in protein S levels with females having higher than adult values at all ages and males having higher than adult values only in the 16–17 year old age group (101–103% in females 7–17 years old vs. 98% in female adults; 116% in males 16–17 years old vs. 108% in male adults) (51).

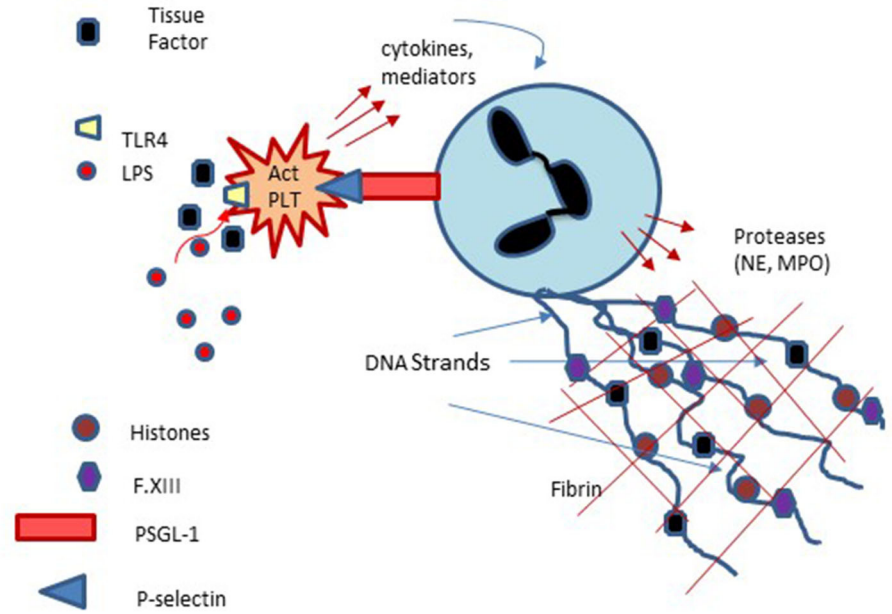

FIGURE 4 | Platelet involvement in NETosis and Immunothrombosis: Platelets interact with neutrophils via platelet membrane expressed P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) expressed on neutrophils with subsequent enhancement of neutrophils, extrusion of neutrophil DNA and formation of Neutrophil Extracellular Traps (NETs) which can serve as a nidus of thrombus formation. Platelet activation via lipopolysaccharide (LPS) binding to platelet Toll-like receptors may also participate in NET formation and NETosis.

Monagle et al. described elevated D-dimer, reduced TFPI, and reduced endogenous thrombin potential in healthy neonates and children compared to established adult values (52). Appel et al. described variation in development of vWf and factor VIII across blood groups, specifically (1) a higher median vWf antigen in non-O compared to O groups at 1–5 years old (97 vs. 71%) and >19 years old (118 vs. 99%), (2) a higher median ristocetin cofactor activity in non-O compared to O groups at 1–5 years old (82 vs. 66%) and >19 years old (106 vs. 82%), and (3) a higher median factor VIII in non-O compared to O groups at 1–5 years old (121 vs. 100%) and >19 years old (131 vs. 116%) (53). Attard et al. combined the results of several cohorts of infants at different ages and found maturation patterns that mirrored prior studies along with a high level of interindividual variability in protein levels (54). Finally, Toulon et al. looked across seven centers and found values that were not significantly different between centers when using the same analysis techniques and reagents (55). In contrast to other reports in which primarily activity of clotting proteins is measured, Attard tracks changes in antigenic levels of hemostatic factors over age (54). It is assumed that the relationship between antigen and activity is (relatively) fixed and that a decrease in antigen implies a commensurate decrease in activity with clinical decisions being made accordingly. However, one cannot rule out the possibility that changes in antigenic level or protein activity noted with age reflect an element of post-translational protein modification.

Taken together, these studies show that most coagulation parameters are highly dependent on age and undergo the greatest changes during the 1st year of life. These changes require the use of age-specific reference intervals in the clinical assessment of hemostasis and the diagnosis of pediatric bleeding and thrombotic disorders. Despite these age-dependent differences, the changing coagulation system throughout infancy, childhood, and adolescence should be viewed as physiologic, i.e., "balanced," with decreased factor levels and prolonged PT and aPTT in infants compared to normal adolescents and adults not necessarily reflecting an increased risk for hemorrhage. In fact, the pediatric hemostatic system may favor a reduced risk of thrombosis without an increased risk of bleeding (52, 56). While generation of thrombin in infants has been shown to be less than that in adults, this is counterbalanced by the reduction in AT and the fact that that the major physiologic inhibitor of thrombin in infants, α_2-macroglobulin, is less efficient than is AT. However, other studies have shown that thrombin generation (endogenous thrombin potential; ETP) is "normal" in infants if measured in the presence of added thrombomodulin and that thrombin generation in very pre-term newborns is not different than that in near term infants (57, 58). That infants exhibit a "balanced" hemostatic system is supported by clinical findings such as decreased thromboembolic disease following surgery or prolonged immobilization in children compared to adults with similar risk factors (52, 59, 60). That said, the molecular basis underlying age-related numerical changes in coagulation measures as well as the clinical manifestation of these changes is not fully understood. Animal studies have demonstrated structural differences in coagulation proteins in newborn compared to adult models but this work has yet to be translated to humans (61). Further translational study is necessary to elucidate

TABLE 1 | Study details of the Andrew et al. series describing the developing hemostatic system during infancy, childhood, and adolescence (45–47).

Year	Study details	Coagulation parameters	Notable findings
1987 (45)	• Study population: Consecutively born term infants (37–42 weeks) at a single center over a 3-month period • Sample size: 118 total infants, 61–77 at each study timepoint • Timepoints: Day 1, 5, 30, 90, and/or 180 after birth • Relevant methods: ACL analyzer, chromogenic assays, ELISA	All studies: • Global: aPTT, fibrinogen, PT • Coagulation: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIIIa, FXIIIb, HMWK, PK, vWf • Fibrinolysis: α_2-AP, plasminogen • Inhibitors: α_1-AT, α_2-M, AT, C1-INH, HCII, protein C, protein S	General findings: • Normal values from birth to 6 months old in the healthy term infant and preterm infant, and from 1 to 16 years old in the child and adolescent were determined • Coagulation tests varied with age and different parameters showed different patterns of maturation Selected specific findings: • Global: • aPTT was prolonged at birth, remained prolonged throughout the postnatal period in preterm infants, and reached adult levels by 3 months in term infants • PT demonstrated variability at birth and shortened to adult levels by 1 month in both term and preterm infants • Despite an aPTT and PT similar to adults, BT was prolonged in children and adolescents at all ages compared to adults
1988 (46)	• Study population: Preterm infants (born at 30–36 weeks) at a single center over a 3.25-year period • Sample size: 137 total infants, 40 to 96 at each study timepoint • Timepoints: Day 1, 5, 30, 90, and/or 180 after birth • Relevant methods: ACL analyzer, chromogenic assays, ELISA	Term/preterm infant studies: • Global: TT Children/adolescents study: • Global: BT, INR • Fibrinolysis: PAI, TPA	• Coagulation: • Vitamin K-dependent factors (FII, FVII, FIX, FX, protein C, and protein S) were low throughout the postnatal period in term and preterm infants as well as in children and adolescents through age 16 compared to adults • FXI, FXII, HWMK, and PK were lower in term and preterm infants in the postnatal period compared to adults • FXI and FXII decreased at 11 to 16 years old compared to adults • FV decreased throughout childhood and was lowest in adolescence • FVIII and vWf were similar in term and preterm infants and reached near or above adult values by 6 months • Factor XIIIb was elevated in early childhood and decreased with age
1992 (47)	• Study population: Healthy children and adolescents (1–16 years old) at a single center over a 2-year period • Sample size: 246 total patients, 4–7 at each age • Relevant methods: ACL analyzer, chromogenic assays, and ELISA		• Fibrinolysis: • Plasminogen gradually rose to near adult levels by 6 months in term infants but remained persistently low in preterm infants • In children and adolescents, TPA levels were low and PAI levels were increased compared to adults • Inhibitors: • All inhibitors were low at birth in term and preterm infants but rose to or near to adult levels by 6 months of age • In preterm infants, α_2-M which was above adult levels at birth and rose to twice adult levels by 6 months of age • Protein C and S remained below adult levels until adolescence

aPTT, activated partial thromboplastin time; α_1-AT, α_1-antitrypsin; α_2-M, α_2-macroglobulin; α_2-AP, α_2-antiplasmin; AT, antithrombin or antithrombin III; BT, bleeding time; C1-INH, C1 inhibitor or C1 esterase inhibitor; ELISA, enzyme-linked immunosorbent assay; F, factor; INR, international normalized ratio; HCII, heparin cofactor II; HMWK, high-molecular-weight kininogen; PK, prekallikrein; PAI, plasminogen activator inhibitor; PT, prothrombin time; TPA, tissue plasminogen activator; TT, thrombin clotting time; vWf, von Willebrand factor.

the important connections between coagulation parameters and clinical thrombotic and bleeding risk.

KEY LABORATORY TESTS OF HEMOSTASIS

Evaluation of abnormalities in pediatric hemostasis requires (1) knowledge of different hemostatic testing options, (2) an understanding of the strengths and weaknesses of each test, and (3) an awareness of utility in the pediatric population, particularly in the context of the developmental hemostasis consideration discussed above. The following are key tests of pediatric hemostasis with specific focus on platelet evaluation, clotting assays, and global measures. Logistics of testing as well as strengths and weaknesses are summarized in **Table 4**.

Platelet Evaluation

While the important role platelets play in the initiation and regulation of clot formation has been recognized for over 100 years, the ability to measure how well-platelets function in these processes remains limited (59). Initial evaluations were confined to determining platelet number and morphology in peripheral blood and did not provide any specific information regarding platelet function (62). However, with the development of light transmission aggregometry (LTA), direct assessment of how platelets respond and aggregate to agonists *ex vivo* was possible (63). While admittedly non-physiologic, this methodology has remained the cornerstone of platelet function assessment for the past 60 years (discussed further below).

Platelet enumeration can be performed in a variety of ways and remains a first-line screening test in platelet evaluation. In

TABLE 2 | Mean values and 95% confidence intervals of key hemostasis measures in term infants, preterm infants, children, and adolescents compared to normal adult values based on the Andrew et al. series (46–48).

Test	GA	1 day	30 days	180 days	1–5 years	6–10 years	11–16 years	Adult
PT (s)	Term	13.0 (12.6–13.4)	11.8 (11.5–12.1)	12.3 (12.1–12.5)				12.4 (10.8–13.9)$^{+}$
	Preterm	13 (10.6–16.2)	11.8 (10–13.6)	12.5 (10–15)				
					11 (10.6–11.4)	11.1 (10.1–12.1)	11.2 (10.2–12)	12 (11–14)$^{++}$
aPTT (s)	Term	42.9 (41.4–44.4)$^{*\#}$	40.4 (38.6–42.2)*	35.5 (34.4–36.6)				33.5 (26.6–40.3)$^{+}$
	Preterm	53.6 (27.5–79.4)$^{*\#}$	44.7 (26.9–62.5)*	37.5 (21.7–53.3)				
					30 (24–36)	31 (26–36)	32 (26–37)	33 (27–40)$^{++}$
TT (s)	Term	23.5 (22.9–24.1)	24.3 (23.6–25)	25.5 (24.6–26.4)				25 (19.7–30.3)
	Preterm	24.8 (19.2–30.4)	24.4 (18.8–29.9)	25.2 (18.9–31.5)				
Fibrinogen (g/L)	Term	2.83 (2.68–2.98)$^{\#}$	2.7 (2.57–2.83)	2.51 (2.32–2.7)	2.76 (1.7–4.05)	2.79 (1.57–4)	3 (1.54–4.48)	2.78 (1.56–4)
	Preterm	2.43 (1.5–3.73)$^{\#}$	2.54 (1.5–4.14)	2.28 (1.5–3.6)*				
BT					6 (2.5–10)*	7 (2.5–13)*	5 (3–8)*	4 (1–7)
Factor II (u/mL)	Term	0.48 (0.45–0.51)*	0.68 (0.64–0.72)$^{*\#}$	0.88 (0.84–0.92)*	0.94 (0.71–1.16)*	0.88 (0.67–1.07)*	0.83 (0.61–1.04)*	1.08 (0.7–1.46)
	Preterm	0.45 (0.2–0.77)*	0.57 (0.36–0.95)$^{*\#}$	0.87 (0.51–1.23)*				
Factor V (u/mL)	Term	0.72 (0.68–0.77)$^{*\#}$	0.98 (0.94–1.02)*	0.91 (0.86–0.96)*	1.03 (0.79–1.27)	0.9 (0.63–1.16)*	0.77 (0.55–0.99)*	1.06 (0.62–1.5)
	Preterm	0.88 (0.41–1.44)$^{\#}$	1.02 (0.48–1.56)*	1.02 (0.58–1.46)*				
Factor VII (u/mL)	Term	0.66 (0.61–0.71)*	0.90 (0.84–0.96)*	0.87 (0.81–0.93)*	0.82 (0.55–1.16)*	0.85 (0.52–1.2)*	0.83 (0.58–1.15)*	1.05 (0.67–1.43)
	Preterm	0.67 (0.21–1.13)*	0.83 (0.21–1.45)*	0.99 (0.47–1.51)				
Factor VIII (u/mL)	Term	1 (0.9–1.1)	0.91 (0.83–0.99)$^{\#}$	0.73 (0.68–0.78)$^{*\#}$	0.9 (0.59–1.42)	0.95 (0.58–1.32)	0.92 (0.53–1.31)	0.99 (0.5–1.49)
	Preterm	1.11 (0.50–2.13)	1.11 (0.5–1.99)$^{\#}$	0.99 (0.5–1.87)$^{\#}$				
vWf (u/mL)	Term	1.53 (1.32–1.74)*	1.28 (1.1–1.46)*	1.07 (0.94–1.2)*	0.82 (0.6–1.2)	0.95 (0.44–1.44)	1 (0.46–1.53)	0.92 (0.5–1.58)
	Preterm	1.36 (0.78–2.1)*	1.36 (0.66–2.16)*	0.98 (0.54–1.58)				
Factor XI (u/mL)	Term	0.38 (0.35–0.42)$^{*\#}$	0.53 (0.5–0.56)$^{*\#}$	0.86 (0.79–0.93)*	0.97 (0.56–1.5)	0.86 (0.52–1.2)	0.74 (0.5–0.97)*	0.97 (0.67–1.27)
	Preterm	0.3 (0.08–0.52)$^{*\#}$	0.43 (0.15–0.71)$^{*\#}$	0.78 (0.46–1.1)*				
Factor XII (u/mL)	Term	0.53 (0.48–0.58)$^{*\#}$	0.49 (0.45–0.53)*	0.77 (0.72–0.82)*	0.93 (0.64–1.29)	0.92 (0.6–1.4)	0.81 (0.34–1.37)*	1.08 (0.52–1.64)
	Preterm	0.38 (0.1–0.66)$^{*\#}$	0.43 (0.11–0.75)*	0.82 (0.22–1.42)*				
Plasminogen CTA, (u/mL)	Term	1.95 (1.85–2.05)$^{*\#}$	1.98 (1.88–2.08)*	3.01 (2.9–3.12)$^{*\#}$				3.36 (2.48–4.24)$^{+}$
	Preterm	1.7 (1.12–2.48)$^{*\#}$	1.81 (1.09–2.53)*	2.75 (1.91–3.59)$^{*\#}$				
					0.98 (0.78–1.18)	0.92 (0.75–1.08)	0.86 (0.68–1.03)*	0.99 (0.77–1.22)$^{++}$
AT (u/mL)	Term	0.63 (0.6–0.66)$^{*\#}$	0.78 (0.74–0.82)$^{*\#}$	1.04 (1.01–1.07)$^{\#}$				1.05 (0.79–1.31)$^{+}$
	Preterm	0.38 (0.14–0.62)$^{*\#}$	0.59 (0.37–0.81)$^{*\#}$	0.9 (0.52–1.28)$^{*\#}$				
					1.11 (0.82–1.39)	1.11 (0.9–1.31)	1.05 (0.77–1.32)	1 (0.74–1.26)$^{++}$
Protein C (u/mL)	Term	0.35 (0.32–0.38)$^{*\#}$	0.43 (0.4–0.46)$^{*\#}$	0.59 (0.56–0.62)*	0.66 (0.4–0.92)*	0.69 (0.45–0.93)*	0.83 (0.55–1.11)*	0.96 (0.64–1.28)
	Preterm	0.28 (0.12–0.44)$^{*\#}$	0.37 (0.15–0.59)$^{*\#}$	0.57 (0.31–0.83)*				
Protein S (u/mL)	Term	0.36 (0.32–0.4)$^{*\#}$	0.63 (0.58–0.68)*	0.87 (0.83–0.92)				0.92 (0.6–1.24)$^{+}$
	Preterm	0.26 (0.14–0.38)$^{*\#}$	0.56 (0.22–0.9)*	0.82 (0.44–1.2)*				
					0.86 (0.54–1.18)	0.78 (0.41–1.14)	0.72 (0.52–0.92)	0.81 (0.6–1.13)$^{++}$

aPTT, activated partial thromboplastin time; AT, antithrombin or antithrombin III; BT, bleeding time; PT, prothrombin time; TT, thrombin clotting time; vWf, von Willebrand factor. *Values that differ statistically from adult values. $^{\#}$Values that differ statistically in term and preterm infants. $^{+}$Adult reference value specific for term/preterm infant studies. $^{++}$Adult reference value specific for children/adolescents study.

manual methods, platelets are observed under light microscopy and counted by hand, a process which is time consuming and carries a coefficient of variation between 15 and 25% (64). Automated counts are more typically utilized as they are rapid, reproduceable and require less tech time. However, while they are highly accurate in most circumstances, cellular debris may

TABLE 3 | Key articles in developmental hemostasis following the Andrew et al. series (48–52).

References	Study details	Coagulation parameters	Notable findings

Flanders et al. (48)	• Study population: Healthy older children and adolescents (7–16 years old) at a single center
	• Sample size: 887 total patients, 75–245 patients in each 2–3 year age cohort
	• Relevant methods: STA-R coagulation analyzer
		• Global: Fibrinogen
		• Coagulation: FII, FV, FVII, FX,
		• Fibrinolysis: α_2-AP, plasminogen
		• Inhibitors: AT, protein C, protein S
			General findings:
			• Similar maturation patterns but different absolute values were identified compared to the Andrew et al. series
			Unique findings (median values):
			• Protein S:
			  • Females: 101–103% between 7 and 17 years old compared to 98% in adults*
			  • Male: 116% at 16–17 years old compared to 108% in adults*

Monagle et al. (49)	• Study population: Newborns (day of life 1, 3), infants, children, and adolescents (1 month to 16 years old) at a single center
	• Sample size: 159 newborns and 458 patients 1 month to 16 years of age
	• Relevant methods: STA compact analyzer, chromogenic assays, functional clotting assays
		• Global: aPTT, fibrinogen, INR, PT, TT
		• Coagulation: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII
		• Fibrinolysis: D-dimer
		• Inhibitors: AT, protein C, protein S
		• Others: ETP, TFPI (free, total)
			General findings:
			• While mirroring the trends described by Andrew et al. differences in the absolute values of coagulation assays with most measures proportionally increased in all age-groups were demonstrated
			Unique findings (mean values):
			• D-dimers:
			  • 1.34–1.47 in neonates compared to 0.18 in adults*
			  • 0.25–0.27 between 1 and 16 years old compared to 0.18 in adults*
			• ETP:
			  • 4,429–5,363 between 1 month and 10 years old compared to 8,475 in adults*
			• Free TFPI:
			  • 7.13 between 1 month and 1 year old compared to 10.7 in adults*
			  • 6.69–7.66 between 6 and 16 years old compared to 10.7 in adults*

Appel et al. (50)	• Study population: Full term infants and children age 0–17 years old and adults between the ages of 20 and 49 years old
	• Sample size: 218 children and 52 adults
	• Relevant methods: Sysmex CA-1500 System, Behring BCS System
		• Global: aPTT, BT, fibrinogen, PT, TT
		• Coagulation: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, vWf
		• Fibrinolysis: α_2-AP, D-dimer, plasminogen
		• Inhibitors: AT, protein C, protein S
			General findings:
			• Differences in coagulation markers were most significant in infants <12 months compared to older children / adults
			• vWf antigen and activity were higher in infants compared to older children
			  • Levels in non-O blood groups reached their nadir at 12 months then gradually increased toward adult levels, while these nadirs were less distinct in the O blood group
			Unique findings (median values using the BCS system):
			• vWf antigen:
			  • 97% in non-O blood groups compared to 71% in the O blood group at 1–5 years old#
			  • 118% in non-O blood groups compared to 99% in O the blood group at >19 years old#
			• vWF, ristocetin cofactor:
			  • 82% in non-O blood groups compared to 66% in the O blood group at 1–5 years old#
			  • 106% in non-O blood groups compared to 82% in the O blood group at >19 years old#
			• Factor VIII:
			  • 121% in non-O blood groups compared to 100% in the O blood group at 1–5 years old#
			  • 105% in non-O blood groups compared to 90% in the O blood groups at 6–10 years old#
			  • 131% in non-O blood groups compared to 116% in the O blood group at >19 year old#

Attard et al. (51)	• Study population: Newborns (day of life 1, 3), infants, children, and adolescents (1 month to 16 years old) • Sample size: 120 patients • Relevant methods: ELISA	• Coagulation: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII • Fibrinolysis: Plasminogen • Inhibitors: AT, protein C, protein S (total, free)	General findings: • Combined results from the study cohorts demonstrated differences in line with prior studies along with a high level of interindividual variability in specific protein levels

(Continued)

TABLE 3 | Continued

Reference	Study details	Coagulation parameters	Notable findings
Toulon et al. (52)	• Study population: Infants, children, and adolescents between 15 days and 17 years old across 7 study centers • Sample size: 1,437 total infants, children, and adolescents • Relevant methods: Clotting assays, latex agglutination, chromogenic analysis, colorimetric analysis	• Global: aPTT, fibrinogen, PT • Coagulation: FII, FV, FVII, FVIII, FIX, FX, FXI, FXII, FXIII, vWf • Fibrinolysis: D-dimer, plasminogen • Inhibitors: AT, protein C, protein S	General findings: • Values followed similar patterns as prior studies and were not significantly different between the seven study centers

*aPTT, activated partial thromboplastin time; α_2-AP, α_2-antiplasmin; AT, antithrombin or antithrombin III; BT, bleeding time; ETP, endogenous thrombin potential; F, factor; INR, international normalized ratio; PT, prothrombin time; TFPI, tissue factor plasmin inhibitor; TT, thrombin clotting time; vWf, von Willebrand factor. *Indicates findings statistically significant from adult values. #Indicates statistically significant findings between blood groups.*

result in overestimation or underestimation of platelet number in samples with platelet clumping or in patients with enlarged platelets (6, 66). Optical counting methods, in which platelets are recognized by light scattering or fluorescent dye, increase accuracy by better measurement of different sized platelets and better discrimination of platelets from cellular debris and other cell populations (66). In flow cytometric methods, platelets are incubated with fluorescent monoclonal antibodies directed against an antigen on the platelet membrane. While determination of platelet number may be different depending on the analyzer, all have been shown to be accurate with platelet counts as low as 10,000/μL (64, 67, 68).

Despite these advances, all platelet counting methods may be problematic in severely thrombocytopenic patients, an inaccuracy that may have substantial clinical impact around bleeding assessment and transfusion. A common cause of artifactual underestimation of platelet count occurs when patient plasm contains EDTA reactive antibodies causing *in vitro* platelet clumping. Most clinical laboratories will identify the presence of platelet clumps or cellular debris and not report an actual platelet number due to the possibility of a spurious result. The clinician must be aware of this possibility when an unexpected high or low platelet count is reported.

Assessment of platelet function through measurement of *in vitro* platelet aggregation, developed in the 1960's, significantly improved the ability to identify alterations in platelet function (63). In this technique, referred to as Light Transmission Aggregation (LTA), platelet-rich plasma is stirred in a cuvette between a light source and a measuring photocell. Agonist-induced platelet aggregation results in increased transmission of light through plasma over time. Multiple agonists are commonly employed and trigger a highly characterized response for each agonist in normal platelets. Deviation from that agonist-specific response enables diagnosis of platelet disorders based on the profile of aggregation (69). In addition to aggregation, shape change, adenosine triphosphate secretion, and (with special equipment) an increase in cytoplasmic calcium ions can also be measured. While much information can be derived from this classical method, turbidimetric platelet aggregometry has several important barriers that limit its practical use. First, it is labor intensive and requires a significant degree of technical expertise to carry out (70, 71). Second, this method is restricted in its ability to assess small aggregates and therefore may have limited sensitivity to both preexisting aggregates or the early phase of aggregation (72). Third, platelet adhesion and change in surface expression of cytoadhesion receptors are also not assessed (70), and fourth, it requires large volume blood sample and (generally) a platelet count over 100,000–200,000/υL limiting its use in small infants and in those with severe thrombocytopenia.

Whole blood aggregometry is another type of LTA and variation of turbidimetric aggregometry. In this technique, whole blood is stirred between two platinum wire electrodes set at a fixed distance (73). The electrode becomes covered with platelets and further adhesion of platelet aggregates is induced after the addition of agonists all of which change the impedance with time (73). Whole blood aggregometry may be superior to turbidimetric aggregometry when monitoring anti-platelet therapy, but is similarly insensitive to smaller platelet aggregates (74). Light scattering methods analyze particle size by flow cytometry and increases the accuracy for counting platelets and for assessing aggregates of all sizes during the early aggregation phase (103). Light scattering may be combined with aggregometry to monitor the continued formation of platelet microaggregates (73). This combined technique accurately assesses primary and secondary aggregation responses and may be of specific use in platelet hyperreactivity disease states (104).

Bleeding time (BT) was the first, and remains one of the only, test to evaluate *in vivo* platelet function. Via the Duke, Ivy, or Mielke method, a "standardized" cut is created and the time until bleeding flow arrests is assessed (75, 76). For the pediatric population, there are commercially available devices based on the Mielke method with age-appropriate templates that take into account the thinner epidermis and size of newborns and infants. While still occasionally used as first line screening for severe hemostatic defects, the perioperative use of the BT has largely disappeared because it, along with many other platelet function tests, does not accurately predict the risk related to bleeding for surgical procedures unless grossly abnormal (77, 105). This suggests that either the test is insensitive or mild platelet defects are not clinically important. Advantages of bleeding time include study of platelet function *in situ* and the ability to perform the test without complex equipment. Disadvantages include its manual nature with variable reproducibility, the invasiveness of the procedure, and that it is time consuming (77, 105).

TABLE 4 | Overview of selected testing in hemostasis.

Test category	Test	Blood volume required	Venue performed	Advantages and limitations
Platelet evaluation	Platelet count (58–62)	• Clinical laboratory: 1 mL whole blood • Sample test volume: ≤ 250 μL whole blood	Clinical laboratory	Advantages: • Has common clinical use with validated standardized procedures • Is simple to perform either manually or through automated techniques Disadvantages: • Does not provide information about platelet function • Can be time intensive (primarily manual counts) • Overestimates when cellular debris or other cell populations are present • Underestimates in samples with enlarged platelets or platelet clumping • Has limited accuracy, particularly very low platelet counts (< 10,000/μL)
	Light transmission aggregometry (57–70)	• Clinical laboratory: 20 mL whole blood • Sample test volume: 3–5 mL platelet-rich plasma or whole blood	Clinical laboratory	Advantages: • Tests specific platelet function responses to a panel of agonist • May be augmented with light scatter techniques to better capture the early phase of aggregation and assess aggregates of different sizes Disadvantages: • Is time and resource intensive • Requires special expertise and training to interpret • Requires large blood volumes, particularly for the pediatric population • May be unreliable if the initial specimen contains platelet aggregates
	Bleeding time (71–74)	NA	Point-of-care	Advantages: Evaluates *in vitro* platelet function through *in vivo* test Does not require specialized equipment to perform Disadvantages: Is time and resource intensive Requires an invasive procedure Requires specialized training to perform (manual method) Has variable reproducibility Has unclear ability to predict bleeding risk unless grossly abnormal
	PFA-100/PFA-200 (74–76)	• Clinical laboratory: 3–5 mL whole blood • Sample test volume: 1–3 mL whole blood	Point-of-care	Advantages: • May provide a more standardized approach than other platelet function testing • Measures platelets at high (physiologic) shear rates Disadvantages: • Is time and resource intensive • Provides unclear association of platelet function and bleeding risk
	Cone and plate analyzer (74, 77)	• Clinical laboratory: 3–5 mL whole blood • Sample test volume: 150–250 μL whole blood	Clinical laboratory	Advantages: • Provides platelet function information using a small blood volume • Uses a variety of specific agonists which allows for a variety of applications • Yields results rapidly (within 15 min) • Allows for platelet function to be measured even at low platelet counts Disadvantages: • Is influenced by red cell count • Is manually conducted and may have significant operator variability • Provides unclear association of platelet function and bleeding risk

(Continued)

TABLE 4 | Continued

Test category	Test	Blood volume required	Venue performed	Advantages and limitations
Clotting assays	aPTT, PT, TT (78–82)	• Clinical laboratory: 3 mL whole blood • Sample test volume: 1 mL of plasma	Clinical laboratory	Advantages: • Has common clinical use that is well-standardized and validated • Are helpful as a screening assessment for bleeding and thrombotic disorders • Are often utilized in diagnostic decision support and therapeutic algorithms • Are easy to perform either manually using the tilt-tube technique or automatedly using high throughput analyzers Disadvantages: • Are non-physiologic tests that oversimplify coagulation pathways • Are affected by *in vitro* and *in vivo* factors that do not have any effect on *in vivo* clot formation • Provides unclear assessment of bleeding risk
	Factor assays (78, 81)	• Clinical laboratory: 3 mL whole blood • Sample test volume: 0.5 mL of plasma	Clinical laboratory	Advantages: • Has common clinical use that is well-standardized and validated • Is useful in the diagnosis of specific protein alterations that may cause or contribute to bleeding and thrombosis disorders Disadvantages: • Requires a large blood volume for pediatric patients, particularly if testing multiple factor levels • Affected by *in vitro* and *in vivo* factors that do not have any effect on *in vivo* clot formation
Global measures	Viscoelastic testing (83–96)	• Clinical laboratory: 3 mL whole blood • Sample test volume: $\leq$ 400 μL whole blood	Point-of-care	Advantages: • Has rapid turn-around time and may be performed at the bedside • Is more cost-effective than standard measurements of hemostasis • May decrease use of blood products in certain populations Disadvantages: • Requires operator training in interpretation • Requires rapid processing of sample (within 3 min) for some measures • Has poor precision with high coefficient of variance • Is unclear in ability to predict bleeding risk
	Exogenous thrombin potential (97, 98)	• Sample test volume: $\leq$ 500 μL platelet-rich or platelet-poor plasma	Primarily research	Advantages: • Is reflective of the physiologic state, assessing activation, clot formation, and fibrinolysis • Allows for different antibiotics, proteins, and enzymes to interrogate different aspects of hemostasis Disadvantages: • Does not have fully established and validated reference ranges • Is not readily available for clinical use
	Sonic estimation of elasticity resonance (99–102)	• Sample test volume: 1.5 mL whole blood	Primarily research	Advantages: • Provides a direct estimate of mechanical clot properties Disadvantages: • Does not have fully established and validated reference ranges • Is not readily available for clinical use

aPTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin clotting time.

In addition to bleeding time, there are number of other tests that have been developed to mimic the processes that occur *in vivo* following vessel injury. The hemostatometer, a commercially available clot signature analyzer, punches holes within a tube containing flowing anticoagulated blood. Under controlled conditions, the punched holes stimulate formation of the primary platelet plug and allow for the assessment of platelet function and of involvement of GPIb, GPIIb/IIIa, and vWf (19, 106). The thrombotic status analyzer pulls whole blood through a capillary tube and the resulting force induces platelet

activation, formation of the platelet plug, and eventually capillary tube occlusion (107). The platelet function analyzer (PFA-100 or updated PFA-200) exposes citrated whole blood to high shear in a capillary tube with a center membrane coated with an agonist. As platelets are activated and aggregate, the device measures the drop in flow rate between the capillary tube and center membrane and ultimately measures tube occlusion or closing time (78, 105). While referred to as an *in vitro* bleeding time, its ability to identify platelet disorders is poor and currently this test may serve as a test for screening and monitoring of von Willebrand disease in the pediatric population (78, 79). The cone and plate analyzer exposes a whole blood sample to a plate under arterial flow conditions and assesses platelet adhesion and aggregation on the surface of the plate (80, 105). For the pediatric population, the main advantage of this system is that it requires only a small volume of blood to perform (150–250 μL) (80). Platelet mapping can be performed using thromboelastography (TEG) though its clinical utility and correlation with other platelet function assays has not been determined (81). In general, none of these tests have been shown to have clinical utility in the routine assessment of platelet function.

Finally, platelet function may be assessed through the identification of markers of platelet activation and reactivity in plasma and/or on circulating platelets. This may be evaluated through measurement of platelet release products such as platelet factor 4 and β-thromboglobulin in platelet-poor plasma. Whole blood flow cytometry can be used to measure activated platelets, platelet hypo-reactivity or hyper-reactivity, platelet-leukocyte aggregates, platelet microparticles, and platelet turnover which is inferred through the measurement of reticulated (i.e., young) platelets (82). Nucleic acid-specific dyes can be used to measure young platelets that contain residual RNA and aid in the assessment of platelet turnover or thrombopoiesis (83).

Clotting Assays

The most commonly utilized tests measuring the effectiveness of *in vivo* clotting measure the conversion of fibrinogen to fibrin and utilize the time to formation of a fibrin clot as determined by one of several methodologies each of which have a different degree of sensitivity. The most common of these tests are PT, aPTT, and thrombin time (TT) (84). A few important aspects of nomenclature for these tests should be noted. First, in contrast to the older PTT, aPTT is an updated, modified, test that includes the addition of an activator to accelerate clotting time resulting in a narrower reference range and increased sensitivity (84, 85). Second, because it is calculated from PT, International Normalized Ratio (INR) is frequently employed as a surrogate PT. However, INR has only been validated as a measure of intensity of vitamin K antagonist induced anti-coagulation and has not been validated as a measure of bleeding risk (86). While the Activated Clotting Time (ACT) is generally performed to monitor anticoagulation during cardiopulmonary bypass surgery and during ExtraCorporeal Membrane Oxygenation (ECMO), outside of these clinical settings it has not gained widespread use.

PT, aPTT, and TT are reported as time to clot formation while fibrinogen is reported as mg/dL determined by a lab-specific calibration curve (84, 87). In regards to assays utilized for the quantification of protein levels of specific clotting assays, time to clot formation is measured and then reported as a percent in relation to time to clot formation using pooled normal adult plasma. By convention, the amount of a specific clotting factor contained in normal plasma is defined as 100%, equivalent to 1 unit/mL in normal plasma. From this assumption, time to clot formation is converted to a U/mL concentration. All of these assays where the end point is the time to a fibrin clot are referred to as "functional assays." An advantage of these assays is that they are relatively simple and easy to standardize, but they can be affected by *in vitro* and *in vivo* factors that do not have any effect on *in vivo* clot formation (84, 87). One such circumstance is common in neonates, particularly premature neonates, and in infants with cyanotic heart disease who manifest a reactive polycythemia with hematocrit >55%. Under this condition, blood collected into standard coagulation testing vacuum tubes (for example, "blue top tubes") may produce PT or aPTT with values that are spuriously prolonged as these samples contain less plasma and are therefore over-anticoagulated (88). This can be remedied by collecting blood in tubes that contain a reduced amount of anticoagulant.

Global Measures of Hemostasis

There are several important global measures of hemostasis that are gaining use and clinical application. This includes viscoelastic testing, a method of assessing clot formation dynamically in whole blood. Viscoelastic testing is designed to imitate sluggish venous blood flow and triggers clot formation to derive measurements of hemostasis (89). Developed in 1948, but not adopted in the clinical setting until the 1980's, viscoelastic testing now has more broad use primarily in trauma, surgery, and cardiopulmonary bypass (89–91).

Currently available methods for viscoelastic testing include thromboelastography (TEG) and the related rapid thromboelastography (r-TEG) as well as rotational thromboelastography (ROTEM) (89). In both, a small sample of patient blood is placed into a cup and a pin that serves as a sensor is inserted. In TEG and r-TEG, the cup is oscillated with a specified rotational cycle to precipitate clot formation. With clot formation, the torque of the rotating cup is transmitted to the pin and the degree of pin rotation is converted to an electrical signal and recorded. The device tracing reflects this change in electrical signal reflecting change in clot strength resulting from clot formation or fibrinolysis. ROTEM uses similar methods but the sensor pin instead rotates within the cup (91).

Viscoelastic testing measures clot initiation or time to initial clot formation, kinetics or growth of the clot, clot strength, and clot lysis. All components are identified by specific measurements on a tracing produced by the device over time. Clot initiation or clot time provides an assessment of early activity of the clotting cascade before clot amplification and the burst of thrombin production. Clot kinetics or clot formation time assesses clot potentiation by activation of platelets and thrombin-mediated cleavage of soluble fibrinogen. Clot strength describes maximal clot strength achieved via GPIIb/IIIa-mediated platelet-fibrin interactions. Finally, clot lysis provides an assessment of activation of the fibrinolytic system (89, 92, 93). In addition

to standard viscoelastic testing, specific elements of the clotting cascade can be interrogated by the addition of specific clotting activators or inhibitors (94).

The diagnostic and prognostic role of viscoelastic testing is being studied in a range of clinical areas (89, 92, 93). The majority of these studies have been in adult perioperative cardiac surgery patients as well as other surgical patients at risk for hemorrhage (89). A recent Cochrane review of 17 studies in a largely cardiac surgery population, only 2 of which included pediatric patients, addressed the utility of thromboelastic testing in bleeding patients (95). They found that while TEG/ROTEM-guided transfusion strategies might decrease transfusion requirements and support a tendency toward improved mortality outcomes, conclusive recommendations could not be made because of limitations in study design and power (88). Subsequent studies in pediactric patients (largely neonates) has continued to investigate the utility of viscoelastic testing as a modality to better manage patients with acquired coagulopathies or requiring anticoagulation (96, 97). Reference range values have been established in term, preterm and very low birth weight infants (98, 99). In the trauma population, TEG-guided resuscitation is growing in use, though current studies are insufficient to determine if thromboelastography-based transfusion is better than existing transfusion practices (100, 101). Other populations of potential interest include liver transplantation, sepsis, obstetric hemorrhage, perioperative thromboembolism, and management of anticoagulation on mechanical circulator support, though evidence from large trials required to formulate consensus recommendations in these areas is lacking (89). This work is even more limited in pediatric specific populations, though studies have suggested a benefit in similar disease populations as to adults (102, 108). That said, viscoelastic testing may be particularly advantageous in pediatric populations since only a small volume of blood is required for sample analysis. Further, in limited use TEG has been shown to be reproducible in some infant populations, and cost-effectiveness studies seem to clearly favor viscoelastic testing compared to standard laboratory run measures of hemostasis (102, 109–113). Taken together, viscoelastic testing remains a novel method that may be advantageous in certain populations including children.

Endogenous thrombin generation is a measure of the global efficiency of coagulation as thrombin sits at the key intersection of endothelial cell, platelet, and coagulation activation, clot formation, and fibrinolysis. Thrombin generated can be quantified in platelet-rich or platelet-poor plasma using the calibrated automated thrombogram (CAT) method. Specifically, CAT monitors the cleavage of a fluorogenic substrate that is compared to the known thrombin activity in a non-clotting plasma sample (100). The CAT system is open, allowing for different antibiotics, proteins, and enzymes to be introduced to evaluate specific aspects of hemostatic process (114). Validation of reference ranges are just now being established with preliminary studies suggesting children and adolescents have lower exogenous thrombin potential compared to adults (115); although as previously stated, low thrombin generation potential noted in infants may reflect in part how the assay is performed (58). While these assays have great clinical potential, standardization and validation need refining as does a more rapid technical process.

Another potentially promising method is sonic estimation of elasticity via resonance. This technology applies acoustic radiation force to induce shear wave resonance within a rigid test chamber. The characteristics of this resonance are compared to numerical or analytical models to quantify the sample filling the chamber (116, 117). The technique may be performed rapidly and shows promise as a point-of-care test and may rival TEG/ROTEM in assessment of clot stiffness and fibrinogen contribution (117–119).

SPECIAL CONSIDERATIONS

There are a number of additional considerations in the assessment of hemostasis in the pediatric population. First, there are numerous technical challenges. This includes practical difficulty drawing sufficient blood volume from small infants and children given their small vessels for certain tests (e.g., LTA) and the overall smaller total blood volume infants and children which limits the quantity of blood that can safely be obtained to perform comprehensive diagnostic testing. Consequently, tests that use small volumes may be preferred and careful consideration is necessary to parsimoniously select appropriate tests given the clinical situation. While central lines help with the technical difficulties of obtaining adequate testing volumes, samples can be contaminated by anticoagulation or other medications running through the line itself. In fact, samples collected through central lines may require 10 mL of blood be withdrawn prior to sample collection for the result to be considered valid. Techniques to mitigate this waste include using the initial sample collection for other labs or reinfusing blood that would otherwise be discarded.

There are also a number of infant and pediatric conditions and diseases that require special testing considerations. Pediatric trauma patients, particularly infants, demonstrate significant endothelial injury, present with acute traumatic coagulopathy and/or coagulopathy related to iatrogenic causes, and, while they may be at less risk of bleeding than their adult counterparts, they are also at risk of thrombosis during recovery from their injury (120–123). While there have been several pediatric-focused studies around damage control resuscitation and thromboelastographic-guided transfusion, there are still gaps in how these tests account for the developmental changes in hemostasis noted in infants and children (99, 116). Pediatric patients with liver dysfunction may have significant hemostatic abnormalities from impaired synthetic function including coagulation factor defects, thrombocytopenia and platelet dysfunction, alterations in the fibrinolytic system, and alterations in endogenous inhibitors of coagulation. While clotting times may be significantly elevated in these patients, these abnormal results often do not correlate with bleeding risk (124, 125). Indeed, assessment of hemostatic balance in patients with liver disease generally demonstrates a neutral or mildly pro-thrombotic hemostatic system (126, 127). The concept of "hemostatic balance" has also been conceptually addressed as alternatively being a condition of a loss in "hemostatic reserve"

by some hematologists, a concept that carries some merit (128). In the oncologic population, patients may be at risk of hyperviscosity, thrombosis, and cytopenias that may increase bleeding risk depending on the type of cancer and therapies. The hemostatic system is frequently monitored in these patients, but transfusion and other therapeutic decisions necessitate an understanding of the limitations and normal variations of these standard hematologic measurements (129).

Patients on extracorporeal life support present with unique hemostatic risks as both the extracorporeal circuit and the underlying disease process induce important dysregulation of the hemostatic system that may favor either hemorrhage or thrombosis (119, 120). Hematologic monitoring of these patients may be one of the most active parts of their management and typically includes frequent evaluation for bleeding, consumptive coagulopathy, hemolysis, and adequacy of anticoagulation. In addition to aPTT to assess anticoagulant effect of unfractionated heparin (UFH), anti-Xa assay (assessing effect of UFH or low molecular weight heparin [LMWH] or some of the new direct acting oral anticoagulants [DOACs]) or the Activated Clotting Time (ACT) are utilized to assess bleeding/thrombosis risks (130, 131) Increasingly, the anti-Xa assay is becoming the preferred test to monitor UFH as well as anti-Xa inhibitors due to its insensitivity to low levels of AT. However, therapeutic range for anti-Xa has been extrapolated from those for UFH by matching aPTT result to anti-Xa level in plasma samples to which heparin was added *ex vivo*. This practice does not account for the primarily anti-IIa action of UFH potentially introducing a degree of uncertainty regarding the extrapolated therapeutic threshold (132, 133). ACT is the preferred monitoring test for UFH given during cardiopulmonary bypass surgery with "therapeutic" (i.e., desired) values being surgeon and device specific (134, 135).

Finally, trauma patients often present in the field or to community hospitals and must be transported to the regional trauma center for care. The use of point of care (POC) tests of hemostasis have only recently been investigated in this patient population (136, 137). While some studies suggest the feasibility of these devices, there are as of yet insufficient data to adequately assess their use.

CONCLUSIONS

Understanding of hemostatic dysfunction remains a unique challenge in critically ill infants and children. It requires knowledge of physiologic hemostatic pathways, comprehension of the development of the hemostatic system by age, an understanding of logistics, advantages, and limitations in hematologic testing, and an awareness of the specifics of application to pediatric populations. Taken together, this knowledge will enable the practitioner to more accurately assess hemostatic dysfunction and determine bleeding and thrombosis risk resulting in more successful diagnosis and management of this vulnerable population.

AUTHOR CONTRIBUTIONS

AN drafted all tables included. RP created all figures included in manuscript. Both authors participated in reviewing appropriate published literature, writing all drafts, and the final submitted draft manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 27 January 2021
doi: 10.3389/fped.2021.611680

Bleeding in Critically Ill Children—Review of Literature, Knowledge Gaps, and Suggestions for Future Investigation

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Clinically significant bleeding complicates up to 20% of admissions to the intensive care unit in adults and is associated with severe physiologic derangements, requirement for significant interventions and worse outcome. There is a paucity of published data on bleeding in critically ill children. In this manuscript, we will provide an overview of the epidemiology and characteristics of bleeding in critically ill children, address the association between bleeding and clinical outcomes, describe the current definitions of bleeding and their respective limitations, and finally provide an overview of current knowledge gaps and suggested areas for future research.

Keywords: bleeding, hemorrhage, scale, epidemiology, critical ill children

OPEN ACCESS

Edited by:
John McGuire, University of Washington, United States

Reviewed by:
Jhuma Sankar, All India Institute of Medical Sciences, India
Martin Kneyber, University Medical Center Groningen, Netherlands

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *29 September 2020*
Accepted: *04 January 2021*
Published: *27 January 2021*

Citation:
Aran AA, Karam O and Nellis ME (2021) Bleeding in Critically Ill Children—Review of Literature, Knowledge Gaps, and Suggestions for Future Investigation. Front. Pediatr. 9:611680. doi: 10.3389/fped.2021.611680

INTRODUCTION

Clinically relevant bleeding can commonly affect critically ill patients, either as an admission diagnosis or as a complication of underlying critical disease. In one large, prospective cohort of critically ill adults, approximately one-fifth had significant bleeding during their admission to the ICU (1). One-tenth of critically ill children were reported to be bleeding on admission in a retrospective study in the PICU. In addition, a similar number developed clinically relevant bleeding during their stay (2).

Physiologically, clinically relevant bleeding (CRB) may lead to reduced oxygen delivery in two ways: (1) decreased blood oxygen content due to lower red blood cell mass and (2) decreased cardiac output due to hypovolemia. Poorer clinical outcomes have been associated with bleeding in subpopulations of critically ill patients. These include increased mortality in bleeding adults with acute coronary syndrome (3) and in those following bone marrow transplant (4). Adults who undergo cardiac surgery and have significant post-operative bleeding are more likely to be mechanically ventilated longer and stay longer periods in the ICU (5). Likewise, poorer clinical outcomes have been observed in critically ill children who bleed including more exposure to vasoactive medications and longer lengths of stay in both the ICU and the hospital as compared to those who do not bleed (2). Nevertheless, until recently, there was no published definition accurately describing the severity of bleeding in critically ill children. We lack large epidemiologic studies documenting prevalence of CRB, as well as risk factors and effective prevention.

In this review, we will provide an overview of the existing evidence regarding the epidemiology and characteristics of bleeding in critically ill children, describe the association between bleeding and clinical outcomes, discuss the current definitions of bleeding and their respective limitations,

and, in doing so, provide an overview of current knowledge gaps and suggestions for areas of future research.

EPIDEMIOLOGY OF BLEEDING IN THE GENERAL PICU POPULATION: INCIDENCE, IDENTIFIED RISK FACTORS, AND OUTCOME

Despite the potential morbidity of CRB for critically ill children, less than a handful of studies depict the epidemiology and potential risk factors of CRB in the general Pediatric Intensive Care Unit (PICU) population. Current epidemiologic studies are typically limited to certain subsets of critically ill patients, such as those undergoing cardiac repair with cardiopulmonary bypass (6–8), or to specific anatomical locations of bleeding, such as chest tubes (6–8) or the gastrointestinal tract (9–11). None of the published studies to date used a validated pediatric definition of CRB and all of the definitions varied.

In a single-center retrospective cohort study conducted over 6 months in a tertiary care children's hospital, Moorehead et al. reported CRB occurring in 9% (19/214) of their patients (12). The authors defined CRB in relation to hematoma formation, the development of hemorrhagic shock, a defined drop in hemoglobin concentration, the cessation of anticoagulant medication due to bleeding CRB, or fluid resuscitation with either crystalloid or red blood cell (RBC) transfusions to control bleeding. Thrombocytopenia (platelet count $< 100 \times 10^9$/L), mechanical ventilation, the use of either antibiotic or antacid medications, and cardiac surgery were patient factors that were each associated with increased risk of CRB. The study was limited by significant disagreement between those reviewing the charts as to which patients met their defined criteria for CRB (kappa statistic = 0.26).

To follow up on this study and account for its limitations, Moorehead et al. applied their definition of CRB to a prospective cohort of critically ill children (13). In particular, they reported on the associations between laboratory values and CRB. Two hundred and thirty-four children were enrolled and a similar incidence of CRB was reported. There was a suggestion of decreasing platelet count being associated with increased bleeding. However, the association was not significant when accounting for confounders. Irregularities in other measures of coagulation, such as international normalized ratio (INR) and activated partial thromboplastin time (aPTT), were not associated with increased bleeding. The study was limited by incomplete laboratory data for each patient enrolled.

In a cohort study in a single-center PICU, White et al. reported CRB in 9% (37/405) of the children admitted to the PICU (14). They modified a previously developed tool from the International Society on Thrombosis and Hemostasis (ISTH) and characterized bleeding as either major, clinically relevant non-major, or minor. The majority of children, ∼80%, had only one bleeding event. Children receiving invasive mechanical ventilation or stress ulcer prophylaxis were more likely to have CRB. Though mortality was no different, those with CRB had longer lengths of stay both in the ICU and the hospital as a whole.

Pinto et al. used the same bleeding tool (known as the ISTH-BAT) to assess the epidemiology of CRB in critically ill adolescents with increased risk for venous thromboembolism (VTE) (15). The frequency of CRB was 30% (95% CI, 20–40%). The high incidence of CRB reported in this study probably reflects the inclusion of bleeding events occurring on the day of admission that were excluded from the previous studies discussed. As a result, the proportion of surgical and trauma patients in this study was also higher. CRB was more commonly seen in adolescents with VTE, likely reflecting increased risk of bleeding due to anticoagulation. Younger age, higher predicted risk of mortality score, and admission for trauma or surgery were also associated with increased risk for CRB.

In conclusion, there is limited data on the epidemiology of CRB in the general PICU population. Reported incidence of bleeding in single center studies was 9–30%, depending on inclusion criteria and based on different bleeding assessment scales. Risk factors identified were admission due to cardiac surgery or trauma, young age, mechanical ventilation, receipt of antacid medications and thrombocytopenia. Patients with CRB had longer ventilation days, increased requirement for vasoactive drugs and blood products, and longer PICU length of stay.

BLEEDING IN SELECTED HIGH RISK POPULATIONS OF CRITICALLY ILL INFANTS AND CHILDREN: CHARACTERISTICS AND ETIOLOGIES

Several subsets of critically ill children may be particularly prone to bleeding complications. ICU diagnoses associated with increased frequency of bleeding include trauma, cardiac surgery, orthopedic surgery, hematologic malignancies, sepsis, respiratory failure (requiring invasive mechanical ventilation), renal failure requiring renal replacement therapy, and extracorporeal membrane oxygenation (ECMO) (16, 17).

As seen in epidemiologic studies, younger age is associated with an increased risk of bleeding. This may be secondary to the characteristics of the normal development of the coagulation system in infancy. Prior to 6 months of age, infants have lower concentrations of vitamin K–dependent pro-coagulant clotting factors and reduced thrombin potential (18), ∼70% of the levels as compared to adults. Prothrombin levels are also significantly lower as compared with that in adults (19). It is unclear whether these lower levels are actually associated with more episodes of CRB.

In addition to patient characteristics, there are several physiologic derangements that occur during critical illness that may worsen bleeding. These include hypothermia, acidosis, excessive fibrinolysis, and consumption of coagulation factors and platelets (20). The activity of coagulation factors, as well as necessary enzymes, are inhibited during these physiologic states (21).

We will further discuss selected groups of PICU patients who may be particularly prone to medical and surgical CRB (see **Table 1**).

TABLE 1 | Medical and surgical conditions associated with clinical relevant bleeding in children.

Medical condition	Surgical condition
Renal failure and hemodialysis	Spinal surgery
Gastrointestinal bleeding	Cardiac surgery
Sepsis and disseminated intravascular coagulation	Extracorporeal membrane oxygenation
Idiopathic pulmonary hemorrhage	
Central nervous system bleeding	

MEDICAL BLEEDING

Renal Failure and Hemodialysis

Acute kidney injury (AKI) is a common complication of critical illness, affecting almost 30% of the children admitted to pediatric intensive care units (22). Many of these patients develop significant enough AKI to require dialysis, often secondary to fluid overload. In prospective studies in adults, bleeding has been reported in 14–50% of patients with dialysis-dependent AKI, and is described as a major risk factor for mortality (23, 24). Similar to previously mentioned populations, there is very little published data concerning dialysis-dependent AKI and bleeding in the pediatric population.

Kreuzer et al. published a retrospective study of bleeding in nearly 200 children with dialysis-dependent acute kidney injury (25). Slightly over a quarter of the children had bleeding events with 62% of the episodes considered life-threatening. Several factors were independently associated with a higher risk of bleeding including the underlying primary disease leading to AKI, presence of multiorgan dysfunction syndrome (MODS), recent surgery or bleeding prior to the onset of AKI, and hemodialysis or continuous veno-venous hemofiltration (as compared to peritoneal dialysis). In addition, the presence of bleeding was independently associated with mortality in this patient population.

Patients with uremia may be more prone to bleed due to several factors including platelet dysfunction, vitamin K deficiency, and/or slower clearance of unfractionated or low molecular weight heparin which is usually given during dialysis (26). Children with dialysis-dependent AKI are an understudied population in hemostatic research.

Upper Gastrointestinal Bleeding

Although perceived as a prevalent complication in the PICU, there is limited data on the incidence, risk factors and outcome of upper gastrointestinal bleeding in critically ill children. In a single-center prospective study, Chaibou et al. reported upper GI bleeding in 10% (103/1006) of their cohort, with only two percent (16/1006) considered CRB (9). CRB was adjudicated by three authors and had to be associated with significant hemoglobin drop, RBC transfusion, hypotension, development of MODS, surgery, or death. Three independent risk factors for clinically relevant upper GI bleeding were respiratory failure, coagulopathy, and pediatric risk of mortality score >10.

In a more recently published retrospective study, Sahin et al. reviewed records of 136 eligible children consecutively admitted to their tertiary care PICU. The overall incidence of upper GI bleeding was 15%. However, the study was limited by small sample size in that only one child developed what was considered significant bleeding. Approximately 30% of the children who received antacid prophylaxis developed any upper GI bleeding. Mechanical ventilation, trauma, coagulopathy, thrombocytopenia, higher severity score at admission, renal and hepatic failure, hypotension, heart failure, and arrhythmia were found to be associated with the development of any upper GI bleeding (27).

Sepsis and Disseminated Intravascular Coagulation (DIC)

Sepsis is commonly associated with the development of thrombocytopenia, hypofibrinogenemia, and prolongation of prothrombin time (28) which may manifest as multisite bleeding including petechiae, hematomas, and pulmonary hemorrhage. However, sepsis can also trigger the extrinsic coagulation cascade and result in microthrombi (29). In a recently published small cohort of children with septic shock, the incidence of DIC was estimated to be 42–66% (depending on the definition of DIC applied) (30). Though the presence of DIC in children with septic shock has been associated with higher mortality (31), the incidence of bleeding vs. clotting with DIC in these children is not clear. The incidence of bleeding and thrombotic complications in one cohort of Thai children with gram negative sepsis and DIC was 59 and 20%, respectively (32).

Idiopathic Pulmonary Hemorrhage (IPH)

Bleeding into the lower respiratory tract can occur acutely (diffuse alveolar hemorrhage) or more chronically (idiopathic pulmonary hemosiderosis). IPH is a diagnosis of exclusion and other causes, such as gastrointestinal bleeding, cows' milk allergy, cystic fibrosis, or vasculitis must be excluded. The incidence of both acute and chronic IPH is rare. In a 10-years retrospective study of infants at Boston Children's Hospital, only 157 cases were identified (33). Those infants most commonly had associated congenital heart disease, chronic lung disease of prematurity, congenital or acquired lung disorders, or congenital or acquired coagulopathies.

Central Nervous System (CNS) Bleeding

The etiologies of bleeding into the central nervous system of children is varied and includes trauma (both accidental and non-accidental), prematurity, underlying congenital or acquired coagulopathies, idiopathic thrombocytopenia purpura, rupture of arteriovenous aneurysms, or hemorrhage into CNS tumors. Given the diverse pathologies, the incidence of and risk factors for CNS bleeding in pediatrics varies.

SURGICAL BLEEDING

Pediatric Spinal Surgery

Children undergoing spinal surgery, most often correction of scoliosis, are at high risk of bleeding given the length of surgery

and the surgical technique required (involving bone dissection and possible disruption of vertebral blood vessels) (34). It is not uncommon for children to lose their entire circulating blood volume. In a large database analysis of blood utilization during spinal surgery from 2000 to 2009, nearly one-third of children required RBC transfusion (35). Survey studies have suggested that the rate of massive bleeding has decreased over time (36), though these studies may be confounded by response bias. The use of antifbrinolytics during surgery (such as tranexamic acid or aminocaproic acid) may have driven the trend of decreased bleeding and decreased post-operative blood product exposure in children undergoing spinal repair (37).

Cardiac Surgery

Children undergoing cardiac repair are also at significant risk of perioperative and post-operative bleeding. Several pre-operative factors may contribute to the bleeding risk. These repairs are often performed during the first year of life. At baseline, infants with congenital heart disease may have lower levels of clotting factors (38). Additionally, cyanotic heart disease has been associated with thrombocytopenia and platelet dysfunction (39–41). Intra-operative blood loss may be pronounced due to coagulopathy induced by the cardiopulmonary bypass circuit, as well as induced hypothermia, use of high dose anticoagulation, and hemodilution (42–47).

Post-operative bleeding is a common complication in pediatric patients undergoing cardiopulmonary bypass (CPB) and is associated with increased morbidity and mortality (46–48). The receipt of post-operative blood products is independently associated with longer lengths of mechanical ventilation and length of PICU stay (49). Post-operative bleeding may be predicted by several factors including thrombocytopenia and hypofibrinogenemia (6, 8).

The Network for the Advancement of Patient Blood Management, Haemostasis, and Thrombosis (NATA) recently published guidelines to minimize blood product exposure in these vulnerable children (50). Their recommendations include the diagnosis and treatment of peri-operative anemia, prophylactic use of either tranexamic acid or aminocaproic acid in the operating room, priming of the cardiopulmonary bypass pump with colloid (as opposed to crystalloid), use of modified ultrafiltration and cell salvage during bypass, and the development of institution-specific transfusion algorithms.

Outside of the post-operative period, critically ill pediatric patients with heart disease can also experience CRB. Patients receiving any form of anti-coagulation (e.g., unfractionated heparin [UFH], low molecular weight heparin [LMWH], and warfarin) are at risk for bleeding secondary to decreased thrombin generation. Acquired von Willebrand disease (vWD) should be considered in patients with excessive mucosal bleeding and the presence of a vascular lesion that creates high shear stress, such as aortic stenosis or a left ventricular assist device (VAD). Definitive treatment of acquired vWD is the correction of the underlying cardiac abnormality, but this is not always possible. Alternatively, treatment with a von Willebrand factor (vWF) containing replacement product can be considered (51).

Extracorporeal Membrane Oxygenation (ECMO)

ECMO may be used to rescue children with profound respiratory and/or heart failure and its use in pediatrics is increasing (52). Like CPB, ECMO is associated with bleeding and is multifactorial secondary to hemodilution, circuit induced coagulopathy, and platelet dysfunction, among other causes. Bleeding and thrombosis, including intracranial hemorrhage and stroke, are frequent and challenging complications in the management of pediatric ECMO patients and associated with increased risk for mortality (53–55). Dalton et al. conducted the first multicenter prospective observational study of children on ECMO examining the incidence, risk factors and associated outcomes of bleeding and clotting (56). Bleeding complications were defined as those requiring RBC transfusion and those occurring in the intracranial space. In a cohort of 514 children followed over 28 days, the incidence of bleeding was 70% (16% of which were intracranial) and the incidence of thrombosis was 38%. Age, indication for ECMO, organ failure at the time of ECMO initiation, and clinical site were all independent predictors of bleeding. Two of three discharge functional status scales were worse in patients who experienced bleeding in comparison to those who did not.

We conducted a secondary analysis of this cohort to evaluate the association between quantifiable bleeding (as measured from chest tubes) and clinical outcomes in children supported by ECMO (57). Over half of the patients had bleeding documented from the chest tube. The median amount of bleeding from the entire population over the course of ECMO was slightly over 120 mL/kg [interquartile range 47–319 mL/kg]. A "dose-dependent" response was observed in the independent association between amount of bleeding and mortality, as well as PICU length of stay. Whereas, some coagulation measures, such as fibrinogen level, were independently associated with chest tube bleeding, other measures, such as platelet count, were not.

BLEEDING SCALES APPLICABLE TO CRITICALLY ILL CHILDREN—WHERE ARE WE TODAY?

As we portrayed thus far, bleeding represents a common complication in the critically ill child, with significant morbidity. In addition, the interventions used to treat CRB, such as blood product, medication administration, and/or surgical procedures, may also be associated with worsened outcomes. Research is needed to guide the clinician as to the correct situations when interventions are needed to treat CRB. However, a standardized definition is needed to conduct these studies.

There are certainly common elements within many of the currently published definitions of bleeding. Definitions often include the amount and site of bleeding (58–61), physiologic or laboratory effects of bleeding (60, 62) and the need for transfusion or other medical intervention to treat the bleeding (59–62). The latter criteria, the need for transfusion or intervention, can add significant variability to the measurement of bleeding incidence as these measures are subjective and may be based on clinician preference. Most definitions have been developed to be

specifically applicable to certain patient populations including those with cancer (58, 59, 62), receiving anticoagulation (60) or with inherited bleeding disorders (61). These do not address the specific risk factors for and characteristics of bleeding in critically ill children (e.g., bleeding from an endotracheal tube). Until quite recently, the HEmorrhage MEasurement (HEME) score was the only diagnostic criteria developed to evaluate CRB in critically ill patients (1) and included specific physiologic (such as heart rate) and laboratory (such as hemoglobin) measures. However, it was developed for critically ill adults, and therefore cannot be applied to critically ill children due to different age specific norms.

We conducted a systematic review in order to identify current bleeding scales and their validity to assess bleeding in critically ill children (63). Studies were included if they contained a bleeding score, bleeding measurement tool or clinical measurement of hemorrhage. Of the 18 studies identified, only two described critically ill children. As with previously mentioned scales in adults, the majority of identified bleeding scales included the need for medical or surgical intervention as a part of the definition. In addition, the majority of scales did not report measures of reliability or validation to clinical outcomes. This review highlighted the need for the development of an objective, validated, operational definition that included characteristics of bleeding particular to critically ill children. The National Institute of Health, National Heart, Lung and Blood Institute (NHLBI) and the Biomedical Excellence for Safer Transfusion (BEST) Collaborative recognized this need as well and identified the development of a validated bleeding measurement scale in critically ill children (as well as other vulnerable patient populations) as a high priority (64, 65).

As the next step in the development of a definition of bleeding in critically ill children, we performed a web-based international survey to qualify the clinical significance of different bleeding characteristics in critically ill children (66). We identified four domains of bleeding (site, amount, severity, and progression) and asked pediatric intensivists what they considered clinically relevant vs. irrelevant. Two hundred and twenty-five pediatric intensivists from 16 countries responded. They identified the following characteristics as clinically relevant: bleeding in critical locations (such as the pericardium, pleural space, central nervous system, and lungs), bleeding requiring interventions (such as chest tube insertion or the administration of blood products), bleeding leading to physiologic repercussions (such as organ failure or hypoperfusion); and bleeding lasting more than 6 h. In particular, quantifiable bleeding of more than 5 mL/kg/h for more than 1 h was frequently considered clinically relevant. In addition, providers identified several specific clinical scenarios of bleeding as being clinically irrelevant, including streaks of blood in the endotracheal tube and non-coalescing petechiae.

Based on these identified and characterized bleeding elements, we conducted a Delphi consensus process with over 30 multidisciplinary experts in bleeding and hemostasis in order to design a standard definition for CRB in critically ill children (67). The results of the process, the Bleeding Assessment Scale in Critically Ill Children (BASIC), are presented in **Table 2**. The experts divided bleeding into severe, moderate and minimal and described clinical characteristics of each category. Qualifiers were made to describe fatal and progressive bleeding. In order to incorporate clinically relevant physiologic consequences of bleeding into the scale, the experts used organ dysfunction as measured by the Pediatric Logistic Organ Dysfunction (PELOD)-2 score (68). Given the PELOD-2 scale includes physiologic derangements within nearly every system, the experts chose not to include each and every possible location of bleeding within the definition. Instead, they only included three sites (intraspinal, intraorbital and intraarticular) that though rare, would not be

TABLE 2 | Consensus definition of clinically relevant bleeding (59).

BLEEDING ASSESSMENT SCALE IN CRITICALLY ILL CHILDREN
Any of the following criteria define severe bleeding:
• Bleeding that leads to at least one organ dysfunction, using PELOD-2 score criteria of organ dysfunction, within 24 h of the previous assessment (if there is no previous assessment, the baseline results are presumed to be normal). The organ dysfunction should be associated with the bleeding, in absence of other causes.
• Bleeding that leads to hemodynamic instability, defined as an increase in HR by >20% from baseline or a decrease in BP by >20% from baseline. The hemodynamic instability should be associated with the bleeding, in absence of other causes.
• Bleeding leading to a drop in hemoglobin >20% within 24 h. The drop in hemoglobin should be associated with the bleeding, in absence of other causes.
• Quantifiable bleeding ≥5 mL/kg/h for ≥1 h (e.g., chest tube, drain).
• Intraspinal bleeding leading to loss of neurologic function below the lesion, non-traumatic intra-articular bleeding leading to decreased range of movement, or intraocular bleeding leading to impaired vision.
All the following criteria must be present to define moderate bleeding:
• Bleeding more than minimal bleeding but without criteria for severe bleeding.
• Bleeding not leading to organ dysfunction, as measured by the PELOD-2 score.
• Bleeding not leading to hemodynamic instability, i.e., change in HR >20% or drop of BP <20% from baseline.
• Bleeding leading to a drop in hemoglobin ≤20%.
• Quantifiable bleeding ≥1 but < 5 mL/kg/h.
Any of the following criteria define minimal bleeding:
• Streaks of blood in ETT or during suctioning only.
• Streaks of blood in nasogastric tube.
• Macroscopic hematuria, or ≤1+ (≤1+) RBCs present on urine dipstick if available.
• Subcutaneous bleeding (including hematoma and petechiae) <5 cm (2 inches) in diameter.
• Quantifiable bleeding <1 mL/kg/h (e.g., chest tube, drain).
• Bloody dressings required to be changed no less than each 6 h, or weighing no >1 mL/kg/h if weighed, due to slow saturation.
Progressive bleeding: progressive bleeding is bleeding that either progresses to a higher severity category (e.g., from minimal to moderate bleeding, or from moderate to severe bleeding) or to a higher number of criteria within the same category (e.g., hemodynamic instability progressing to organ failure, or streaks of blood in the ETT and subsequent slightly blood-tinged urine).
Fatal bleeding: bleeding that is the direct cause leading to death will be characterized as fatal bleeding.

BP, blood pressure; ETT, endotracheal tube; HR, heart rate; PELOD, Pediatric Logistic Organ Dysfunction.

reflected in the PELOD-2 scoring system. In addition, changes in heart rate, blood pressure and hemoglobin were described by proportional differences, as opposed to whole numbers, in order to account for age-related standards. Lastly, the experts chose to exclude clinical interventions within the definition, based on both the subjectivity of clinical decision-making, as well as retaining the ability of the use of the definition in studies to assess the value of such interventions.

The BASIC definition underwent preliminary internal validity testing as it was applied to 40 critically ill children with 46 distinct bleeding episodes in a single center (67). When comparing the application of the definition to describe the bleeding episodes by two independent observers, there was substantial inter-rater reliability [kappa statistic 0.74 (95% CI, 0.6–0.9)]. The BASIC definition represents the first diagnostic criteria for bleeding derived via expert consensus applicable to critically ill children. It must be further validated, both in larger patient cohorts and in relation to clinical outcomes, in order to be applied in clinical trials.

CONCLUSIONS AND FUTURE DIRECTIONS

Pediatric intensivists will encounter bleeding on a relatively frequent basis in their patient population. In critically ill children, bleeding has been associated with significant morbidity and mortality. There is paucity of data on CRB acquired in PICU patients, stemming in part from the lack of a standardized pediatric bleeding score. The BASIC definition should be validated to clinical outcomes. This will facilitate the accurate identification of subpopulations of critically ill children at risk for CRB. The data generated from large scale epidemiologic studies can be used to development a numerical bleeding score which includes patient characteristics and laboratory values. This can be used, in turn, to plan monitoring strategies and prevention measures. A standardized and well-validated score can be used to define inclusion criteria or outcome and will aid in the development of large multicenter clinical trials to test current hemostatic interventions, such as platelet or plasma transfusion thresholds in children who are prone to bleed, such as those treated with anticoagulation, those in DIC or supported on ECMO and test new and investigational therapies, such as anti-fibrinolytics, factors replacements, use of whole blood and cold stored platelets.

AUTHOR CONTRIBUTIONS

AA, OK, and MN conceived of the format of the review and contributed to its writing. All authors reviewed the manuscript and edited it prior to submission.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 26 February 2021
doi: 10.3389/fped.2021.625632

Hemostatic Challenges in Pediatric Critical Care Medicine—Hemostatic Balance in VAD

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Ventricular assist devices (VAD) are used more in children. Safe and effective anticoagulation is required for successful management of children supported with ventricular assist devices. Developmental hemostasis, device hemocompatibility, plastic to body ratio, surgical variable techniques, lack of knowledge on pharmacokinetics of anticoagulants, and wide variability in anticoagulation protocols have all contributed to increased incidence of bleeding and thromboembolic complications. New collaborative learning networks, such as the ACTION network, provide opportunities to define best practices, optimize, and reduce anticoagulation related adverse events. ACTION was established Dec 2017. It consists of expert clinicians in heart failure, as well as researchers, parents, and patients, with goals to improve outcomes, share data, improve education and standard practice for children with heart failure ([1], n.d). Changes in pediatric VAD anticoagulation strategy from using mainly heparin to DTI such as bivalirudin have helped reduce bleeding and clotting complications.

Keywords: hemostasis, ventricular assistance device, anticoagulation, thrombosis, bleeding, heparin, bivalirudin, TEG-PM

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *03 November 2020*
Accepted: *06 January 2021*
Published: *26 February 2021*

Citation:
Ghbeis MB, Vander Pluym CJ and Thiagarajan RR (2021) Hemostatic Challenges in Pediatric Critical Care Medicine—Hemostatic Balance in VAD. Front. Pediatr. 9:625632. doi: 10.3389/fped.2021.625632

INTRODUCTION

Since FDA approval of the Belin Heart EXCOR ventricular assist device (VAD) in North America over a decade ago (1) pediatric VAD use has increased with favorable reduction (>50%) in waiting list mortality and improved survival following heart transplantation (2). The 4th Annual Pediatric Interagency Report for Mechanical Circulatory Support (Pedimacs) published in 2019 reports that 1,031 devices were placed in 856 children at 44 centers during September 2012 through December 2019 (3). Increase in VAD use is largely due to increasing use of intracorporeal continuous flow devices (4). Despite increasing VAD use, hemocompatibility related adverse event, including bleeding and thrombosis with current antithrombosis agents remain among the significant challenges in children supported on VAD (5). Safety and efficacy of antithrombotic agents in these patients are in ongoing need of evaluation. While the use of antithrombotic therapies worldwide was consistent, variability in practices among VAD centers was noted (6). Achieving optimal anticoagulation and antithrombosis in VAD patients requires a balanced control of thrombin and platelets inhibition against physiologic hemostasis. In infants and young children, achieving this balance has been challenging due to several unique physiologic factors including developmental hemostasis as originally described by Monagle et al. (7). This report summarizes these challenges and describes the current antithrombotics use in children supported with VAD.

[1] Available online at: www.actionlearningnetwork.org/collaborative.

Pediatric VAD Device Options

a. Berlin Heart EXCOR, a pulsatile paracorporeal pump, is the first and only Food and Drug Administration durable VAD for use in children as a bridge to transplantation. The Berlin Heart device is used as a single or biventricular assist. Placement requires median sternotomy and use of cardiopulmonary bypass. Berlin Heart EXCOR ventricular assist device can be used to support children weighing >3 kg up to adulthood, in the form of univentricular and biventricular support. With previous heparin based anticoagulation regimen (Edmonton Anticoagulation and Antiplatelet Protocol), the rate of stroke was consistently ∼ 30%, but has since decreased over the last 5 years with the adoption of multiple interventions, including use of steroids to mitigate inflammation, (8) use of consistent team to manage anticoagulation (9), and adoption of DTI for primary anticoagulation (10).

b. Paracorporeal continuous flow devices include temporary circulation support devices such as Pedimag®/Centrimag®, Rotaflow®, and the Revolution® pumps (11). These are placed *via* median sternotomy and use of cardiopulmonary bypass is limited to supporting the most challenging patients whose size and anatomy necessitate use of this paracorporeal pump. Despite the described continuous flow technology increased risk of thrombosis in children with body surface area (BSA) <1.0 m^2 (12), it's applications continue to evolve with tendency toward longer term support in select populations (13). TandemHeart is also a paracorporeal continuous flow device which is used more in the adult populations. One main advantage of this device is that cannulas are placed percutaneously so no need exists for thoracotomy, however the size of the cannulas is the limiting factor and thus pediatric experience remains limited (14).

c. Intracorporeal VADs are currently the most common VAD type placed in older children, composing around 50% as per the most recent annual Pediatric Interagency Registry for Mechanical Circulatory Support (Pedimacs) report. This shift in the use of VAD for children is attributed to advancements in device design with associated lower adverse event profile (3). Per Morales et al., 34% of Intracorporeal patients are still supported with the device at 6 months and nearly 20% at 1 year, which is in stark contrast to paracorporeal device types, which have close to zero patients remaining on device support at 1 year. They also estimated 50% of the patients are receiving Intracorporeal VAD as a bridge to candidacy (3). The HeartWare_ HVAD system (Medtronic, Mounds View, MN) is one of the earlier intracorporeal continuous flow pump to be used in larger children and adolescents even before FDA approval for adults only in 2012. The smallest known patient with this device is 2.7 years old and 13.1 Kg. It is reported that close to 50% of children with HeartWare devices are discharged home (15). The HeartMate VAD represents the newest in centrifugal continuous-flow VAD technology. Its optimized hemocompatibility profile, particularly HM3, and shear stress reduction makes it with the least side effects profile among all the devices. The smallest reported patient supported with this device is 8.8 years and 19.1 Kg. Per O'Connor et al., adverse events related to the HM3 device were uncommon so far (median 78 days, 2–646), with no episodes of pump thrombosis, pump dysfunction requiring operative exchange, or stroke (16). The Jarvik 2015 VAD is the most recent and promising intracorporeal continuous flow pump for infants and smaller children. The preliminary enrolment weight range is 8–20 kg with BSA range of 0.4–1.0 m^2. As described in its website, www.pumpkintrial.com, the PumpKIN trial is a prospective, multi-center, single-arm feasibility study that will evaluate the safety of the Jarvik 2015 as a bridge to transplant or recovery in children. Of note, patients eligible for the PumpKIN trial are limited severe heart failure with two-ventricles (17).

   It is worthy to note that pediatric patients on intracorporeal devices are generally less ill, older, and are less likely to have congenital heart disease compared with patients on paracorporeal pulsatile or paracorporeal centrifugal flow devices (3).

d. Impella is a percutaneous ventricular assist device that is a micro-axial pump. Impella has recently been utilized more in pediatrics for short-term circulatory support. The most common indication of its implant was cardiogenic shock with variable underlying pathophysiology including cardiac allograft rejection, myocarditis, or cardiomyopathy (18, 19). It is inserted and secured percutaneously (or through a chimney graft) into the axillary or femoral artery, and then positioned in the LV across the aortic valve. The continuous blood flow that is pumped can vary based on the Impella generation, but most recently up to 5.5 l/min.

Hemostasis and Age

Coagulation Cascade

Maturation related differences in the coagulation cascade in infants and children presents unique physiologic challenges compared to adults (7, 20). Clinicians are faced with the need to explore age related changes with each component of the coagulation cascade, both in physiologic or pathophysiologic states. These normal physiological differences in maturation of the coagulation cascade are referred to as "developmental hemostasis." During fetal life, maternal coagulation factors are unable to penetrate the placental barrier. Coagulation factors synthesis in the fetus starts as early as 5.5 weeks (21). After birth, coagulation factors have different postnatal patterns of maturation toward adult values for most components by 6 months of age (22, 23). Some factors such as fibrinogen, Factors V, VIII, von Willebrand (vWF), and XIII are similar to mature adults even at birth, while Kuhle et al. reported levels of vWF and high-molecular weight multimers as well as vWF collagen binding activity remain increased during the first 2 months of life and then gradually decrease to adult levels (21). While at birth, plasma concentrations of the direct inhibitors AT (antithrombin)

Abbreviations: ACTION, Advanced Cardiac Therapies Improving Outcomes Network; AT, Antithrombin 3; BMP, basic metabolic panel; CRP, C-Reactive Protein; DTI, Direct Thrombin Inhibitor; HIT, heparin-induced thrombocytopenia; INR, international normalized ratio; LDH, Lactate dehydrogenase; PT, prothrombin time; PTT, Partial thromboplastin time; TEG-PM, Thromboelastography with Platelet Mapping; VAD, Ventricular assist devices.

and HCII (heparin cofactor II) are ∼50% of adult values. Then at about 6 months of age, plasma concentrations of AT and HCII reach adult levels, α2-M levels are nearly twice those of adult values and remain increased throughout childhood (21). It is however also worth noting that variations in AT activity are not fully explained by age and other factors may play a role (24, 25).

Platelets

The only available evidence for altered platelet function in children is in neonates and in *in vitro* studies. Michelson and colleagues reported neonatal platelets to be hyporeactive to the platelet-activating agents such as thrombin, adenosine, phosphate/ epinephrine, and thromboxane A2. Yet, the bleeding time in neonates, a reflection of platelet function, is normal due to increased red blood cell size, hematocrit, and vWF multimers (26).

Hemostasis and Congenital Heart Defects

Congenital heart disease (CHD) is known risk factor for intravascular thrombosis due to altered flow dynamics that can predispose to areas of flow stasis, and/or higher shear stress, with platelet activation, and placement of thrombogenic artificial material. Patients who undergo cardiac surgery are at risk of both thrombosis and bleeding. These risks are higher in the < 1 year age group (27). Additionally, delay in age based normalization of coagulation factors in children with CHD has also been documented. In children with CHD, maturation in coagulation factors occurs after 48 months of age (28). Given the body and vessels size for young children, there is an increased chance for chest re-exploration and post-operative bleeding following heart surgery. Increased bleeding may be secondary to an acquired dilutional coagulopathy in these patients with the use of large priming solution compared with the blood volume during cardiopulmonary bypass, as well as delayed maturation of coagulation factors (28). Other clinical factors associated with coagulation system abnormalities in patients with congenital heart disease may be related to the physiological effects of heart failure, which may include decreased hepatic coagulation factor production (25). Finally, other unique coagulation factor abnormalities in patients with single ventricle congenital heart disease is the reported increase in factor VIII following Fontan operation with increased thromboembolic risk (29).

These issues illustrate challenges to providing antithrombotic therapies to children supported with VAD. Careful consideration of developmental hemostasis is required in constructing safe and efficient antithrombosis protocols in children, particularly children with congenital heart disease.

THROMBOTIC COMPLICATIONS AND VAD

Thrombotic complications are among the most common serious adverse events in VAD patients, despite different attempts to protocolize antiplatelet and anticoagulant therapies with different ways to accurately monitor its effects (5, 30). Different reasons are believed to cause thrombosis in VAD patients that mainly involve contact mechanism from artificial surfaces of the device as well as high shear stress, both of which activate vascular endothelial cells as well as platelets, and white cells. Inflammation which has been observed in VAD patients is also believed to contribute to the prothrombotic state in these patients (31). Other proposed factors include turbulence at anastomosis sites, and heat generated by the device.

In the recent Pedimac registry report, the incidence of hemorrhagic stroke was noted to be 11%. This was lower compared to prior times and did not vary by device type (3). In the prospective trial of the Berlin Heart pediatric VAD device, stroke occurred in 29% (1). Similar to paracorporeal pulsatile flow devices, paracorporeal continuous flow devices, such as the Rotaflow (Maquet, Wayne, NJ) and the Centrimag/Pedimag (Abbott, Abbott Park, IL), were found to have a high rate of neurologic events at 24% (13). In pediatric HeartWare HVAD patients, neurologic events were reported as 15.8% of all causes of death. When compared to young adults, children had more early bleeding and more early and late neurologic dysfunction.

The recent increased use of DTI for paracorporeal VAD patients has further reduced the incidence of stroke on DTI therapy for all devices types to as low as 1.7 events per 1,000 days of support. The Stanford Modified Antithrombotic Guideline that utilized triple antiplatelet agents in addition to enoxaparin was shown to reduce the incidence of stroke in children supported with Berlin Heart EXCOR devices to 0.8 events per 1,000 days of support utilizing.

In terms of time to first neurologic event and ischemic stroke, intracorporeal continuous flow devices performed better than both types of paracorporeal devices, pulsatile and continuous flow. The incidence of stroke was not different for paracorporeal pulsatile or continuous flow devices (3). In the early HeartMate 3 VAD experience of pediatric and patients with congenital heart disease, stroke or pump thrombosis were not detected for median of 78 days of follow-up.

In comparison to children supported with VAD, the incidence of stroke in adults supported with intracorporeal devices is reported to be around 17% (32). Similar to children, HeartMate 3 (HM3) VAD, had a lower reported stroke than other devices with an incidence of 0.46 events per patient-year in the 1st month and down to 0.04 events per patient-year in the 2 year period (33).

PUMP THROMBOSIS

VAD pump thrombosis represents an increased risk for morbidity and mortality given association with device malfunction, thromboembolic events, and hemolysis. Factors related to pump thrombosis can be related to patient, device, or anticoagulation management. In adult patients, Bartoli et al. reported LVAD thrombosis in 2–13% patients with a continuous-flow LVAD, 4–13% with axial-flow devices, and 2% with centrifugal-flow devices (34). The incidence of pump thrombosis reported in adult patients on LVAD continuous flow devices ranged from 0.014 to 0.03 events per patient-year (35), The HeartMate III device had no reported pump thrombosis for the first 6 months in a large prospective trial (36), and in another retrospective large study (37) though there are case reports of pump thrombosis at 1 year (38).

In Pediatric VADs, device thrombosis occurs in 18% of pediatric patients with a paracorporeal pulsatile device (39). Pump thrombosis event rate on DTI was 3.7 per 1,000 patient-days for the Berlin Heart EXCOR VAD, and 13.7 per 1,000 patient-days of centrifugal-flow VAD support (11). Pump thrombosis in HeartWare device in children was reported as 11% in a multicenter study using the Pediatric Interagency Registry for Mechanical Circulatory Support (Pedimacs) registry (16). In the early experience of HM3 in the pediatric and young adult population, there was no reported pump thrombosis (16).

Overall, time to first device malfunction/thrombus in children was significantly better in intracorporeal devices types, than paracorporeal device types, with the paracorporeal pulsatile pumps performing significantly better than paracorporeal continuous flow devices (3).

Pump thrombosis can start as either fibrin-rich (red) or platelet-rich (white) thrombi. Fibrin thrombi form around stagnant blood flow whereas platelets thrombi form in areas of turbulence. Management of pump thrombosis included surgical or pharmacological. Some pharmacological options are intravenous thrombotic agents such as fibrinolytics (e.g., alteplase) and glycoprotein IIb/IIIa (GPIIb/IIIa) inhibitors (e.g., eptifibatide) (40). Most reports of tPA use for pump thrombosis is inconclusive with evidence of increased bleeding complications (35). Similarly, GPIIb/IIIa inhibitor also increased risk of bleeding complications (40). A common practice in adult VAD institutions for pump thrombosis includes intensifying antiplatelet therapies, increasing the International Normalized Ratio (INR) goal when warfarin therapy is resumed, and/or DTI targeting a high activated partial thromboplastin time (aPTT) (40). With increasing reports of HIT among VAD adult patients, some institutions now use Bivalirudin as the agent of choice for pump thrombosis (40). Other studies have shown that bivalirudin therapy for management of pump thrombosis was associated with high recurrence rates and suggest need for other therapies including surgical pump replacement (41). Similarly others found that surgical intervention resulted in less mortality, stroke, and resolution of hemolysis than a medical strategy alone (42).

Pediatric VAD pump thrombosis treatment is based on adults' experience with case reports of using bivalirudin and low-dose systemic tissue plasminogen activator (TPA). When there are concerns of hemolysis impact on kidney function or if the patient is close to a previous surgery, device exchange should be considered (43). ACTION is currently working on settings shared guidelines for pediatric VAD management including pump thrombosis protocols (44).

Bleeding

Bleeding is similarly among the most common complications for VAD patients. Some known factors are the historic lack of a standardized approach to anti-coagulation and anti-platelet dosing or monitoring in children, surgical techniques due to size and complexity, as well as developmental hemostasis in children.

In adults, major bleeding events are the most common adverse event within the first 3–12 months of continuous flow LVAD implantation. Gastrointestinal hemorrhage was the most common site of bleeding (up to 40%, particularly after 3 months) (45, 46). Based on the Pediatric Interagency Registry for Mechanical Circulatory Support (PediMACS) report for outcomes of children supported with temporary devices, the most frequent early adverse events were bleeding (28%) (13). In the Australian retrospective report for children on HeartWare HVAD and Berlin Heart EXCOR VADs, major bleeding occurred in 39% of the patients, with the majority of these events happening while on unfractionated heparin and on more than one antiplatelet agent (47). In a meta-analysis for children on durable Ventricular Assist Devices (87% of which were Berlin Heart), the incidence of bleeding overall was 37%. Reported bleeding events included gastrointestinal bleeding 15%, intracranial hemorrhage 16% or chest re-exploration 23% (6). In children and young adults with HeartWare devices, the event rate of early bleeding was 2-times higher in children than in young adults, but the overall bleeding incidence was the same, 23% (16). In the early HeartMate 3 VAD experience of pediatric and patients with congenital heart disease, significant post-operative bleeding was uncommon and was reported in only two patients (6%) with a median age of 15.7 years (8.8–47.3) (16). These findings may help understand bleeding risk better given the fact that these are larger devices in smaller size patients.

The role of improved understanding of anticoagulation and antithrombosis management for pediatric VAD patients was more recently noted. In patients on Berlin EXCOR VAD, using the Sanford protocol, bleeding events incidence rate was 8.6 per 1,000 days of support (48). VanderPluym et al. reported in the largest multicenter experience of DTI use for anticoagulation therapy in pediatric paracorporeal VAD support, major bleeding present in 16% of the cases (2.6 events per 1,000 patient days of support on DTI) (10).

Additionally, bleeding events are minimized by following certain management tips. Achieving prompt hemostasis following the VAD placement is of importance, and was a fundamental requirement for patients in the DTI therapy protocol for example with 90% of patients starting DTI 12 h postoperatively or after (10). Cognizant anticoagulation management and monitoring in the early post-operative periods, as well as during switching between agents is highly recommended. Other management tips for bleeding while on VAD are by decreasing intensity of the antithrombotic regimen and/or discontinuing the antiplatelet agents.

Anticoagulation Management

Hemostasis in pediatric patients on VAD is a common challenge, and the optimal antithrombotic therapy for children with VADs is unknown. Pediatric VAD anticoagulation and antithrombotic management is widely variable with a shift toward more aggressive antithrombotic therapy. This shift could be in part due to previous experience of high rates of thrombotic complications in children with paracorporeal pulsatile-flow devices. Furthermore, developmental hemostasis in children, and coagulation challenges brought on by the presence of CHD, a population at risk for needing VAD, continues to challenge clinicians managing VAD in the pediatric populations (27).

TABLE 1 | Pediatric VAD anticoagulation protocols.

	Post op	Long term	Anti-platelets	Monitoring	Other
Edmonton protocol (5)	Heparin	Enoxaparin ≤12 months Warfarin >12 months	Dual antiplatelet therapy: Dipyridamole 4 mg/kg/day Aspirin 1 mg/kg/day	TEG-PM	
Stanford protocol (49)	Heparin	Enoxaparin ≤12 months Warfarin >12 months	Triple anti-platelet therapy: Aspirin 30 mg/ kg/day Dipyridamole 15 mg/kg/day Clopidogrel 1 mg/kg/day	None	Steroids for signs of inflammation
DTI (ACTION) harmonization protocol (10)	Bivalirudin	Bivalirudin transitioned to Couamdin	Aspirin	TEG-PM or VerifyNow	Steroids for signs of inflammation

TEG-PM, Thromboelastography with Platelet Mapping; ACTION, Advanced Cardiac Therapies Improving Outcomes Network; DTI, Direct Thrombin Inhibitor.

The three main known pediatric anticoagulation pediatric VAD protocols based on the historic timeline are illustrated below **Table 1**:

a. Edmonton Protocol: includes initiating unfractionated heparin when bleeding is minimal at 24–48 h post-implantation with long-term anticoagulation using Enoxaparin for patients ≤12 and warfarin for patients >12 months of age. Dual antiplatelet therapy starts at 48 h with dipyridamole begun at 4 mg/kg/day, followed by Aspirin 1 mg/kg/day divided twice daily after chest tubes removal. Thromboelastography with Platelet Mapping (TEG-PM) is used for both anticoagulation and antiplatelet titration.
b. Stanford Protocol: includes similar anticoagulation strategy starting with heparin and converting to Enoxaparin or Warfarin, but adds a 3rd anti-platelet agent and targets a weight based dose (Aspirin 30 mg/ kg/day, Dipyridamole 15 mg/kg/day, Clopidogrel 1 mg/kg/day), with no titration to effect based on TEG-PM, and recommends using use of prednisone for signs of inflammation (fibrinogen > 600 mg/dL) (48).
c. ACTION Direct Thrombin Inhibitor (DTI) Harmonization Protocol: Includes starting bivalirudin once surgical and coagulopathic bleeding has resolved with evidence of normalizing coagulation laboratories. Bivalirudin is titrated to achieve an aPTT goal of 60–90 s in patients with standard risk bleeding, and 50–60 s for those at high risk bleeding. Antiplatelet agents are also used, and include Aspirin, with confirmation of therapeutic range by TEG-PM or VerifyNow. Steroids (prednisone) is used for signs of inflammation as in the Stanford protocol.

Heparin

Unfractionated heparin (UFH) is the first-line anticoagulant therapy in the children with heart disease. It is the most common anticoagulant agent for the immediate post-operative VAD placement period. The criteria to start it are typically absence of bleeding and surgical hemostasis. Heparin dose is typically titrated thereafter using a target activated partial thromboplastin time (aPTT), most commonly between 50 and 80 s (6).

However, heparin has many known challenges but mostly related to the heterogeneous biochemical composition of different molecular weight glycosaminoglycans as well as its dependence upon AT. The heterogeneity in composition of commercial formulations of UFH can result in a wide inter-patient variability in dose–response. The differences in AT levels that change from fetal to adult life is a key additional variant that needs to be considered in infants and children managed with heparin. Heparin-induced thrombocytopenia (HIT) and the osteopenia with its long term use are reported and known risks of heparin exposure (15). There have also been reports of potentially genetic variances in the AT protein with point mutations at the glycosylation sites where AT binds to heparin, which may impair the strength of the covalent bonding (50).

Measuring the heparin response to titrate heparin dose is a known area of controversy. Activated clotting time (ACT) is a point of care assay which measures time to clot initiation of whole blood. This test has several limitations including variability in technical methods for clot activation and detection. Additionally, ACT is not specific to the effects of heparin and reflect other physiologic factors that impact coagulation (51).

Although aPTT is the most commonly used blood test to titrate heparin anticoagulation effect in pediatrics VAD patients, anti-factor Xa activity is increasingly used (6). The anti-factor Xa activity (anti-Xa) assay chromogenically quantifies the heparin–AT complex, and is therefore highly dependent upon serum AT levels; hence anti-Xa activity is frequently low in neonates and infants (52). AT replacement during the perioperative period to augment heparin response is reported in a number of studies. The threshold for replacing AT is variable and ranges <70% in older children and <60% in neonates (6).

BIVALIRUDIN

Bivalirudin is an intravenous DTI that inhibits both circulating and fibrin-bound thrombin. It is relatively new and semisynthetic drug. It does not bind to plasma proteins and inhibits both free and clot-bound thrombin. It is not dependent on AT and is less immunogenic than heparin. It may also inhibit platelet adhesion. Bivalirudin has a short half-life of 25–35 min secondary

to its intravascular proteolytic degradation and minimal renal clearance (∼20%). Clinical studies in pediatric showed safety and efficacy of bivalirudin in different settings including procedural anticoagulant, post cardiac surgery ECMO, heart transplant (53, 54). In a single institution experience in 54 children placed on ECLS for a total of 56 runs, Bivalirudin use showed no differences in outcomes compared to heparin, however resulted in longer freedom from first circuit intervention (55). Bivalirudin has also been used safely in pediatric in cases of contraindications to heparin (i.e., HIT) (56) or when unreliable heparin monitoring exists (i.e., severe hemolysis and hyperbilirubinemia) (57). The DTI experience in children is growing. The collective experience of 10 pediatric VAD centers across North America using bivalirudin in children supported with paracorporeal VADs was recently described by VanderPluym et al. Bivalirudin use in this settings was associated with a lower or a comparable rate of stroke to other known anticoagulation and antiplatelet regimens. The risk of bleeding was also comparable in older children supported with intracorporeal continuous flow VADs (10).

The starting dose of bivalirudin is 0.3 mg/kg/h, which is halved in patients with renal dysfunction **Table 2**. Measuring aPTT is the standard test to monitor anticoagulation with DTI with goal ranging between 2 and 3 times baseline aPTT value for standard bleeding risk patients, and 1.5–2 times baseline aPTT value for high bleeding risk patients. Patients are initially monitored every 4 h until a stable Bivalirudin dosing is achieved, and then daily **Table 3**. In a small retrospective case-control study for pediatric patients on ECMO, time to reach goal therapeutic anticoagulation level was shorter in bivalirudin compared to heparin (11 vs. 29 h, $P = 0.01$) (58). INR is frequently elevated in patients on Bivalirudin. This is believed to be a false elevation due to the interaction between DTI and the thromboplastin and tissue factor contained in the PT assay, along with the high molar concentrations of the DTIs needed to achieve their anticoagulant effect (59). aPTT sample contamination is infrequently encountered. While hepzyme can be used to neutralize contaminating heparin, concomitant INR is used to identify contamination since INR will not increase with heparin contamination alone. INR monitoring can also be utilized more when bivalirudin dose is escalated which is observed to happen over time (10) at which time there would also be a more "plateau" effect rather than linear aPTT to dose response effect. Some explanation of this phenomenon was related to the increased in fibrinogen levels over time in these patients, which in turn competes with the bivalirudin for the thrombin binding sites (58).

The cost effectiveness of bivalirudin remains an area of controversy since the drug by itself is far more expensive than heparin. Ongoing efforts are undergoing to understand this more, however in ECMO patients and when including the cost of AT replacement and the various laboratory monitoring, the overall cost for anticoagulation was decreased in patients receiving bivalirudin, particularly in children younger than 1 year (58).

TABLE 2 | ACTION initial bivalirudin dosing.

Goal: aPTT Standard risk (of bleeding): aPTT 60–90 (∼2-3x baseline)* High risk (of bleeding): aPTT 50–60 (∼1.5-2x baseline)**	
Renal function (GFR)	Initial dosing
Normal (>60 ml/min/1.73 m^2)	0.3 mg/kg/h IV infusion
Mild-moderate (30–60 ml/min/1.73 m^2)	0.2 mg/kg/h IV infusion
Severe (<30 ml/min/1.73 m^2)	0.1 mg/kg/h IV infusion

Baseline aPTT may be elevated if previously on AC agent, therefore use age/intuitional normative range.

***Given the risk of early bleeding, most of patients will begin in the "High risk" category for the 1st several days on support, then transition to "Standard risk".*

TABLE 3 | ACTION maintenance bivalirudin titration.

Goal: aPTT Standard risk (of bleeding): aPTT 60–90 (∼2-3x baseline) High risk (of bleeding): aPTT 50–60 (∼1.5-2x baseline)	
If aPTT 5–15 s out of range:	Increase or decrease by 15% (round up to closest 2nd decimal) Recheck 2–3 h after dose change
If aPTT in target range, no change	Recheck 2–3 h, then can decrease frequency when stable
If aPTT ≥15–30 s out of range	Increase or decrease by 25% (round up to closest 2nd decimal Recheck 2–3 h after dose change
If aPTT >3x baseline or ∼120 s	With normal renal function: hold 15 min and reduce by 30% With mild to moderate renal dysfunction: hold for 45 min and reduce by 40% With severe renal dysfunction: hold 2 h and recheck PTT before restarting

Antiplatelet Agents

Antiplatelet therapy has been the main focus for evolving pediatric VAD anticoagulation protocols which could have been driven based on the early Berlin Heart EXCOR experience. Dual and triple antiplatelet therapy had dominated most of the earlier regimens, however with the evolving of improved and consistent anticoagulation agents, i.e., DTI, the emphasis on the dosing with multi antiplatelet agents' nature has been less popular. We use primarily one agent at our institution with consideration to switch or add another agent if there is evidence of sub-therapeutic effect based on the available laboratory tests (Platelets mapping and VerifyNow). TEG-PM is a functional assay to test for pathologic bleeding etiology or to confirm therapeutic anticoagulation and antiplatelet effects. In this testing technology, the thrombus integrity is measured mechanically and in real time. It is then represented by a characteristic curve, which is interpreted based on normal reference. The clot strength is measured by the maximal amplitude (MA) on the curve, which is also used to evaluate ADP (adenosine-diphosphate) or AA (arachiodonic acid) pathways inhibition, which form the platelets mapping test portion (60). VerifyNow is a functional assay point-of-care testing which evaluates platelet aggregation by a turbidimetric-based optical detection (61).

While antiplatelet therapy is historically the main focus for pediatric VAD anticoagulation, and it remains an essential part of it, there is some evidence that TEG-PM in pediatric VAD patients have high intraindividual variability (62). There are also some preliminary reports of a significant role for timing of drug administration and interval frequency which might help improve our understanding of cases of suboptimal response. Adult VAD literature reports a common practice for addition of antiplatelet agents to the anticoagulation regimen, with acknowledgment for treatment resistance and consideration for routine assessment for such.

VITAMIN K ANTAGONIST (VKA)

Most adult VAD patients supported on intracorporeal devices are maintained on vitamin K antagonist (VKA) such as warfarin with goal INR that varies mostly between 2 and 3 (63). Similarly, pediatric VAD patients with intracorporeal devices are primarily maintained on VKA with goal INR ranging from 2 to 3.5, which may vary based on patients and device factors. VKA are typically started when the patient is extubated, with advancing diet. VKA effect is heavily influenced by diet, status of illness, and polypharmacy. Studies to estimate time in therapeutic range for pediatric patients are lacking, but reports from adult studies show the time in therapeutic range as only 30–50% (64).

DOAC

Direct oral anticoagulants (DOAC) are increasingly used in place of VKA. DOAC medications include factor Xa inhibitors (apixaban and rivaroxaban) and DTI (dabigatran). Few positive features are ease of administration with oral formulations, lack of dependency on AT, lack of dietary interaction, and the requiring less monitoring. Indications for use in the adult populations include venous thromboembolism prophylaxis and atrial fibrillation. A prospective, randomized, open label phase II multi-national clinical trial of a direct oral anticoagulant (DOAC), Apixaban, in children and infants with congenital and acquired heart disease is currently underway (48).

DOAC use in adult VAD patients is not well-established. Two examples of such experience are a small study of seven patients using dabigatran with no excess rate of bleeding or thrombosis (65) and a single center, randomized trial of dabigatran vs. warfarin which was terminated early due to increased thromboembolic events associated with dabigatran (66). Additionally, given the negative experience with dabigatran use in mechanical heart valves, routine use could not be recommended without more reassuring clinical trials (67).

PRE-OP MANAGEMENT

Prior to VAD device placement, laboratory assessment is recommended to identify potential risk factors for adverse events related to immediate and long-term anticoagulation use. These can include platelet count, prothrombin time and International Normalized Ratio (INR), partial thromboplastin time, fibrinogen, basic metabolic panel (BMP). Other optional labs include Thromboelastography with platelet mapping (TEG with PM), C-reactive protein CRP, Lactate dehydrogenase (LDH), cystatin C, screening for heparin induced thrombocytopenia (HIT screen). In addition to these labs, past medical history is obtained to identify prior thrombotic and bleeding events along with any family history that predisposes the patient to complications.

Thrombophilia evaluation can be completed if there is past or family history of thrombotic events suggesting either an acquired or inherited thrombophilia. These labs include: anticardiolipin, beta-2 glycoprotein, lupus anticoagulant, factor V Leiden, prothrombin gene 20210A mutation, AT3, and proteins C and S.

POST OP MANAGEMENT

Postoperative management details are critical in establishing a stable and sustained VAD circulation. Cognizant anticoagulation management and monitoring in the early post-operative periods, as well as during switching between agents is highly recommended. The majority of bleeding events and thromboembolic events in pediatric VAD patients occurred while patients were on unfractionated heparin or transitioning between heparin and warfarin (47).

At our institution, postoperative inpatient management include antiplatelet and anticoagulation strategies in the early postoperative period **Table 4**. This is initiated and managed by the VAD team in consultation with the CICU. Standard heparin anticoagulation for cardiopulmonary bypass is utilized with full protamine reversal in the operating room. Following admission to CICU, once hemostasis is achieved (chest tube output <2 cc/kg/h for 4 consecutive hours) and following correction of acquired coagulopathy (coagulation labs normalizing with platelet count $>100{,}000$, INR <1.4, aPTT <40 s and fibrinogen >100), we start bivalirudin with initial aPTT goal of 50–60, and escalate the goal to 70–90 over the next few days, based on the appearance of the pump. Antiplatelet agents are usually introduced based on appearance of the pump (started sooner if formation of fibrin or clot). We utilize TEG with platelet mapping, and delay initiation of antiplatelet agents until clot strength is >70 mm. As such, antiplatelet agents are generally started 1–2 weeks after VAD implantation for patients on bivalirudin and 5–7 days for patients on warfarin therapy supported on intracorporeal devices. Patient supported on paracorporeal devices remain on bivalirudin for the duration of VAD support, based on data supporting the low rate of hemocompatibility related adverse events using DTI. Patients supported on intracorporeal devices are bridged with bivalirudin or unfractionated heparin to VKA, with target INR goal of 2–3.5. A dedicated Multidisciplinary Anticoagulation Management Team is crucial to optimal anticoagulation management in VAD patients and should include a pharmacist, Cardiac Critical Care, and VAD physicians.

Procedure Anticoagulation Management

Given that the majority of bleeding and thromboembolic events in pediatric VAD patients occur while patients were

TABLE 4 | Inpatient anticoagulation management of pediatric VAD patients.

Prior to VAD placement	
Baseline labs	
Minimum	Platelet count, PT, INR, PTT, fibrinogen, basic metabolic panel (BMP)
Additional	TEG with PM, CRP, LDH, cystatin C, HIT screen
Medical and family history	Thrombotic and bleeding events
If + medical or family history	Anticardiolipin, beta-2 glycoprotein, lupus anticoagulant, factor V Leiden, prothrombin gene 20210A mutation, antithrombin 3, and proteins C and S
Post VAD placement	
Post-operative day 0	Following hemostasis[a], Bivalirudin is started at 0.1–0.3 mg/kg/h with initial aPTT goal of 50–60, and escalating the goal to 70–90 over the next few days
Post-Operative day 1 and after	
Paracorporeal	Bivalirudin remains for the duration of VAD support Antiplatelet agents are started within 1–2 weeks, and are delayed until clot strength is >70 mm based on TEG-PM. They may start sooner if formation of fibrin or clot on the pump
Intracorporeal	Bivalirudin or unfractionated heparin is bridged to VKA, with target INR goal of 2–3.5 Antiplatelet agents are started within 5–7 days

[a]*Hemostasis: chest tube output <2 cc/kg/h for 4 consecutive hours, correction of acquired coagulopathy with platelet count >100,000, INR <1.4, aPTT <40 and fibrinogen > 100.*

on unfractionated heparin or transitioning off heparin, it is imperative that the anticoagulation and antithrombosis around any procedure is carefully managed. Antithrombosis considerations surrounding procedures are specific to the clinical state of the patient and the pump, and the bleeding risk of the procedure. As such, decisions to be made in conjunction with all team members, as risk of thrombotic and bleeding events is naturally highest during procedures, even those deemed relatively minor. These decisions need to be individualized taking into consideration the thrombotic risk of the patient and pump, weighed against the bleeding risk of the procedures. Nonessential procedures should be deferred till after VAD support, and essential procedures require discussion with all team members to understand bridging plan so that cessation of antithrombotic agents is minimized, while procedural hemostasis is maximized.

As a general principle we recommend the following:

- For procedures deemed low risk of bleeding

Continue all antiplatelet agents, and dose reduce anticoagulation agent for lower therapeutic target around the procedures

- For procedures deemed high risk of bleeding

* Hold antiplatelet agents around 3 days prior to procedure
* Hold bivalirudin ∼ 1–4 h prior to skin incision (depending on renal function)
* Hold unfractionated heparin 4 h prior to skin incision
* VKA, start holding 3 days prior to procedure with daily INR measurement. Once INR <2, then start LMWH or continuous infusion of DTI or UFH
* LMWH, hold night before and morning of procedure. Consider bridging with UFH or DTI depending on thrombotic risk of pump and patient.

Prompt re-initiation of anticoagulation is required once hemostasis achieved and in discussion with all team members to ensure risk of re-bleeding is minimized, with hourly pump examinations for paracorporeal pumps for 24 h post procedure.

HEMOLYSIS

Hemolysis is a common phenomenon seen after VAD implantation, particularly in children. Patient size and anatomy, Device hemocompatibility profile, and shear stress, as well as anticoagulant management all contribute to increased hemolysis. Hemolysis can also be an important marker of device thrombosis, and thus early recognition is important. Routine laboratory monitoring with plasma free hemoglobin (product of erythrocyte destruction), lactate dehydrogenase (LDH), or haptoglobin should be considered. Increasing plasma free hemoglobin is associated with renal dysfunction. Supplemental iron, folic acid, and erythropoietin, along with a lower threshold for transfusions were recommended by (11).

VAD Acquired Hemostatic Pathophysiologies

High shear stress effects result in loss of high molecular weight von Willebrand (vWF) multimers which significantly reduces its size which in turn provides less hemostasis. Acquired von Willebrand syndrome (AVWS) is reported in patients following VAD placement. In a prospective cohort for 37 adult patients, significant loss of HMW vWF multimers was reported within 30 days of CF-VAD implantation, yet only 10 of the 37 patients experienced bleeding complications. This suggested that loss of HMW vWF multimers alone could not predict bleeding risk (68). Device hemocompatiblity is likely to be a factor in this syndrome, as AVWS in patients with HM III was less severe than in patients with HMII, which also correlated with less bleeding symptoms (69).

Platelets aggregation function is also believed to be impaired in VAD patients. In adult VAD patients, and unlike AVWS, platelet function defects were found equally present with both VAD types, HM II, and HM III (69).

FUTURE DIRECTIONS

Ventricular assist devices are increasingly used in children, not only as a bridge to transplantation, but also as bridge to decision and many patients are starting to discharge to home. Safe and effective anticoagulation is required for successful management of children supported with ventricular assist devices. Developmental hemostasis, device hemocompatibility, plastic to body ratio, surgical variable techniques, lack of

knowledge on pharmacokinetics of anticoagulants, and wide variability in anticoagulation protocols have all contributed to increased incidence of bleeding and thromboembolic complications. New collaborative learning networks, such as the ACTION network, provide opportunities to define best practices, optimize and reduce anticoagulation related adverse events. Changes in anticoagulation strategy from the use of heparin to a DTI such as bivalirudin have helped reduce bleeding and clotting complications. The future of this field lies in the development of VAD with improved biocompatibility profiles that can help reduce the need for anticoagulants therapies and reduce the risk of bleeding and thrombosis complications.

AUTHOR CONTRIBUTIONS

The main content of the article was provided by MG. CV and RT reviewed, minimally edited, and confirmed the article materials provided. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 02 March 2021
doi: 10.3389/fped.2021.627715

Hemostatic Challenges in Neonates

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The neonatal hemostatic system is strikingly different from that of adults. Among other differences, neonates exhibit hyporeactive platelets and decreased levels of coagulation factors, the latter translating into prolonged clotting times (PT and PTT). Since pre-term neonates have a high incidence of bleeding, particularly intraventricular hemorrhages, neonatologists frequently administer blood products (i.e., platelets and FFP) to non-bleeding neonates with low platelet counts or prolonged clotting times in an attempt to overcome these "deficiencies" and reduce bleeding risk. However, it has become increasingly clear that both the platelet hyporeactivity as well as the decreased coagulation factor levels are effectively counteracted by other factors in neonatal blood that promote hemostasis (i.e., high levels of vWF, high hematocrit and MCV, reduced levels of natural anticoagulants), resulting in a well-balanced neonatal hemostatic system, perhaps slightly tilted toward a prothrombotic phenotype. While life-saving in the presence of active major bleeding, the administration of platelets and/or FFP to non-bleeding neonates based on laboratory tests has not only failed to decrease bleeding, but has been associated with increased neonatal morbidity and mortality in the case of platelets. In this review, we will present a clinical overview of bleeding in neonates (incidence, sites, risk factors), followed by a description of the key developmental differences between neonates and adults in primary and secondary hemostasis. Next, we will review the clinical tests available for the evaluation of bleeding neonates and their limitations in the context of the developmentally unique neonatal hemostatic system, and will discuss current and emerging approaches to more accurately predict, evaluate and treat bleeding in neonates.

Keywords: neonate, bleeding, platelet function, hemostasis, platelet transfusion, FFP transfusion

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *10 November 2020*
Accepted: *18 January 2021*
Published: *02 March 2021*

Citation:
Davenport P and Sola-Visner M (2021) Hemostatic Challenges in Neonates. Front. Pediatr. 9:627715. doi: 10.3389/fped.2021.627715

CLINICAL OVERVIEW OF BLEEDING IN THE NEONATE

Neonates, especially those born pre-term, are at high risk of bleeding, making this a commonly encountered problem in the Neonatal Intensive Care Unit (NICU). A recent study utilizing a standardized and validated neonatal bleeding assessment tool (NeoBAT) found that 25% of all neonates admitted to eight different NICUs experienced an episode of bleeding during their hospitalization, with 11% of episodes categorized as major/severe bleeds, 1% as moderate, and 13% as minor bleeds (1). In the same study, pre-term neonates <28 weeks' gestational age were found to have a higher incidence of bleeding compared to infants born at >28 weeks' gestation, highlighting the increased bleeding risk associated with a lower gestational age at birth.

This high incidence of bleeding, particularly among pre-term neonates, is at least partially related to factors specific to the neonatal population. These unique risk factors include the trans-placental passage of some hemostatically active vitamins (i.e., vitamin K) or maternal

anti-platelet antibodies, and the developmental stage of blood vessels, the gastrointestinal tract, and the hemostatic system at varying gestational ages. In addition to these developmental stage-specific risk factors for bleeding, neonates are also at risk for more universal causes of bleeding due to their high incidence of sepsis, DIC, and frequent need for mechanical ventilation and critical care after birth. As such, the differential diagnosis for neonatal bleeding is broad and a thorough understanding of developmental stage, risk factors and underlying pathophysiology is critical to appropriately treat and try to prevent major bleeding in this vulnerable patient population. The goals of this review are to describe common clinical presentations of bleeding in neonates admitted to the Neonatal Intensive Care Unit (NICU), discuss the main developmental differences between neonatal and adult platelet function and hemostasis, review the tests available to evaluate and predict bleeding in neonates and their limitations, and evaluate current approaches to the management and prevention of clinically significant bleeding in infants. Of note, this review will not cover the hemostatic challenges of neonates on ECMO or neonates requiring cardiac surgery with cardiopulmonary bypass.

Intraventricular Hemorrhage (IVH)

Intraventricular hemorrhage (IVH) is one of the most serious complications of prematurity owing to the critical window for brain development that occurs during fetal and neonatal life. IVH puts infants at risk for long term neurodevelopmental morbidity and mortality and, despite improvements in IVH rates over recent decades, its incidence remains high, affecting 15–25% of very- and extremely- premature infants (<32 and <28 weeks' gestational age, respectively) in the first week of life (2). Intraventricular hemorrhages often originate in a highly vascularized collection of neuronal-glial precursor cells called the germinal matrix (3). This region is selectively vulnerable to hemorrhage in premature infants due to its developmental paucity of pericytes, immature basal lamina, and deficiency of glial fibrillary acidic protein, all of which result in vascular fragility (3). When this fragile vasculature encounters disturbances in cerebral blood flow due to the impaired cerebral autoregulation of premature infants, hemorrhage can result. If the hemorrhage in the germinal matrix is substantial, the immature ependyma breaks and the cerebral ventricles fill with blood, becoming visible on head ultrasound evaluation (3). A number of antenatal, perinatal, and post-natal factors have been found to be associated with disturbances in cerebral blood flow and possibly IVH (most importantly gestational age), but most times the specific clinical circumstance or factor leading to hemorrhage remains unidentified. Currently, the only proven intervention to decrease the rate of IVH is the administration of antenatal steroids to the mother. A recent study demonstrated that administration of antenatal steroids decreased the rate of IVH by 16% in 24-25+6/7-week gestation infants, by 12.9% in the 26-27+6/7-week gestation infants, and by 19.4% across the entire cohort (4). Research is ongoing to find better strategies to prevent and manage this significant complication of prematurity.

Pulmonary Hemorrhage

Pulmonary hemorrhage (PH) occurs in 3–5% of mechanically ventilated pre-term infants and in up to 8.6% of those born extremely premature (23–24 weeks' gestation) (5, 6). Additional risk factors include small for gestational age status, low Apgar scores, sepsis, presence of a patent ductus arteriosus (PDA), and severe respiratory distress syndrome (7). It most commonly occurs in the first 2 days of life and is associated with an increase in mortality overall and at 7 days of life (5). It has been hypothesized that PH occurs due to the rapid lowering of intrapulmonary pressure after administration of exogenous surfactant, which allows for an increase in pulmonary blood flow as blood shunts from the systemic to pulmonary circulation across the PDA (6). Currently, PH is managed with ventilator titration (increased positive end-expiratory pressure), administration of epinephrine via the endotracheal tube, and (after the acute phase) additional doses of surfactant. Platelet and/or FFP transfusions are frequently given to neonates with active PH, sometimes empirically in the setting of active bleeding and sometimes in response to decreased platelet counts or prolonged PT/PTT. However, the contribution of thrombocytopenia and/or coagulopathy to these hemorrhages remains uncertain.

Lower Gastrointestinal (GI) Hemorrhage

Bloody stools can be seen in well-appearing newborns due to common causes such as swallowed maternal blood during delivery, the presence of an anal fissure, or allergic colitis. These cases are not associated with disorders of hemostasis, and typically resolve with close monitoring and the removal of cow's milk protein from the diet (in the case of allergic colitis). In contrast, in critically ill premature infants, frank rectal bleeding is most frequently seen in the setting of necrotizing enterocolitis (NEC). Necrotizing enterocolitis is one of the most common and devastating diseases in pre-term neonates with an estimated incidence of 7% among infants with a birth weight between 500 and 1,500 g (8) and an estimated incidence of death of 20–30%, with the highest mortality seen in those infants who require surgery (9). The typical presentation of NEC is feeding intolerance, abdominal distension, and bloody stools in a pre-term infant after 8–10 days of life, associated with bowel wall ischemia and bacterial overgrowth. These symptoms progress rapidly over hours often leading to systemic hypotension and respiratory failure, and can culminate with bowel perforation requiring surgery. Infants with severe disease also frequently develop severe thrombocytopenia ± coagulopathy associated with disseminated intravascular coagulation, further predisposing them to bleeding in the GI tract and other sites. Recent work in an animal model of NEC identified thrombin generation as an early event in the pathogenesis of NEC (10), which might trigger platelet activation as well as intravascular thrombosis. In this mouse model, thrombin inhibition as well as platelet depletion attenuated the severity of the intestinal injury and reduced mortality, pointing to a contribution of this pathway to the pathophysiology of the disease and highlighting potential mechanisms through which platelets could worsen the disease process.

Minor Bleeding Events

In addition to the serious etiologies of neonatal bleeding mentioned above, neonates frequently experience minor bleeding events throughout their hospitalization. The presentations are numerous but include cephalohematoma sustained at birth, blood tinged endotracheal tube secretions in mechanically ventilated infants, and oozing from the umbilical stump or from sites of blood draws. It is unclear whether minor bleeding events are harbingers of more serious bleeding, but they often resolve with only close monitoring.

DEVELOPMENTAL DIFFERENCES IN HEMOSTASIS

Platelet Counts and Platelet Function in Neonates

The largest study on neonatal platelet counts conducted to date, which included ∼47,000 infants delivered between 22 and 42 weeks' gestation (11), showed that platelet counts increased during gestation by $\sim 2 \times 10^9$/L per week. Importantly, the mean platelet count was $\geq$200 x 10^9/L (well within the normal adult range) even in the most pre-term infants, but the 5th percentile was 104×10^9/L for those $\leq$ 32 weeks gestation and 123×10^9/L for late-pre-term and term neonates (11). This suggested that platelet counts between 100 and 150×10^9/L might be more frequent among extremely pre-term infants than among full term neonates or older children/adults, and that perhaps different definitions of thrombocytopenia should be applied to neonates at different gestational ages. Nevertheless, even in the most pre-term neonates –just like in adults- platelet counts <100 x 10^9/L are considered abnormal.

While platelet counts are similar in neonates and adults, there are substantial developmental differences in regard to platelet function. In *in vitro* platelet function assays, platelets from neonatal (full term) cord blood activate and aggregate less than adult platelets in response to traditional platelet agonists such as adenosine diphosphate (ADP), epinephrine, collagen, thrombin, and thromboxane analogs (12, 13). More recently, neonatal platelets were also found to exhibit a pronounced hyporesponsiveness to collagen-related peptide (CRP) and to the snake venom toxin rhodocytin, which activate the collagen receptor GPVI and the C-type lectin-like receptor 2 (CLEC-2), respectively (14). The few studies that have investigated platelet activation in pre-term neonates suggest that the platelet hyporeactivity might be more pronounced in premature infants compared to those born at term (13–15).

Different mechanisms contribute to the reduced responses of neonatal platelets to various agonists: 1. the hyporesponsiveness to epinephrine is due to a marked reduction in the number of α2-adrenergic receptors, the binding sites for epinephrine, on the surface of neonatal platelets; 2. the decreased responsiveness to thrombin is related to reduced expression of the thrombin receptors PAR-1 and PAR-4 in neonatal platelets (16); 3. the decreased response to thromboxane results from reduced signaling downstream from the receptor (17); 4. the reduced responses to collagen and rhodocytin result from mildly reduced expression of GPVI and CLEC-2, respectively, coupled with an intracellular signaling defect evidenced by reduced Syk and PLCγ2 phosphorylation following stimulation (14). Recently, developmental differences have also been described in regard to platelet *inhibitory* pathways, specifically a hypersensitivity of neonatal platelets to the inhibitory effects of prostaglandin E1 (PGE_1) during ADP- and collagen-induced platelet aggregation, associated with a functional increase in the PGE_1-cAMP-PKA axis (18).

Upon activation, in addition to experiencing a conformational change in the surface receptor GPIIb/IIIa that increases its affinity to fibrinogen, platelets secrete the content of their dense and alpha granules. In comparison with adult platelets, agonist-induced secretion of platelet granule content is reduced in both term and pre-term human neonates. In the case of dense granules, this might be related to the presence of fewer dense granules in neonatal compared to adult platelets (19). However, the 10-fold more abundant α-granules are present in similar numbers in neonatal and adult platelets (19–24). Recently, Caparros et al. demonstrated that the reduced exocytosis of alpha granules in neonatal platelets was associated with significantly reduced levels of syntaxin-11 and its regulator, Munc18b, which are SNARE proteins that mediate the fusion between vesicles and plasma membranes required for exocytosis (23). This provided a potential additional mechanism to explain the reduced degranulation of neonatal platelets in response to agonists.

Surprisingly, while the hypofunctional *in vitro* platelet phenotype would predict impaired primary hemostasis and a bleeding tendency, bleeding times (BTs) in healthy term neonates (using a device and technique modified to make a smaller skin incision) were found to be paradoxically ***shorter*** than BTs in adults (25). Similarly, studies using the Platelet Function Analyzer (PFA-100®, an *in vitro* test of primary hemostasis that measures the time it takes to occlude a small aperture, or Closure Time) found that cord blood samples from term neonates exhibited ***shorter*** closure times (CTs) than blood samples from older children or adults (26–29). These paradoxical findings, in the setting of platelet hyporeactivity, are explained by the presence of multiple factors in the blood of healthy neonates that enhance platelet/vessel wall interactions, including higher concentrations of circulating vWF with enhanced adhesive activity due to a predominance of ultralong polymers (30–33), higher hematocrits, and a higher MCV (34). These factors effectively counteract the platelet hyporeactivity, with the net effect of shorter bleeding times in neonates compared to adults (**Figure 1**). In support of the importance of the hematocrit on neonatal primary hemostasis, hematocrits below 28% in pre-term neonates were associated with longer bleeding times, which improved following red cell transfusion (35).

These compensatory mechanisms might be less well-developed in pre-term infants, whose platelets are also more hyporeactive than those of full-term infants, potentially leading to a more vulnerable primary hemostatic system. In fact, BTs performed on the first day of life were longer in pre-term compared with term infants, with neonates <33 weeks gestation exhibiting the longest BTs (36). PFA-100 CTs from

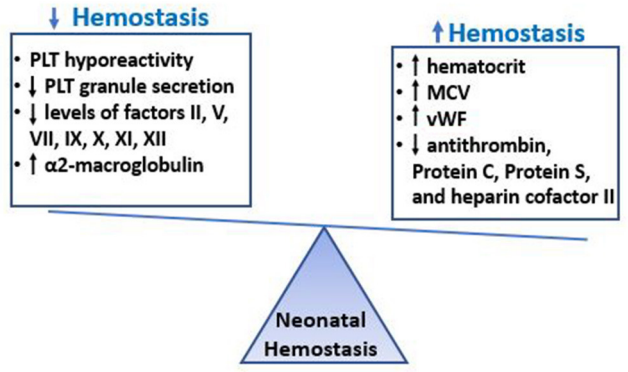

FIGURE 1 | Neonatal Hemostasis. The hemostatic system of neonates is characterized by decreased platelet reactivity and decreased levels of multiple coagulation factors, which would predict a bleeding phenotype. However, these findings are exquisitely equilibrated by other factors in neonatal blood that promote clotting, such as the increased hematocrit, MCV, VWF levels and the low levels of natural anticoagulants. The overall hemostatic balance in healthy term neonates is different from that of adults but well-balanced, and perhaps slightly tilted toward thrombosis, with shorter bleeding times and faster initiation of coagulation compared to healthy adults.

non-thrombocytopenic neonates were also inversely correlated to gestational age in both cord blood and neonatal peripheral blood samples obtained on the first day of life (37). However, CTs in pre-term neonates were still near or within the normal range for adults, suggesting that healthy pre-term neonates also have adequate primary hemostasis.

Neonatal platelet function, measured as platelet activation by flow cytometry, improves significantly post-natally and nearly normalizes by 10–14 days, in term as well as pre-term infants (13, 15). Consistently, Del Vecchio et al. found that all infants had shorter BTs by day of life 10 than at birth, and that early gestational age-related differences disappeared by then. Moreover, little or no further shortening occurred between days 10 and 30 (36). PFA-100 CTs are also significantly longer in the blood of neonates as early as on day of life 1–2 compared to cord blood, but remain similar to or shorter than those of adults (37).

Taken together, while the decreased platelet function of neonates has been invoked as a potential contributor to the high incidence of bleeding among neonates, particularly those born pre-term, the evidence suggests that–under normal circumstances- this platelet hyporeactivity is not a developmental defect, but rather an integral part of a developmentally distinct, but well-balanced neonatal primary hemostatic system. Key points related to neonatal primary hemostasis are summarized in **Table 1**.

Ontogenetically, these differences in platelet reactivity might be an important mechanism to prevent unwanted platelet activation and thrombosis during birth, a process frequently associated with tissue injury and epinephrine surges. However, how disease processes perturb this delicate system, and whether these disturbances contribute to bleeding, are unanswered questions.

The Neonatal Hemostatic System

There are also substantial differences between the fetal/neonatal and the adult coagulation system. In the classical coagulation cascade model, clotting factors interact and undergo a series of enzymatic reactions via the contact activation (intrinsic) and tissue factor (extrinsic) pathways, which converge on a final common pathway that culminates in thrombin formation. Coagulation factors do not cross the placenta because of their size, and are produced in the fetus starting at 11 weeks gestation. The levels of most coagulation factors increase during gestation and post-natally, and therefore are lower in pre-term compared to term neonates, and in term neonates compared to older children and adults. In full term infants, levels of most coagulation factors (particularly the vitamin K-dependent ones) are ∼50% of the adult levels, and increase to near-adult values by 6 months of age (38, 39). The activity of vitamin K-dependent factors is further reduced in pre-term infants, to ∼30% in 24–29 weeks' gestation infants. In contrast to vitamin K-dependent factors, neonates have normal levels of factor VIII, factor XIII and fibrinogen, and elevated levels of vWF, as described above (40). Keeping in mind these developmental differences in the activity levels of the different coagulation factors is essential to adequately interpret laboratory results in neonates.

The PT and PTT, the two most commonly used tests to evaluate coagulation status, are measures of the intrinsic and the extrinsic clotting pathways, respectively. Both tests were developed specifically to investigate coagulation factor deficiencies, and thus are longer in healthy pre-term and term neonates compared to adults, reflecting the lower activity levels of most coagulation factors in these populations. Andrew *et al.* published the first PT, PTT and fibrinogen reference ranges for healthy near-term and term infants in 1987 (30), followed by a study in pre-term neonates 30–36 weeks in 1988 (31). These studies included reference ranges for infants on days of life 1, 5, 30, 90, and 180, which showed rapid changes in the first few days after birth and through infancy. In a subsequent study, the same group measured the concentration of 33 components of the hemostatic system in children ages 1 to 16 and showed important physiologic differences between the hemostatic system of children and that of adults (41). Taken together, these observations led to the concept of "developmental hemostasis" to describe a series of age-related physiological changes of the coagulation system from fetal to neonatal to pediatric and ultimately to adult life.

Focusing on more pre-term infants, Christensen et al. published reference ranges for infants <34 weeks' gestation, using cord blood samples (42), and Neary et al. reported PT, PTT and fibrinogen reference ranges obtained on day of life 1 in a cohort of 183 infants born at <27 weeks' gestation, the group at highest risk of clinically significant bleeding. In this high-risk cohort, the median (range) PT was 20.2 (14.4–36.7) s, PTT was 67.4 (34.9–191.6) s and fibrinogen 1.4 (0.5–4.8) g/L (43). These values were higher than those reported by Christensen et al., which could have been related to the source of the blood (cord blood vs. neonatal blood) and/or to the use of different reagents and testing systems (44). Taken together, these studies indicated that the upper limits for both PT and PTT values are

TABLE 1 | Key points regarding neonatal primary hemostasis and platelet transfusions.

Platelet count and function	• Mean platelet counts are within the normal adult range even in the most pre-term neonates • Neonatal platelets are hyporeactive in response to most agonists *in vitro* (pre-term > term) • Platelet reactivity improves to near-adult levels by day of life 10–14
Compensatory factors	This platelet hyporeactivity is balanced by factors in neonatal blood that enhance platelet/vascular wall interaction: • Increased hematocrit • Increased MCV • Increased vWF and predominance of ultralong multimers
Tests of primary hemostasis	• Bleeding times are shorter in healthy neonates than in adults • PFA-100 closure times are shorter in term cord blood samples than in adult blood samples. • These suggest enhanced primary hemostasis in neonates, despite their platelet hyporeactivity.
Effects of platelet transfusions	• Platelet transfusions should be administered to thrombocytopenic neonates *with active bleeding* • Non-bleeding pre-term neonates who received prophylactic platelet transfusions for PC $<50 \times 10^9$/L had significantly *higher* bleeding and mortality compared to neonates transfused only for PC $<25 \times 10^9$/L. • High risk, critically ill neonates benefit from the lower transfusion threshold as much as low risk stable neonates.

TABLE 2 | Neonatal reference ranges for common coagulation tests measured on day of life 1, by gestational age.

	PT (s)	PTT (s)	Fibrinogen (mg/dL)
<27 weeks*	14.4–36.7	40.5–158.5	70–480
28–34 weeks**	13.9–20.6	30–57	87–470
30–36 weeks*	10.6–16.2	27.5–79.4	150–373
Full Term***	10.1–15.9	31.3–54.5	167–399

Reference ranges reflect 2.5th–97.5th percentiles (31, 43).
***Reference ranges reflect 5th–95th percentiles (42).*
****Reference ranges calculated from mean ±SD (2SD below and above the mean) (30).*

higher among healthy extremely pre-term neonates than among moderately pre-term or term infants (**Table 2**), consistent with the developmental differences in clotting factor levels. However, both PT and PTT decrease rapidly in the first few days after birth, with significantly lower levels noted on day of life 3 compared to day of life 1 in extremely pre-term neonates (45).

In 2006, Monagle et al. published comprehensive reference ranges for coagulation tests measured in healthy term neonates on days of life 1 and 3 and in children ranging from 1 month to 16 years, using more modern coagulation testing systems (44), and confirmed the concept of developmental hemostasis initially described by Andrew et al. The physiologic changes in the coagulation system over the course of development and their implications for clinical practice have been described in detail in recent reviews (39, 46). However, it is important to keep in mind that the actual values of the various tests vary depending on the reagents and analyzer system utilized, in addition to the patient's age (44). Thus, individual coagulation laboratories need to develop age-related reference ranges using their own testing systems, in order to interpret the results adequately.

The "prolongation" of the PT and PTT in neonates and particularly in extremely pre-term neonates, who have the highest incidence of major bleeding, has been frequently interpreted as a hemostatic defect and has led to the practice in many Neonatal Intensive Care Units (NICUs) of routinely checking coagulation tests and administering FFP to non-bleeding pre-term neonates with "abnormal" values to try to prevent bleeding, particularly IVH (43, 47). Paradoxically, however, studies by Cvirn et al. using assays with physiologic amounts of tissue factor found adequate and *faster* thrombin generation (the final product of the coagulation cascade) in neonates compared to adults (48). More recently, Neary et al. found that thrombin generation was similar in very pre-term compared to term cord blood samples, despite differences in PT and PTT levels. Interestingly, lag time and time to peak thrombin generation were ***shorter*** in pre-term compared to term cord blood samples, indicating *faster* thrombin generation in the pre-term infants (45).

While the reasons for the discrepancy between prolonged coagulation times and adequate/faster thrombin generation in pre-term compared to term infants (and in neonates compared to adults) are not fully understood, they likely involve the co-existent developmental differences in *anticoagulant* pathways in neonates, which are not well-reflected in the PT and PTT assays. Indeed, just like most coagulation factors are decreased in neonates, most natural anticoagulants are also reduced at birth (30, 31, 41), which results in a balanced neonatal hemostatic system. Specifically, antithrombin (AT), heparin cofactor II and proteins C and S levels are significantly reduced in neonates (both term and pre-term), ∼50% of adult levels at birth (39). Levels of these anti-coagulants increase slowly after birth, and AT and heparin cofactor II reach adult levels by 3 months of age (41). Perhaps to compensate for these low levels, another anticoagulant, α2-macroglobulin, is present at higher levels in neonates than in adults, and further increases over the first 6 months of age (30, 41). Taken together, the evidence suggests that the prolonged PT and PTT found in otherwise healthy pre-term and term neonates should not be interpreted as a developmental deficiency or a bleeding tendency, but rather as a limitation of these tests to reflect the complexities of a developmentally unique but well-balanced neonatal hemostatic system (**Figure 1** and **Table 3**).

EVALUATION OF BLEEDING AND PREDICTION OF BLEEDING RISK IN NEONATES

Platelet Counts

Platelets are essential for primary hemostasis. For that reason, a platelet count is always part of the initial evaluation of a neonate who presents with abnormal bleeding. In that setting,

TABLE 3 | Key points regarding neonatal secondary hemostasis and FFP transfusions.

Coagulation factor levels	• Neonates have reduced levels of most coagulation factors, particularly vitamin K-dependent factors • FVIII, FXIII, and fibrinogen levels are normal • vWF levels are elevated • Coagulation factor levels change in neonatal life, infancy and childhood following specific patterns (developmental hemostasis).
Natural anticoagulant levels	• Neonates have low levels of AT, HCII, protein C and protein S • α2-macroglobulin is present at higher levels in neonates than in adults
Laboratory evaluation of hemostasis	• PT and PTT are longer in healthy neonates than in healthy adults (pre-term > term) and decrease in the first few days after birth • Actual values for tests of coagulation vary depending on the source of the sample (cord blood vs. neonatal blood), reagents and testing systems used. • However, thrombin generation is faster in neonates than in adults (pre-term > term) • Tests of whole blood hemostasis (TEG, ROTEM) show faster initiation and propagation of coagulation in neonates compared to adults
FFP transfusions	• FFP should be administered to neonates who present with bleeding associated with coagulation factor(s) deficiency, if the specific factor is not known or available. • Prophylactic FFP transfusions given empirically to pre-term neonates or in response to "prolonged" PT or PTT do not decrease the incidence or severity of IVH.

vWF, von Willebrand Factor; AT, antithrombin; HCII, Heparin cofactor II; IVH, Intraventricular hemorrhage.

the finding of thrombocytopenia (either isolated or as part of a coagulopathy) is an important diagnostic clue. Severe isolated thrombocytopenia in an otherwise healthy neonate should raise suspicion for Fetal/Neonatal Alloimmune Thrombocytopenia (caused by the transplacental passage of maternal alloantibodies directed against antigens in the fetal platelets) which is associated with an incidence of bleeding of 10–20% (49, 50). Isolated thrombocytopenia can also be found in neonates with a history of intrauterine growth restriction or with viral, bacterial or fungal infections. In critically ill neonates with bleeding in the setting of sepsis, NEC, or severe perinatal asphyxia, thrombocytopenia is frequently present, either in isolation or as part of a picture of disseminated intravascular coagulation (DIC).

The utility of the platelet count as a predictor of bleeding risk in neonates *without* abnormal bleeding is much more controversial, although in neonatal clinical practice thrombocytopenia has been widely considered a risk factor for bleeding, particularly among pre-term infants. In support of an association between thrombocytopenia and bleeding, a recent study of 972 very-low-birth-weight infants (VLBW, <1,500 g at birth) found that having a platelet count <150 $\times$ 10^9/L in the first week of life was associated with a higher incidence of IVH (hazard ratio 2.17; 95% CI, 1.53–3.08; $p <$ 0.001). However, association does not imply causality, and the same study found no correlation between the severity of thrombocytopenia and the risk for IVH (51). The latter finding, consistent with several other studies (52–56), raised serious questions regarding the value of the platelet count as a marker of bleeding risk in pre-term neonates, and the effectiveness of platelet transfusions at preventing hemorrhage in non-bleeding neonates with mild to moderate thrombocytopenia (see Platelet Transfusions below). Interestingly, the same lack of correlation between severity of thrombocytopenia and significant bleeding has been reported in pediatric patients with chemotherapy-induced thrombocytopenia (57), suggesting that factors other than the platelet count might be the main determinants of bleeding risk in thrombocytopenic children as well as neonates.

Recently, other approaches have been suggested to assess bleeding risk in thrombocytopenic pre-term neonates. In a study evaluating platelet function in pre-term and term neonates by flow cytometry, fibrinogen binding and degranulation responses to ADP were significantly reduced in septic compared to healthy neonates, raising the possibility that tests of platelet function might eventually contribute to identify neonates at high risk of bleeding (58). However, whether these platelet functional differences are associated with a higher incidence or severity of bleeding remains to be determined. Given the unique features and dynamic nature of the neonatal primary hemostatic system, we hypothesized that a whole blood test of primary hemostasis, such as the closure time in response to collagen and ADP (CT-ADP) measured in the Platelet Function Analyzer-100 (PFA-100), would be a better marker of bleeding risk in pre-term neonates than the platelet count or the platelet function alone, since it would measure the combined effects of platelet count, platelet function, hematocrit, and vWF levels on a baby's primary hemostatic ability. Indeed, in a cohort of 54 infants with gestational age <27 weeks, we found a significant correlation between median CT-ADP (but not platelet count) and bleeding severity, quantified using a validated neonatal Bleeding Assessment Tool (55). Furthermore, changes in the CT-ADP were strongly correlated with changes in the bleeding score, while changes in platelet counts were not (59). This study suggested that tests of whole blood primary hemostasis might be more useful markers of bleeding risk among thrombocytopenic pre-term neonates than platelet counts, although the relatively high volume of blood required for the CT-ADP (800 μL) precludes its current widespread use in this population. Finally, Fustolo-Gunnink and collaborators developed a dynamic model to predict major bleeding in pre-term neonates at any time-point during the first week after the onset of severe thrombocytopenia, which incorporated the variables gestational age, post-natal age,

intrauterine growth retardation, NEC, sepsis, platelet count and mechanical ventilation (60). While not yet prospectively validated, this is a promising approach to making individualized treatment decisions in this population and has the unique advantage of incorporating *in vivo* risk factors that cannot be captured by any laboratory test.

PT/PTT

As previously described, the PT and PTT can be prolonged in neonates at baseline, especially in those born premature, likely reflecting an aspect of the developmentally unique neonatal hemostatic system. Thus, it is difficult to decide how to interpret and respond to these values in a non-bleeding neonate, and studies have shown no association between results of these tests and IVH rates in pre-term neonates (42, 45). However, when faced with neonatal bleeding of unclear etiology, the PT and PTT can be very useful to evaluate for specific coagulation factor deficiencies, which can present with clinical bleeding and a prolongation of either the PT or PTT beyond that seen in healthy neonates (**Table 4**).

Vitamin K Deficiency of the Newborn (Previously Hemorrhagic Disease of the Newborn)

Clinically, infants suffering from Vit K deficiency present with visible frank bleeding from the nose, umbilicus, skin, urinary tract, GI tract, and from sites of needle pricks. Less visible, but more devastating, they also can suffer from intracranial, pulmonary, and massive GI bleeding (61). Vit K deficiency leads to a reduction in the activity of the Vit K-dependent coagulation factors II, VII, IX, X, and of the anticoagulant protein C and protein S. It should be suspected in a neonate who presents with abnormal bleeding and a marked prolongation of the PT, which measures the activity of three of the four Vit K-dependent factors (II, VII, and X) (61). Vit K deficiency has been classified into early, classical, and late disease, each with unique etiologies and presentations (**Table 5**). Early Vit K deficiency is rare and presents in the first 24 h of life in an infant whose mother took medications during pregnancy that interfere with Vit K metabolism (such as warfarin, phenytoin, rifampin, and isoniazid). Classical Vit K deficiency presents between 2 and 7 days of birth and is likely due to inadequate oral feeding, given how critical the successful establishment of breast feeding is to Vit K status (62). Late Vit K deficiency presents between 8 days and 6 months of life. This form nearly always occurs in exclusively breast-fed infants and has a higher incidence of intracranial hemorrhage, which is often the presenting sign (63). Many of the affected infants have hepatobiliary dysfunction, resulting in cholestasis and impaired secretion of bile salts that lead to malabsorption of Vit K (64). With the nearly universal administration of intramuscular Vit K after birth, the incidence of Vit K deficiency (in particular the classical form) markedly decreased. However, the increasingly frequent refusal to Vit K administration among parents in westernized countries has led to a trend of increasing cases. Some healthcare systems or families opt for a multi-dose oral regimen of Vitamin K after birth, but this is not recommended due to concerns for poor enteral absorption and compliance. Neonates who present with acute bleeding due to Vit K deficiency should be treated upon suspicion with intravenous Vit K, which will reverse the coagulopathy (62). However, due to the time required for Vit K to take effect, FFP should also be administered immediately to prevent devastating intracranial hemorrhage.

TABLE 4 | Typical test result patterns in bleeding disorders that can present in the neonatal period.

Disease	PT	PTT	Platelets
Hemophilia A	Normal	↑↑↑	Normal
Hemophilia B	Normal	↑↑↑	Normal
Factor XIII Deficiency	Normal	Normal	Normal
Vitamin K Deficiency of the Newborn	↑↑	Normal	Normal
vWD type 3	Normal	↑↑↑	Normal
DIC	↑↑	↑↑	↓↓↓

vWD, Von Willebrand Disease; DIC, Disseminated Intravascular Coagulation.

TABLE 5 | Classification of vitamin K deficiency of the newborn.

	Risk factors	Clinical presentation
Early (24 h of life)	Maternal medications: • Vitamin K antagonists • Anticonvulsants • Tuberculosis drugs	• Umbilical stump bleeding • Cephalohematoma • ICH
Classical (1–7 days of life)	Inadequate Vitamin K due to: • Lack of prophylaxis • Poor breastfeeding	• GI bleeding • Mucocutaneous bleeding • Oozing at umbilicus or circumcision site • ICH
Late (8 days to 6 months of life)	• Exclusive breastfeeding • Poor feeding • GI disorders • Liver disease • Pancreatic disease	• GI bleeding • Mucocutaneus bleeding • Very high risk for ICH • Death

ICH, Intracranial hemorrhage; GI, Gastrointestinal.

TABLE 6 | Reasons for diagnostic testing in newborns with hemophilia*.

Number of infants ages 0–2 years	864
Number diagnosed within 1 month of birth	633 (73%)
Reason for diagnosis	
Carrier mother	299 (47.2%)
Other family history	147 (23.2%)
Bleeding event	182 (28.8%)
Unknown	5 (0.8%)

**Data from the Universal Data Collection (UDC) 2010 update (65).*

Hemophilia A and B

Hemophilia A and B, caused by deficiencies of Factor VIII and Factor IX, respectively, are the most common inherited bleeding disorders that present in neonatal life. They are classically inherited in a X-linked recessive pattern, but ∼1/3 of cases are due to spontaneous genetic mutations with no family history. The diagnosis of hemophilia is occurring at earlier ages, with over half of cases now being diagnosed in the neonatal period (65). Reasons prompting testing and leading to the diagnosis in newborn infants are listed in **Table 6**. Unlike older children with hemophilia, who present with hemarthroses, neonates typically present with iatrogenic bleeding (oozing or excessive hematoma formation after venipuncture or intramuscular Vit K administration), excessive bleeding after circumcision, or intracranial/extracranial bleeding (66–68). In a survey of 102 neonates with cranial bleeds and hemophilia, the mean age at diagnosis was 4.5 days, and intracranial hemorrhages (most frequently subdural) were more common than extracranial bleeds (such as cephalohematomas and subgaleal hemorrhages) (69). A clinical suspicion of hemophilia is supported by an isolated prolongation of the PTT, but definitive diagnosis requires measurement of Factor VIII or IX. Since factor VIII levels are within the normal adult range in both pre-term and term neonates, it is possible to diagnose hemophilia A of any severity in the neonatal period. However, this is not true for Factor IX, which shows reduced levels at birth and an even further reduction in infants born pre-term. Thus, severe hemophilia B can be diagnosed at birth, but confirmation of mild cases requires repeat testing at 6 months of age or genetic analysis (if a familial genetic defect is known). The delivery room management of an infant with known or suspected hemophilia has been the subject of multiple retrospective studies (69–72). Current recommendations state that there is no contraindication to a vaginal delivery, but an instrumented delivery (i.e., forceps, vacuum extraction, and the use of scalp electrodes) should be avoided and early transition to cesarean delivery is recommended if there is a failure of labor (73, 74).

Isolated Coagulation Factor Deficiencies

Outside of hemophilia A and B, neonates can inherit deficiencies of other isolated coagulation factors (75). Von Willebrand disease (VWD) is the most frequent inherited bleeding disorder, affecting ∼1% of the population. It is classified into three categories based on the quantitative level or function of von Willebrand Factor (vWF). Type I is due to a quantitative deficiency of vWF and typically has a mild presentation with mucosal bleeding. Type II is due to a qualitative defect in vWF and is divided into four subtypes, which are associated with more severe bleeding phenotypes than Type I. Due to the increased levels of vWF and the presence of high molecular weight vWF multimers in neonates, these types do not typically present in neonatal life. However, type III VWD is the most severe form, due to a complete or almost complete deficiency of vWF, and this form can present in neonates with a phenotype similar to severe hemophilia A (76).

Other factor deficiencies can present in the neonatal period but are rare and diagnosis requires an astute clinician with a high index of suspicion and often assistance by a pediatric hematologist. Deficiency of Factor XIII, which is responsible for cross linking fibrin and stabilizing clots, classically presents with delayed umbilical cord hemorrhage but a normal PT and PTT. Thus, the diagnosis requires a high index of suspicion prompting the measurement of Factor XIII levels. Alpha2-antiplasmin and plasminogen activator inhibitor-1 both act to reduce plasmin activity and deficiency of either is extremely rare, but should also be considered when the PT and PTT are normal in a neonate with abnormal bleeding (76). In afibrinogenemia and in Factor II, V, and X deficiency the PT and PTT are both prolonged (76–78). Finally, Factor VII deficiency is a rare, heritable bleeding disorder with variable presentation and over 250 causal mutations (79). Neonates often present with multifocal spontaneous bleeding in the first few days of life that can range from epistaxis, gum bleeding and hematomas to hemarthrosis and life-threatening cerebral and gastrointestinal hemorrhages (80). Coagulation studies reveal a prolonged PT, and the diagnosis is confirmed by low Factor VII levels (75).

Tests of Global Hemostasis: TEG and ROTEM

Thromboelastography (TEG) and Rotating Thromboelastometry (ROTEM) are both viscoelastic point-of-care tests that offer rapid global assessments of whole blood hemostasis in small volumes of whole blood, making them ideal tests for neonatal patients with small blood volumes. Both assays provide information on platelet function, clot formation, tensile strength of the clot, and subsequent clot lysis. While similar, the values obtained from TEG and ROTEM assays are not interchangeable, but both can help guide the selection of blood products for transfusion in a bleeding patient. Given the developmental differences in neonatal hemostasis suggested by standard coagulation tests (PT/PTT), investigators have been interested in comparing measures of global hemostasis between neonates and adults using these assays. Multiple studies have found contrasting results, likely due to differences in sample collection (umbilical cord blood vs. peripheral venous or arterial blood) and in anticoagulant used. Initial small studies using the TEG assay in neonates did not demonstrate differences in fibrin clot formation, clot strength, or rate of fibrinolysis (81, 82), but more recent studies found that neonates have *faster* initiation and propagation of coagulation (83–86), consistent with the faster thrombin generation described above. This relative pro-coagulant state seen on TEG, despite prolongation of conventional coagulation tests, reinforces the theory that neonatal hemostasis is not defective, but rather carefully balanced in a developmental stage-specific manner. A study comparing ROTEM values in pre-term vs. full-term infants found that maximal clot firmness (MCF) was significantly lower in pre-term compared to full-term neonates (87). However, this and a subsequent study (84) found no association between any TEG parameter and the occurrence of post-natal complications in pre-term infants, including intraventricular hemorrhage (IVH). Conversely, a recent study comparing values of healthy neonates to those of bleeding neonates at different gestational ages found statistically significant differences in assay values

that were associated with clinical bleeding (86). This study also described reference ranges for both citrate-modified and heparinase-modified TEG values, given the frequency of these modifications in neonatal blood samples.

While TEG has been studied more extensively in neonates, ROTEM has only recently begun to be used in this population. One study described ROTEM references ranges in a pediatric cohort from 0 months to 16 years and found differences among all age groups (83). The most striking differences were with infants 0–3 months of age, who exhibited accelerated initiation and propagation of coagulation and increased clot firmness despite prolonged standard plasma coagulation tests (PT/PTT) (83). As reference ranges have become published, investigators have started assessing the utility of the ROTEM to evaluate coagulation in common neonatal clinical conditions, such as sepsis and congenital heart disease (CHD). Initially, one group found that a hypercoagulable ROTEM profile on the first day of neonatal sepsis was associated with disease severity (88). In a more recent study, the same group found that septic neonates were more likely to show fibrinolysis shutdown on ROTEM than non-septic neonates, but the test could not discriminate septic from non-septic neonates and could not predict clinical outcomes (89). It has also been well-described that neonates with CHD often have coagulation abnormalities that result in an increased incidence of bleeding (90, 91). One study evaluated hemostasis in these infants with ROTEM and found that neonates with cyanosis secondary to CHD had inferior clot formation as well as a higher incidence of abnormal parameters when compared to non-cyanotic CHD patients and healthy controls (92), suggesting that cyanosis and/or polycythemia may have an impact on hemostasis.

INTERVENTIONS TO MANAGE AND PREVENT BLEEDING IN NEONATES

Platelet Transfusions

Platelets are essential for hemostasis and contribute substantially to clot formation. For that reason, in the setting of acute bleeding, neonates are frequently transfused platelets if the platelet count is $<100 \times 10^9$/L, or empirically in cases of life-threatening acute hemorrhage (i.e., as part of a massive transfusion protocol). However, the majority of platelet transfusions in the NICU are given to non-bleeding neonates, when the platelet count falls below an arbitrary level below which the risk of bleeding is thought to increase.

Historically, it has been widely accepted that thrombocytopenic pre-term neonates should receive platelet transfusions at higher platelet count (PC) thresholds than older children and adults due to their high incidence of spontaneous intracranial bleeding, particularly intraventricular hemorrhage (IVH). Over the last decade, several surveys and observational studies revealed a striking world-wide variability in neonatal platelet transfusion thresholds, and an overall more liberal approach to platelet transfusions in U.S. compared to European NICUs (93, 94). This variability was at least in part due to the paucity of high-level evidence in the field. Until recently, there was only one randomized trial of platelet transfusion thresholds in pre-term neonates, published 25 years ago (95). That study randomized 152 Very-Low-Birth-Weight (VLBW, <1,500 g at birth) neonates to receive platelet transfusions for platelet counts $<150 \times 10^9$/L or $<50 \times 10^9$/L in the first week of life, and found no differences in the incidence of new IVH or extension of existing IVH (the primary outcome) between the two groups (95). These results likely formed the basis for the use of 50×10^9/L as the most frequent threshold for platelet transfusions in pre-term neonates.

The recently published much larger PlaNeT-2 multicenter trial randomized 660 thrombocytopenic neonates with a median gestational age of 26.6 weeks and a median birth weight of 740 grams to receive platelet transfusions at platelet count thresholds of $<50 \times 10^9$/L (<50 group) or $<25 \times 10^9$/L (<25 group). Unlike in the prior study, infants were randomized at any time during their NICU hospitalization when the platelet count fell below 50×10^9/L, and the primary outcome was a composite of death or new major bleeding within 28 days of randomization (96). Ninety percent of infants in the <50 group and 53% in the <25 group received at least one platelet transfusion. Unexpectedly, infants in the <50 group had a significantly *higher* rate of mortality or major bleeding within 28 days of randomization compared to those in the <25 group (26 vs. 19%, respectively; odds ratio 1.57, 95%CI 1.06–2.32). In a subgroup analysis, findings were similar for neonates <28 weeks' gestation, the group at highest risk of bleeding and death (53, 55).

While these findings might have seemed surprising at first, they were in fact consistent with a number of prior observational studies describing a poor association between severity of thrombocytopenia and bleeding risk (51, 53–55), a lack of effectiveness of platelet transfusions to prevent bleeding in neonates (51, 97), and an association between number of platelet transfusions and neonatal mortality and morbidity (98–101).

The results of PlaNeT-2 provided high-level evidence in support of these concepts, although the possibility that the benefits of the lower transfusion threshold would be limited to clinically stable infants with a low risk of bleeding and/or death led to initial skepticism. This question was largely addressed in a follow-up study in which a multivariable logistic regression model was developed (as described above) (60) and used to predict the baseline bleeding/mortality risk of neonates enrolled in PlaNeT-2 (102). Based on their model-predicted baseline risk, 653 neonates in PlaNeT-2 were divided into four quartiles (very low, low, moderate, and high risk) and the absolute risk difference between the <50 group and the <25 group was assessed within each quartile. Interestingly, the lower transfusion threshold was associated with an absolute risk reduction in all four groups, varying from 4.9% in the lowest to 12.3% in the highest risk group. These results suggested that using a lower ($<25 \times 10^9$/L) prophylactic platelet transfusion threshold is beneficial even in high risk neonates (**Table 1**).

Although these studies provided strong support for the use of lower platelet transfusion thresholds in non-bleeding pre-term infants, some uncertainties remain. First, only 37% of infants in the study were randomized by day of life 5 and 59% by day 10, the period when most clinically significant hemorrhages occur in

pre-term neonates (66). While this might have simply reflected the time of onset of thrombocytopenia in the study population, 39% of infants in PlaNeT-2 received one or more platelet transfusions prior to randomization, for unknown reasons and at non-specified platelet counts. This raises the question of whether these transfusions were given during the first few days of life, the highest risk period for IVH in pre-term neonates.

Nevertheless, the results of PlaNeT-2 provided strong support for the hypothesis that platelet transfusions may have deleterious effects in neonates, which could be mediated by various potential mechanisms. Neonates in PlaNeT-2, consistent with routine neonatal practices, were transfused with 15 mL/Kg of a platelet suspension. This is a substantially higher volume than that used in older children or adults, who usually receive ∼5 mL/Kg of a standard platelet suspension. This high transfusion volume, combined with the fragile vasculature of pre-term neonates (see Intraventricular Hemorrhage above) and a rapid rate of transfusion, raises the possibility that the platelet transfusion itself could have caused or extended intraventricular hemorrhages through rapid volume expansion, thus providing a potential explanation for the higher incidence of major bleeding in the <50 compared to the <25 group. It has also been hypothesized that a "developmental mismatch" occurs when adult platelets are given to neonates. As reviewed above, adult platelets are functionally hyperreactive compared to neonatal platelets, and *in vitro* mixing studies found that adult platelets added to neonatal thrombocytopenic blood (with its higher hematocrit, MCV, and vWF levels) can induce a prothrombotic phenotype (103). Finally, it has become increasingly clear over the last decade that platelets have important functions beyond hemostasis, including as central mediators and modulators of inflammation (104, 105). Thus, it is plausible that some of the pathogenic effects of platelet transfusions on neonates could be mediated through inflammatory pathways. Additional work is needed to elucidate which of these potential mechanisms contribute to the increased mortality and morbidity associated with the liberal use of platelet transfusions in neonates, but in the meantime the data suggest that non-bleeding neonates (regardless of severity of illness) benefit from a restrictive platelet transfusion threshold.

Fresh Frozen Plasma

Fresh frozen plasma (FFP) contains all of the clotting factors, fibrinogen, plasma proteins, electrolytes, protein C, protein S, antithrombin, and tissue factor pathway inhibitor. It is used primarily to replenish coagulation factors and is clinically indicated in the setting of hemorrhage, bleeding or severe coagulopathy due to multiple coagulation factor deficiencies secondary to liver disease, DIC, or congenital factor deficiencies for which there is no concentrate available (i.e., Factor V or XI deficiencies). While these are clear indications, for which FFP administration can be life-saving, FFP is most frequently given to non-bleeding neonates, especially those who are critically ill, either with the goal of preventing bleeding and/or for non-hematological indications (i.e., volume expansion in an infant with massive capillary leak). It has been estimated that 6–12% of all NICU admissions receive at least one FFP transfusion (106, 107), and in a study by Stanworth and colleagues 62% of infants who received FFP did not have signs of clinical bleeding at the time of transfusion, and 14% did not even have coagulation tests prior to FFP administration (108). Studies examining the success rate of FFP transfusion normalizing neonatal clotting times found success rates ranging from 40 to 60%, depending on the dose administered (107–109). The success rate of clotting time correction increased to 59–68% if neonatal reference ranges for coagulation factors were used (42). However, multiple studies have shown that, regardless of clotting time correction, administration of FFP does not change clinical outcomes. One study looking at FFP administration in the setting of DIC found no improvement in coagulation tests or in overall survival (110). Four studies have investigated the use of prophylactic FFP transfusion in pre-term neonates to decrease the incidence of IVH: One found a decrease in IVH with FFP administration, but the other three reported similar IVH rates in their control and treatment arms (111–114) and a meta-analysis found no differences in grade of IVH or mortality (115) (**Table 3**). Additional controlled studies have found no benefit of FFP administration to non-bleeding neonates with sepsis, respiratory distress syndrome, hypotension, or hypoxic ischemic encephalopathy (116–120). Despite this growing evidence, FFP continues to be administered to non-bleeding neonates outside of the evidence-based recommendations, in many instances in response to "prolonged" coagulation tests that might be developmentally appropriate.

Recombinant FVIIa and Prothrombin Complex Concentrate

Recombinant Factor VIIa (rFVIIa) is a genetically engineered coagulation protein initially created for the treatment of bleeding in patients with hemophilia and antibodies against standard coagulation factor replacements. It is extremely effective in activating the final pathway of the coagulation cascade and has been perceived by some as a "universal hemostatic agent," prompting its frequent off-label use in bleeding patients without hemophilia. Since bleeding and coagulation disorders are common in neonates, rFVIIa is an attractive solution to an unsolved problem. Several case reports have described the successful administration of rFVIIa to pre-term and term neonates with intractable hemorrhage and/or severe coagulopathy (121). One retrospective report of 29 neonates found that earlier rFVIIa administration (<24 h from beginning of bleeding) was associated with a statistically significant improvement in survival (121) and with a decreased need for subsequent blood products. However, concerns have been raised regarding the safety profile of rFVIIa, specifically a potential increase in thrombotic events (122). A systematic review of neonates receiving rFVIIa or FFP found no difference in the occurrence of thrombotic events in neonates with bleeding or coagulopathy (123). However, while multiple randomized controlled trials of rFVIIa administration have been performed in adults, only three studies have included children with a total of 11 neonates,

and so high-quality data is lacking to guide the safe use of rFVIIa in this population and concerns over increased risk of thrombosis remain.

Prothrombin complex concentrate (PCC) is a human plasma-derived product containing the vitamin K-dependent coagulation factors and the vitamin K-dependent clotting inhibitor proteins. The current indications for PCC are the treatment and perioperative prophylaxis of bleeding in congenital or acquired deficiency of prothrombin complex coagulation factors. Increasingly, it has been used off-label in hopes of preventing and treating severe bleeding in neonates. In 2002, The Guideline for the Investigation and Management of Neonatal Hemostasis and Thrombosis recommended considering PCC when high volume products (such as FFP) need to be avoided or in the presence of hemorrhage due to depleted factors (124). Due to its smaller volume, PCC can be infused quicker than, and corrects the INR faster than FFP (41 vs. 115 min) (124, 125). As with rFVIIa, there are no randomized controlled studies to guide the safe use of PCC in the neonatal population, but a recent retrospective study examined 37 neonates with intractable bleeding or severe coagulopathy who received PCC as a rescue intervention. In this study, hemostasis was achieved in the majority of infants and there was a statistically significant improvement in PT, INR, and PTT. Thirteen out of 24 neonates survived. PCC had been administered to the neonates who survived within 24 h of bleeding initiation and no thrombotic events were observed (126). As with rFVIIa, randomized controlled trials or prospective controlled studies are needed to determine the efficacy and safety of PCC in the neonatal population before it can become part of the standard care of a bleeding neonate.

CONCLUSIONS

The neonatal hemostatic system is strikingly different from that of adults in that neonates exhibit comparatively hyporeactive platelets and decreased levels of coagulation factors, the latter translating into prolonged clotting times (PT and PTT). Since pre-term neonates have a high incidence of bleeding, particularly IVH, neonatologists frequently administer blood products (i.e., platelets and FFP) based on arbitrary laboratory thresholds in an attempt to overcome these "deficiencies" and reduce the bleeding risk. However, it has become increasingly clear that both the platelet hyporeactivity as well as the decreased coagulation factor levels are effectively counteracted by other factors in neonatal blood that promote hemostasis (i.e., high levels of vWF, high hematocrit and MCV, reduced levels of natural anticoagulants), resulting in a well-balanced neonatal hemostatic system, perhaps slightly tilted toward a prothrombotic phenotype (**Figure 1**). While life-saving in the presence of active major bleeding, the administration of platelets and/or FFP to non-bleeding neonates based on laboratory tests has not only failed to decrease bleeding, but has been associated with increased neonatal bleeding and mortality in the case of platelets. Given the unique features of neonatal hemostasis, there has been interest in exploring the potential use of new tests of whole blood hemostasis (i.e., TEG or ROTEM) or primary hemostasis (i.e., PFA-100 CT-ADP) to predict and/or manage bleeding in neonates. However, more studies are needed to establish the potential value of these tests in the management of neonates of different gestational ages and with different clinical conditions. With an increased understanding of neonatal hemostasis and *in vivo* factors that increase a neonate's bleeding risk, it might be possible to develop novel and more accurate approaches to manage the hemostatic challenges of critically ill neonates.

AUTHOR CONTRIBUTIONS

PD and MS-V reviewed the literature, wrote, and edited the manuscript. Both authors contributed to the article and approved the submitted version.

FUNDING

MS-V work was funded by P01HL046925 and PD was supported by T32HL0079172.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 01 April 2021
doi: 10.3389/fped.2021.629824

Red Blood Cell Contribution to Hemostasis

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Red Blood Cells (RBCs) have been increasingly recognized to play important roles in hemostasis and the mechanisms by which they do so continue to be elucidated. First and foremost, RBC biomechanics are the principal determinant of viscosity and flow dynamics of blood, which strongly influence all features of hemostasis. Of note, morphologic pathology, such as that found in sickle cell disease, leads to increased risk of thrombotic disease. RBC surface interactions govern signaling between platelets and RBCs and also aid in the conversion of prothrombin to thrombin. Additionally, RBCs generate microparticles which have been shown to reduce clotting time. Finally, blood clot structure and maturation are dependent on the inclusion of RBCs in forming thrombi. Here, we review the above mechanisms of RBC contribution to hemostasis.

Keywords: red blood cell(s), shear rate, aggregation, phosphatidylserine, microparticles, hemostasis

OPEN ACCESS

Edited by:
Gemma Crighton,
Royal Children's Hospital, Australia

Reviewed by:
Marianne Nellis,
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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *16 November 2020*
Accepted: *18 February 2021*
Published: *01 April 2021*

Citation:
Gillespie AH and Doctor A (2021) Red Blood Cell Contribution to Hemostasis. Front. Pediatr. 9:629824. doi: 10.3389/fped.2021.629824

INTRODUCTION

Red blood cells (RBCs) have long been known to influence thrombosis through the visco-elastic properties of flowing blood. Moreover, it has become increasingly apparent that RBCs have multiple roles in thrombosis and hemostasis. For example, RBC deformability strongly influences clot structure and biomechanical properties, and cell to cell signaling between RBCs and endothelial cells as well as platelets contribute to both physiologic and pathologic hemostasis. RBCs also influence humoral contributions to thrombosis via RBC and RBC generated microvesicle surface phosphatidylserine interaction with the coagulation cascade. Long thought to have no active role in clot formation, RBCs are now known to be active in clot formation and contraction and to help regulate clot resolution via fibrinolysis (see **Figure 1**).

RED BLOOD CELLS AND RHEOLOGY

Viscosity

There has long been indirect evidence linking thrombosis and elevated RBC volume (Hematocrit), through effect upon blood rheological properties. In 1964, Dintenfass observed that the viscosity of blood taken from patients suffering deep venous thrombosis and coronary disease was much higher than that of healthy donors (1). Epidemiologic studies have also shown higher hematocrit to be associated with deep venous thrombosis and cardiovascular disease (2). In pathologic conditions with increased hematocrit such as cyanotic congenital heart disease and polycythemia vera, thrombosis risk is increased (3, 4). In fact, one of the treatments for polycythemia vera is hematocrit reduction through phlebotomy (4). In addition to this indirect evidence of RBC contribution to thrombosis, RBC transfusion has been reported to promote clot formation via platelet

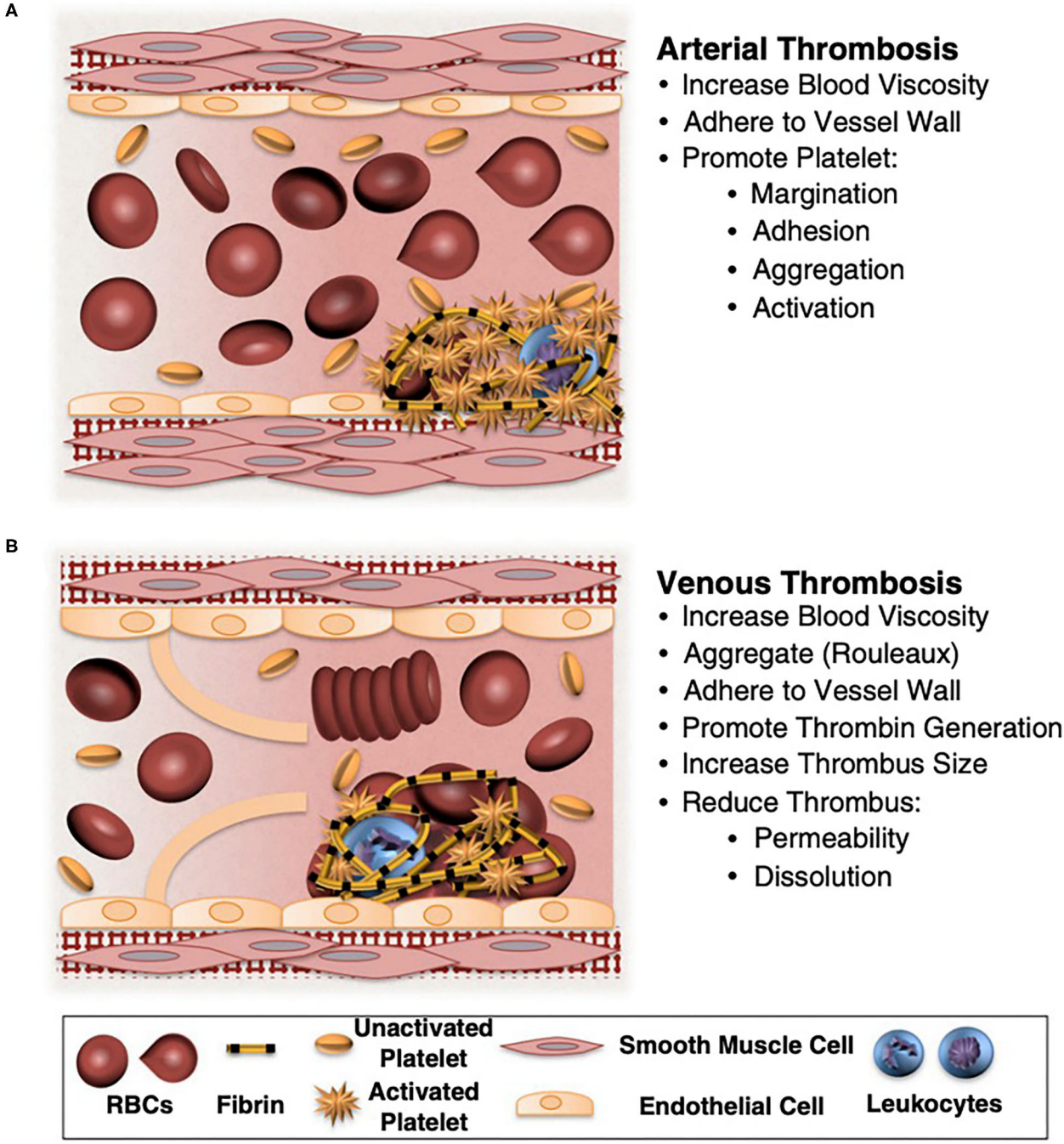

FIGURE 1 | Potential contributions of normal and abnormal RBCs to arterial and venous thrombosis/thromboembolism. **(A)** Arterial thrombi arise in vessels with high shear rates, which promotes the rapid formation of platelet-rich thrombi. During arterial thrombosis, RBCs promote platelet margination, increase platelet-thrombus interactions, and enhance platelet adhesion and activation. Although RBCs increase blood viscosity, this effect is lessened in arteries by high shear-induced shape change. **(B)** Venous thrombi form slowly in stasis or low flow (frequently in venous valve pockets) and are RBC and fibrin rich. In veins, RBC aggregation into stacked rouleaux structures increases blood viscosity. RBCs can also directly or indirectly adhere to the vessel wall and may contribute to thrombin generation within thrombi. Once incorporated into venous thrombi, RBCs increase thrombus size and reduce thrombus permeability and susceptibility to lysis. In disease states, abnormal RBCs and RBC-derived microvesicles may also adhere to the endothelium or extracellular matrix, activate platelets and other cells, and enhance local thrombin generation during thrombosis. Adapted with permission from Byrnes and Wolberg (2).

activation, particularly in the setting of thrombocytopenia (5). Historically, the relationship between RBC abundance and thrombosis was assumed to be related to viscosity; however, the contribution to viscosity by RBCs is only part of their rheologic activity.

In general, an increase in RBC abundance increases viscosity; (6) however, the viscosity-hematocrit relationship is complex. Originally, in 1935, Nygaard et al. (7) reported that the relationship between hematocrit and viscosity is non-linear; i.e., the viscosity increases in an exponential fashion above a threshold hematocrit. Initially for every 10% increase in the hematocrit, blood viscosity increases by 26% (6); whereas at higher hematocrits, a 1% increase (45–46%) increases viscosity by 4% (8). This complex relationship arises from non-Newtonian fluid properties of blood. The classification of fluids into Newtonian and non-Newtonian is based on the relationship between viscosity and shear rate (9), with Newtonian fluids demonstrating constant viscosity over differing shear rates (10) and non-Newtonian fluids having shear-dependent viscosity (9, 11). As such, in blood, RBC-viscosity relationships (and therefore hydrodynamic influence of RBCs upon clot initiation and growth) is not solely a function of the hematocrit, but is also dependent on circulatory context: shear rate, pressure gradients, vessel geometry and blood flow character (laminar vs. turbulent) (12).

Shear Stress and Shear Rate

The forces that govern RBC behavior under flow, particularly with regard to clot initiation and growth are shear stress and shear rate. Shear stress is the applied force per unit area (13), and is directly proportional to flow rate and inversely proportional to vessel diameter (14). Therefore, it is distributed in a concentric gradient within the vessel lumen—in straight segments, this gradient is inversely proportional to the third power of vessel radius (15). Shear rate is the velocity gradient between two adjacent fluid microlayers divided by their distance and also varies across the flow axis (13). *In vivo*, the instantaneous shear rate of blood not only changes along the axis of flow, but also during the cardiac cycle as a function the pulse pressure gradient (6). In a non-Newtonian fluid such as blood, viscosity is defined as the ratio of shear stress to shear rate and as such, the viscosity is lowest and velocity is highest at the vessel center; whereas, viscosity is highest and velocity is lowest at the endothelial surface. This gradient is strongly influenced by hematocrit and RBC biomechanical properties, and this physiology determines RBC aggregation (RBC-RBC association) and adhesion (RBC-endothelium association) at sites of vessel wounding or pathology—which are highly relevant for clot initiation and growth (see **Figure 2**).

Red cell aggregation is promoted when shear rates are low (16). RBCs assemble into stacks (rouleaux) which travel in parallel promoting plasma layering adjacent to the endothelium and preventing RBC adhesion. In addition, the RBC concentration in the vessel core concentrates platelets and coagulation factors to a more radial location in the fluid stream, which promotes interaction with endothelium. Rouleaux formation also diverts single RBCs to the flow-stream periphery and preferentially toward branching vessels. This effectively reduces the hematocrit in smaller vessels. In fact, the spatial distribution of the hematocrit throughout the circulatory tree has a coefficient of variation of 60%—as a consequence, viscosity/shear relationships (and, of course, O_2 content) are spatially non-uniform at any given moment (17).

In smaller vessels, shear stress is higher, which prevents rouleaux formation. Not only does this decrease blood effective viscosity, but increased RBC spacing promotes interaction with the endothelial layer. RBC oxygen release velocity (related to "tank treading" and within-cell hemoglobin mixing) is also increased in smaller vessels due to higher shear stress (18), promoting oxygen release (for any given oxygen gradient) in smaller vessels and capillaries, thereby enhancing transport to metabolically active tissues. Despite the lack of rouleaux formation in small vessels, RBCs still preferentially move in the vessel center, and the more peripheral layer of cell-free plasma is maintained along endothelium (19). Even in capillary vessels (smaller diameter than RBCs), the endothelial glycocalyx (rather than the cell membrane) is the contact interface with RBCs (19). Platelets are marginated to this area which increases their concentration and potential for adhesion. However, platelets increase apparent blood viscosity more so than do red blood cells, further decreasing adjacent wall shear stress (20) and preventing shear stress induced platelet activation which is vital to maintaining vessel patency.

Endothelial cells exhibit classical mechano-transduction signaling responses to shear stress, comprising variation in protein expression and vasoactive factor release (the most notable is shear-induced nitric oxide and prostacyclin release, which link vessel tone to pulse pressure). Not only is site-specific shear stress important, but the pattern of flow informs endothelial cell signaling. The pattern of flow, i.e., laminar vs. turbulent, is mechanically transduced through the cell-free plasma layer that is immediately adjacent to endothelium (glycocalyx) (21–23). As noted above, through effect upon blood viscosity and shear properties, endothelial mechano-transduction is strongly influenced by RBC abundance (hematocrit), biomechanical (RBC deformability), and surface (aggregation and adhesion) properties. With regard to hemostatic related mechano-transduction by endothelium, the synthesis of pro-thrombotic and pro-inflammatory mediators such as Tissue Factor, von Willebrand Factor, endothelin, ICAM-1, and VCAM-1 is highly dependent on both shear stress and laminar flow (24). The expression of anticoagulant mediators such as thrombomodulin are also partially dependent on shear stress. In lower shear stress conditions where thrombosis risk is increased, thrombomodulin expression is increased; thrombomodulin binds to activated Protein C, which binds to Protein S. This protein complex inactivates factors Va and VIIIa, dampening the clotting cascade (25). In addition, platelet activation is attenuated through endothelial expression of both nitric oxide and prostacyclin (26) which also promote vessel dilation. Platelets also exhibit mechano-transduction, and are activated by high shear stress; as such vessel dilation also dampens platelet activation by lowering shear stress. Therefore, the balance between the pro- and anti-thrombotic properties of the vessels are dynamic

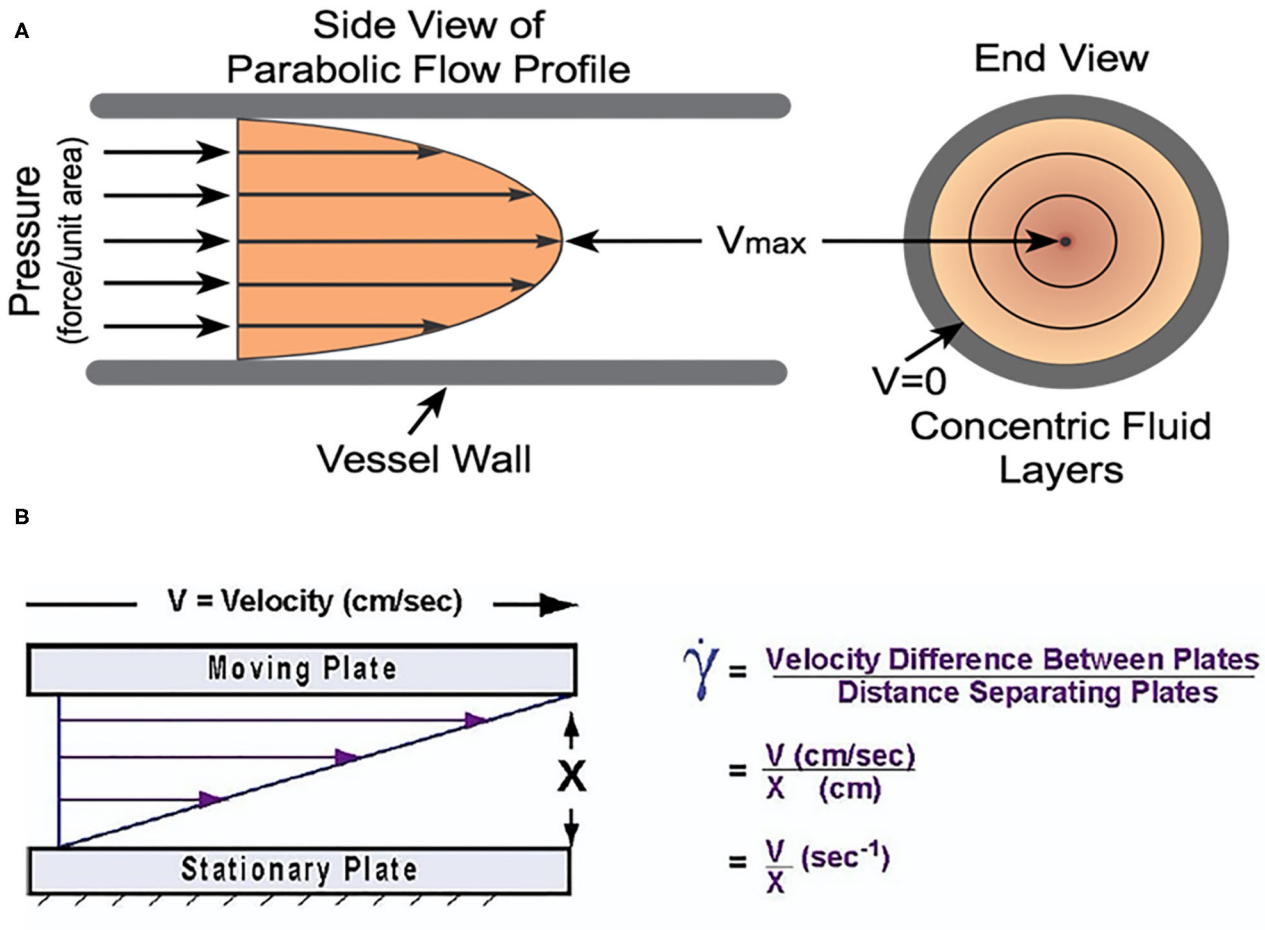

FIGURE 2 | (A) The velocity gradient due to shear forces in blood. The fluid layers have differential velocity according to their position along the radial axis. It is worth noting that the viscosity gradient is the inverse of this gradient with the lowest viscosity at the center of the vessel and the highest nearest the vessel wall. Reprinted with permission from: Cardiovascular Physiology Concepts. cvphysiology.com Richard E. Klabunde, PhD. **(B)** The velocity gradient in blood is due to the differential forces acting upon each fluid layer due to the distance from the vessel wall. Adapted with permission from Papaioannou and Stefanadis (14).

and influenced by blood composition via the rheology-based physiology described above. Healthy vasculature adapts rapidly to the rheology-based changes within the normal dynamic range; however, in disease-related changes to rheology outside the normal dynamic range (very high or low hematocrits, or with very stiff or sticky RBCs), this compensation does not take place and alters the likelihood of both bleeding and thrombosis.

In contrast, turbulent flow (note transition thresholds for laminar vs. turbulent flow are viscosity-dependent and therefore also influenced by RBC number and character) causes endothelial cell activation, promoting RBC and leukocyte adhesion (26). Moreover, endothelium can, in fact, undergo not just biochemical, but structural changes in areas with turbulent blood flow (26). In stenotic vessels, blood flow acceleration increases the shear stress in excess of two orders of magnitude (27). Platelets are activated in areas of high shear stress (27) which then initiates the coagulation cascade. Both changes increase thrombotic risk; the relative risk for abnormal clotting consequence to vessel stenosis, therefore, is also related to RBC number and character (see **Table 1**).

RBC Transfusion and Venous Thromboembolism

The literature examining the influence of RBC transfusion upon hemostasis is mixed, with few formal randomized controlled trials in uniform, defined populations. Several studies in the adult literature demonstrate increased rates of bleeding after RBC transfusions in non-variceal gastrointestinal hemorrhage (28–30). However, multiple other adult studies report association between RBC transfusion and increased venous thromboembolism (VTE) risk, but these are largely retrospective and are missing key data, such as co-incident platelet and fresh frozen plasma transfusions. In the pediatric literature, an association between venous thromboembolism and RBC transfusions has also been reported; (31) similarly, these studies suffer from some of the same issues as the adult studies; a heterogenous patient population and incomplete transfusion

TABLE 1 | Influence of blood flow upon factors relevant to hemostasis.

Parameter	Impact
↑ laminar flow	↑ rouleaux, viscosity, and von Willebrand Factor release
↑ turbulent flow	↑ thrombomodulin, VCAM-1, and endothelin expression
↑ shear rate	↑ RBC release of O_2, ATP and NO, platelet activation, prostacyclin release, and ICAM-1 expression

information. Logically and in practice, the population of patients requiring RBC transfusions is inherently different than those patients who do not; designing robust analyses in this situation is therefore quite challenging.

There is, however, a plausible basis for influence of RBC transfusion upon hemostasis, with regard to the storage lesion. Moreover, as previously discussed, historical studies suggest that significant increase in viscosity alone may be sufficient to increase risk of thrombosis. Additionally, one of the main metabolic derangements that occurs with RBC storage is decreased ATP production secondary to decreased glycolysis (32). A decrease in ATP then inhibits the Na/K ATPase pump which in turn causes an influx of sodium into the RBCs with a correlative increase in size and decrease in deformability (33). The decrease in deformability can then lead to both impaired rheology as well as hemolysis which can in turn decrease nitric oxide availability secondary to scavenging from free heme. In addition to changing the size of RBCs, the loss of ATP production decreases flippase activity, increasing phosphatidylserine (PS) on the outer membrane, which along with other changes related to storage duration is associated with microvesicle (MVs) production, both of which are associated with thrombosis (34). Of note, thrombin generation is accelerated almost 3×10^5 times in the presence of PS (35), leading to increased coagulation.

RED BLOOD CELL MORPHOLOGY

RBC morphology is integral to their function in thrombosis and hemostasis. The mechanical properties of the cytoskeleton and membrane lipid bilayer are both vital to deformation. The cytoskeleton is composed of actin filaments and spectrin tetramers (arrayed in a mesh-like fashion) that allow for deformation under stress (36). The lipid bilayer is tethered to the cytoskeleton; its composition influences fluidity and has a small role in deformability (37). The high surface to volume ratio of the red cell also is important to RBC deformability in small vessels, and changes in this ratio, such as that found in hereditary spherocytosis, determine RBC circulation time (deformability determines the success of splenic transit). Cytoplasmic viscosity and hemoglobin solubility both affect red cell membrane deformation as well (20). Increases in intracellular viscosity such as those mediated by RBC hydration and within-RBC hemoglobin concentration (e.g., the MCHC) both decrease cellular deformation by changing the hydrodynamic effective volume (37).

The RBC membrane has various components that affect cellular interaction and flow principles. For example, band-3 is the major transmembrane membrane protein and plays a role in both ion exchange and in cellular adhesion. In addition, the cytoplasmic domain of band 3 is an assembly point for other membrane proteins through binding sites that regulate the flexibility, stability, and deformability of the red cell (38). PIEZO1, a non-selective cation channel, helps to maintain red blood cell volume homeostasis (38). Defects in this channel result in hereditary xerocytosis which can lead to hemolytic anemia. PIEZO1 interacts with the Gardos channel, otherwise known as KCNN4/IK-1, which is a calcium-activated potassium channel. By governing RBC hydration, the Gardos channel helps to mediate red cell changes in shape and volume that are necessary for passage through small capillaries.

RBC deformation is important from a cell signaling perspective, a physical perspective, and from a gas and ion exchange perspective. In high shear stress situations such as near the vessel wall, RBCs adopt an elliptical shape and travel parallel to blood flow (36). This transformation prevents aggregation which lowers effective viscosity and resistance. From a physical perspective, RBCs are 7–8 microns in diameter, yet traverse 1–3 microns blood vessels (20). The normal RBC deforms to a bullet shape which maintains a high surface area near the endothelium to maximize gas exchange. Even the act of deformation promotes oxygen release (39). Red cells that are unable to deform are more likely to adhere to the vessel wall, which increases vascular resistance. Once adhered, the magnitude of the shear stress required to detach RBCs is an order of magnitude higher than that to prevent aggregation (40). Diseases characterized by abnormal RBC deformation are commonly also associated with increased risk for thrombosis. For example, in sickle cell disease, hemoglobin S polymerizes and sickled RBCs are stiffer than normal (20, 41). Not only is this important in the acute setting with vaso-occlusive crises, but over time, the rheologic properties of RBC membrane are altered leading to more rigid cells and increased thrombotic risk (41).

CELL TO CELL SIGNALING AND HUMORAL INFLUENCES ON HEMOSTASIS

Endothelium and Red Blood Cells

The endothelial glycocalyx is composed of proteoglycans, glycoproteins, and glycosaminoglycans containing heparan sulfate, chondroitin sulfate, hyaluronan, and various other proteins (22). These components are arranged in such a way as to provide both a steric and a charge dependent semipermeable barrier preventing cell adhesion (22). Therefore, under normal conditions, RBCs interact with the endothelial glycocalyx and not with the endothelial cell membrane itself *per se*. The glycocalyx also includes a high density of anticoagulant proteins such as antithrombin, heparin cofactor II, thrombomodulin, and tissue factor pathway inhibitor which prevent thrombosis in healthy vasculature (42). In pathologic conditions such as sickle cell disease and in other conditions which structurally or metabolically alter RBCs, RBC adhesion to the endothelium increases (19). Once adherent, a set of adhesion receptors is externalized that is not found on RBCs in normal conditions. The

exact receptors expressed are dependent on the disease, but they are thought to overall increase the risk of thrombosis (43).

Platelets and Erythrocytes

RBCs interact with platelets in both a mechanical and biochemical fashion. Not only do RBCs promote platelet margination through axial flow, but they also interact directly with platelets through the $\alpha_{IIb}\beta_3$-ICAM4 receptors (43, 44). Moreover, RBCs can biochemically activate platelets through the export of ATP and ADP during high shear conditions, hypoxia, and acidosis (19, 45). Platelet aggregation is also enhanced by RBC export of thromboxane A2 (44). Platelets stimulated by RBC presence exhibit enhanced P-selectin externalization and integrin $\alpha_{IIb}\beta_3$ activation which initiates a positive feedback loop during platelet activation (2). With RBC destruction (hemolysis), free hemoglobin molecules scavenge nitric oxide which leads to platelet disinhibition (19).

Phosphatidylserine Exposure

The RBC membrane is a bilayer with amphiphilic molecules such as phosphatidylserine (PS) concentrated on the inner leaflet (46). Under physiologic conditions, RBC membrane proteins flippases and translocase maintain the negatively charged PS polar head orientation to the cytosolic (rather than the outer surface), which is therefore concealed from coagulation proteins (47). With high shear rates, inflammation and/or oxidative stress, flippase, and translocase are inactivated and the protein scramblase is activated (44). This change causes inversion of the PS polar head asymmetry from the inner to the outer membrane leaflet. On the outer membrane, externalized PS provides a scaffold upon which factor X can be activated to Xa and thrombin can be generated from prothrombin (46). There is a small population of senescent RBCs with PS exposure on their surfaces (∼0.6%) (48). In pathologic conditions this RBC sub-population expands, and RBCs can account for up to 40% of the thrombin generation potential of whole blood (19, 49). Interestingly, however, in patients with sickle cell disease, RBC PS externalization is inversely correlated with thrombin generation (50). PS externalization also enhances RBC adherence to and activation of endothelium (43).

Red Cell -Derived Microparticles

Membrane blebbing is common in activated, apoptotic, and aging cells. In RBCs, these processes cause both loss of membrane asymmetry via PS externalization and the release of RBC-derived microparticles (MPs), also called microvesicles (20, 51). MP release is also increased in inflammatory conditions (52). RBC-derived MPs contain five procoagulant proteins: phospholipid scramblase I, plasminogen precursor, fibrinogen beta chain precursor, complement component C9 precursor, and β2-glycoprotein I (53). MPs can activate coagulation through both the tissue factor and contact pathways of the coagulation cascade (53). Overall, increased RBC MP formation is associated with decreased clotting times (19). They are also assumed to play a role in the increased incidence of deep venous thrombosis associated with the transfusion of RBCs stored for longer duration (19). In addition to their inherent procoagulant activity, MPs are capable of scavenging and internalizing free hemoglobin, and can—through fusion—then transfer their payload to endothelium in a process that leads to vaso-occlusion in sickle cell disease (19). MPs are also released in large amounts during hemolysis; (19) therefore, they promote a pro-thrombotic milieu in conditions such as those described for microangiopathic hemolytic anemias (MAHAs) which commonly are associated with disseminated intravascular coagulation.

Humoral Influences on Red Cells

Multiple plasma proteins found in plasma that interact with RBCs, such as thrombospondin, which is both an extracellular matrix protein and a soluble plasma protein (38) that facilitates bridging between RBCs and endothelial cells via interaction with RBCs carrying exposed phosphatidylserine. Thrombospondin is increased in multiple inflammatory conditions (54) and may play a role in pathologic thrombosis. Von Willebrand Factor is also found in plasma and facilitates endothelial cell-RBC adhesion. The mechanism of this interaction is also thought to be through phosphatidylserine and Annexin V and is shear-dependent (55). Shear stress "uncoils" and activates von Willebrand factor and increases its binding capapcity (55). ADAMTS13 is a protease that regulates the concentration of ultra-long von Willebrand factor multimers that promote a thrombotic state. There are multiple diseases in which the ADAMTS13 level or activity is low predisposing to thrombosis; (55) thrombotic thrombocytopenic purpura (TTP) is a classic example and more recently, Thrombocytopenia Associated Multiple Organ Failure (TAMOF) has been shown to also arise from diminished ADAMTS13 activity. Fibrinogen is a plasma glycoprotein whose asymmetric structure contributes to plasma viscosity and non-Newtonian behavior. It is elevated in inflammatory conditions and has been correlated with thrombotic events-even in the absence of endothelial injury (56). It is thought to be able to bind directly to RBCs, although the exact mechanism by which this occurs is yet to be fully determined. The current hypothesis is that RBCs and fibrinogen are bound through either von Willebrand factor or through an integrin receptor. High fibrinogen levels increase RBC aggregation into rouleaux which increase the local blood viscosity and promote thrombosis in low shear environments (20). Finally, immunoglobulins are found in plasma, the most important being immunoglobulin G (IgG). IgG interacts with band 3 on the red cell cytoskeleton in a non-specific fashion and is part of both physiologic processes such

TABLE 2 | Summary of modalities through which RBCs contribute to hemostasis.

- Effect upon humoral and endothelial mechano-signaling *via* altered blood flow
- RBC membrane surface as physical interface with other hemostatic elements
- RBC membrane surface as biochemical/signaling trigger and accelerant in coagulation cascade
- Direct signaling *via* exported biomolecules from RBCs to endothelial cells and platelets
- Elaboration of RBC microparticles, with both mechanical and signaling effects
- Contribution of intact RBCs to 3D geometry and biophysical properties of thrombi

as the removal of aging RBCs and pathologic conditions such as autoimmune hemolytic anemia (38).

ERYTHROCYTES AND CLOT MECHANICS

Long thought to be an innocent bystander of the clotting cascade, RBCs are more recently recognized as an important and active part of the thrombotic process. First, there are multiple biochemical properties of RBCs which influence the clotting cascade including interactions with platelets, von Willebrand factor, and fibrinogen. The release of RBC-derived MP's offers a surface with phosphatidylserine also is able to initiate thrombin generation in a Factor XII-dependent manner (53). Classic biochemical and flow related environments may initiate thrombosis, that is then completed with RBC incorporation into the clot structure.

Clot structure arises from a growing fibrin matrix that is stabilized by factor XIIIa crosslinking (57). RBCs are entrained in this matrix through such crosslinking. Currently it is believed that there is no bond between factor XIIIa and RBCs, but instead fibrin crosslinking secondarily traps RBCs (58). We also know that RBC presence changes the underlying fibrin structure itself in a concentration-dependent manner (59). As RBC number increases in a forming clot, the fibrin strands become larger and the clots less porous (20). In addition, RBC deformability influences elastic properties of the thrombus (59). RBC presence increases lytic resistance in the clot as well by impairing plasminogen activation and through the above-mentioned changes in fibrin structure (60). Once formed, the clot undergoes platelet-mediated contraction (20), during which entrained RBCs are compressed into shapes called polyhedrocytes, which appear between fibrin layers as "building blocks." Finally, in a contracted clot, RBC presence modulates fibrinolytic activity both by steric inhibition and through the RBC fibrinogen receptor (59).

Influence of Anemia Upon Bleeding Risk

Given the integral role of RBCs in hemostasis, it is important to consider how anemia may affect risk of bleeding in critically ill patients. First, although not necessarily directly correlated with the risk of bleeding, it is important to appreciate the impact of hematocrit on coagulation assay results. For example, with the PFA-100 analyzer, there is an inverse relationship between hematocrit and closure time; in fact, no platelet plug forms in this device with hematocrits <20% and, moreover, for every decrease of 50,000 in the platelet count, an increase in hematocrit by 10% will "normalize" assay output (up to hematocrits of 50%) (61). Similar findings are reported for bleeding time (anemia extends bleeding time); (62–64) however, these differences in coagulation parameters as a function of anemia do not clearly equate to altered risk of clinically significant bleeding in patients. For example, hemoglobin level is reported to independently influence bleeding risk in adults with AML (for each increase in hemoglobin of 1 g/dL, significant bleeding was reduced by 22% (RR, 0.78; 95% CI: 0.61–1); (65) however, a meta-analysis ($\sim$ 12 K subjects) evaluating restrictive v liberal RBC transfusion trials revealed a non-significant trend toward lower risk of bleeding in the restrictive (more anemic) group (66). On the other hand, in a *post-hoc* analysis of a large multicenter trial of prophylactic platelet transfusions in oncology patients (PLADO), a hematocrit $\leq$25 was associated with a non-significant trend toward an increased risk of clinically significant bleeding (OR 1.29; 95%CI: 0.98–1.47); however, there was no observed interaction between RBC transfusion and next-day bleeding, as a function of anemia severity (67). Of note, the above CI barely dips below 1 and as in many situations, it may be reasonable to consider the difference between statistically significant and clinically significant information. As may be apparent from other material in this paper, it is probable that there is a threshold below which anemia is a clinically relevant risk factor for bleeding, likely in synergy with abnormalities in hemostasis, itself (such as low platelets, fibrinogen, etc.). This issue is reviewed thoughtfully in an editorial accompanying the *post-hoc* PLADO analysis (68). Therefore, at this time, while there is no clear empiric support for targeting a specific hemoglobin level to prevent bleeding complications; in patients with clinically significant bleeding that is refractory to appropriate correction of coagulopathy, or in patients with uncorrectable defects in hemostasis (persistent thrombocytopenia or hypofibrinogenemia), there may be plausible biologic justification to target a higher hematocrit goal than would be pursued under other circumstances.

CONCLUSION

Overall, RBCs have a significant impact on the hemostatic process, both actively and passively. Through multiple facets including rheology, morphology, cell signaling and humoral interactions, and physical presence in blood clots, RBCs contribute to hemostasis. Appreciation of the extent of this contribution will likely continue to expand in the future and may possibly offer novel therapeutic targets for modulating hemostasis (see **Table 2**).

AUTHOR CONTRIBUTIONS

AG researched the current literature and completed the first draft of the article. AD oversaw the writing of the article, discussed the relevant research, and edited the draft. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by NIH R01GM113838 (AD).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 21 April 2021
doi: 10.3389/fped.2021.647680

Pediatric Fibrinogen PART II—Overview of Indications for Fibrinogen Use in Critically Ill Children

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Bleeding is frequently seen in critically ill children and is associated with increased morbidity and mortality. Fibrinogen is an essential coagulation factor for hemostasis and hypofibrinogenemia is an important risk factor for bleeding in pediatric and adult settings. Cryoprecipitate and fibrinogen concentrate are often given to critically ill children to prevent bleeding and improve fibrinogen levels, especially in the setting of surgery, trauma, leukemia, disseminated intravascular coagulopathy, and liver failure. The theoretical benefit of fibrinogen supplementation to treat hypofibrinogenemia appears obvious, yet the evidence to support fibrinogen supplementation in children is sparce and clinical indications are poorly defined. In addition, it is unknown what the optimal fibrinogen replacement product is in children and neonates or what the targets of treatment should be. As a result, there is considerable variability in practice. In this article we will review the current pediatric and applicable adult literature with regard to the use of fibrinogen replacement in different pediatric critical care contexts. We will discuss the clinical indications for fibrinogen supplementation in critically ill children and the evidence to support their use. We summarize by highlighting current knowledge gaps and areas for future research.

Keywords: fibrinogen, children, hypofibrinogenemia, cryoprecipitate, fibrinogen concentrate, bleeding, hyperfibrinolysis

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *30 December 2020*
Accepted: *09 March 2021*
Published: *21 April 2021*

Citation:
Crighton GL and Huisman EJ (2021) Pediatric Fibrinogen PART II—Overview of Indications for Fibrinogen Use in Critically Ill Children. Front. Pediatr. 9:647680. doi: 10.3389/fped.2021.647680

INTRODUCTION

Bleeding in Critically Ill Children

Bleeding is a common complication observed in critically ill children and appears to be multifactorial (1). In children admitted to the pediatric intensive care unit (PICU), clinically significant bleeding is reported to occur in ∼10% with higher rates reported in those receiving mechanical circulatory support (1–3). Bleeding is associated with adverse outcomes including longer PICU stays, increased vasopressor support, increased red cell transfusions (1, 2) and in children requiring extracorporeal membrane oxygenation (ECMO) increased mortality (3).

Hypofibrinogenemia in Critically Ill Children

Fibrinogen (Factor I) is an essential hemostatic protein, with a key role in all aspects of normal hemostasis (4, 5). In critically ill children, fibrinogen has not been identified as an independent risk factor for bleeding. Studies looking at risk factors for bleeding in critically ill children have

either not evaluated fibrinogen levels (1) or found the opposite, namely that fibrinogen levels are higher in critically ill children with bleeding (2). This finding most likely reflects that fibrinogen is an acute phase reactant and therefore may be elevated in the critical care context, in the context of sepsis, infection, or inflammation (6). Hypofibrinogenemia has been identified as an important risk factor for bleeding in other pediatric and adult settings (7–9). It is therefore important to appreciate a low fibrinogen level and recognize its potential contribution to bleeding.

See Pediatric Fibrinogen Part I—Pitfalls in Fibrinogen Evaluation and Use of Fibrinogen Replacement Products for further information about the diagnostic tools used to measure fibrinogen in critically ill children and the available fibrinogen replacement products.

Hypofibrinogenemia in Critically Ill Children

Hypofibrinogenemia in the PICU is most commonly acquired, or secondary to an underlying process. See **Figure 1**. It may result from:

A) ***Reduced/absent or abnormal fibrinogen synthesis*** as seen in severe liver disease (10) or, in the rare instance of congenital fibrinogen disorders.
B) ***Fibrinogen loss exceeding fibrinogen production*** for example, during massive blood loss (11).
C) ***Hyperfibrinolysis***, a condition where fibrinolytic activity exceeds fibrin formation, which may be observed in trauma (12), post cardiopulmonary bypass (CPB), with extracorporeal membrane oxygenation (ECMO) (9, 13) and in disseminated intravascular coagulopathy (DIC) (14). See **Figure 1**.

Cryoprecipitate and fibrinogen concentrate are most commonly used to provide fibrinogen supplementation, and both effectively restore fibrinogen levels (15–18). They may be given to provide fibrinogen replacement during active bleeding or as prophylaxis to prevent bleeding. A prospective study of cryoprecipitate use in England, found that most cryoprecipitate given to children was for prophylactic, rather than therapeutic indications, specifically in the context of pediatric cardiac surgery, critical care, or the hematology/oncology setting (19).

THE CRITICAL FIBRINOGEN LEVEL

There are a number of the age-related differences in the hemostatic systems including fibrinogen and fibrinolysis between neonates and older children and adults (20, 21). The critical fibrinogen threshold level and the risk of bleeding is debated in children of all ages.

Nevertheless, it is obvious that there is a strong association between fibrinogen level and bleeding severity in individuals with congenital fibrinogen deficiency, with bleeding rarely reported at fibrinogen levels >100 mg/dL (22). The International Council for Standardization in Hematology considers a fibrinogen level of 50–200 mg/dL to be a critical test result but recognizes the overlap with normal references ranges; a result of 150 mg/dL, may be normal or critical depending on the clinical context (23). In the adult surgical setting a fibrinogen level of <150–200 mg/dL, increases the risk of perioperative and postoperative bleeding tendency (7). Similarly in children undergoing cardiac surgery, post-CPB fibrinogen levels of <150 mg/dL have been shown in both retrospective (9), and prospective (8) studies to be associated with increased rates of post-operative bleeding.

As a result, international clinical guidelines outlining pediatric fibrinogen replacement guidance also, having varying fibrinogen thresholds and targets. See **Table 1**.

In summary, fibrinogen is a key hemostatic protein and hypofibrinogenemia is an important risk factor for bleeding, but the critical threshold is debated. Fibrinogen levels need to be interpreted in their clinical context, e.g., a child's age, the presence of inflammation or active bleeding and any planned surgery.

THE EVIDENCE BASE

Systematic Reviews and Randomized Controlled Trials

There is a lack of high-quality evidence to support the prophylactic or therapeutic use of cryoprecipitate and fibrinogen concentrate in children (24–28).

Existing evidence comes from the 2018 Cochrane systematic review, which evaluated the efficacy and safety of fibrinogen concentrate, with respect to the primary outcomes of mortality and thromboembolic events, in addition to secondary bleeding outcomes (27). The review identified 22 randomized controlled trials (RCT)s (n = 1,535) looking at fibrinogen concentrate of which three were pediatric (n = 143) (27). On review of the trial settings, 15 of the 22 trials were conducted in the elective perioperative setting, three in the trauma setting, two in women with post-partum hemorrhage and one in the context of liver transplantation (27). Ten of the 22 trials looked at prophylactic fibrinogen indications, nine compared fibrinogen concentrate with placebo and one trial evaluated two different fibrinogen concentrate intervention points based on FIBTEM. The remaining 12 trials were therapeutic trials, eight compared fibrinogen concentrate with placebo, and four compared fibrinogen concentrate to an active comparator (fresh frozen plasma [FFP], cryoprecipitate, or platelets) (27). The systematic review was significantly limited by the quality of the data published; trials were conducted in heterogeneous clinical settings, with varying interventions, primary outcomes, and different time points for outcome analysis (27). Therefore, meta-analysis could not be performed. Study samples sizes were small (mean 70 participants [range 20–249]) and none of the studies were powered to detect harm or adverse events, limiting their generalizability to clinical practice. Nevertheless, the systematic review observed that fibrinogen supplementation may reduce a patient's risk of receiving a red cell transfusion when compared with placebo in both cardiac and non-cardiac settings. In addition, prophylactic fibrinogen concentrate may reduce post-operative bleeding (27).

Two other recent systematic reviews have evaluated fibrinogen replacement therapies and included pediatric studies (26, 28). The first systematic review evaluated the

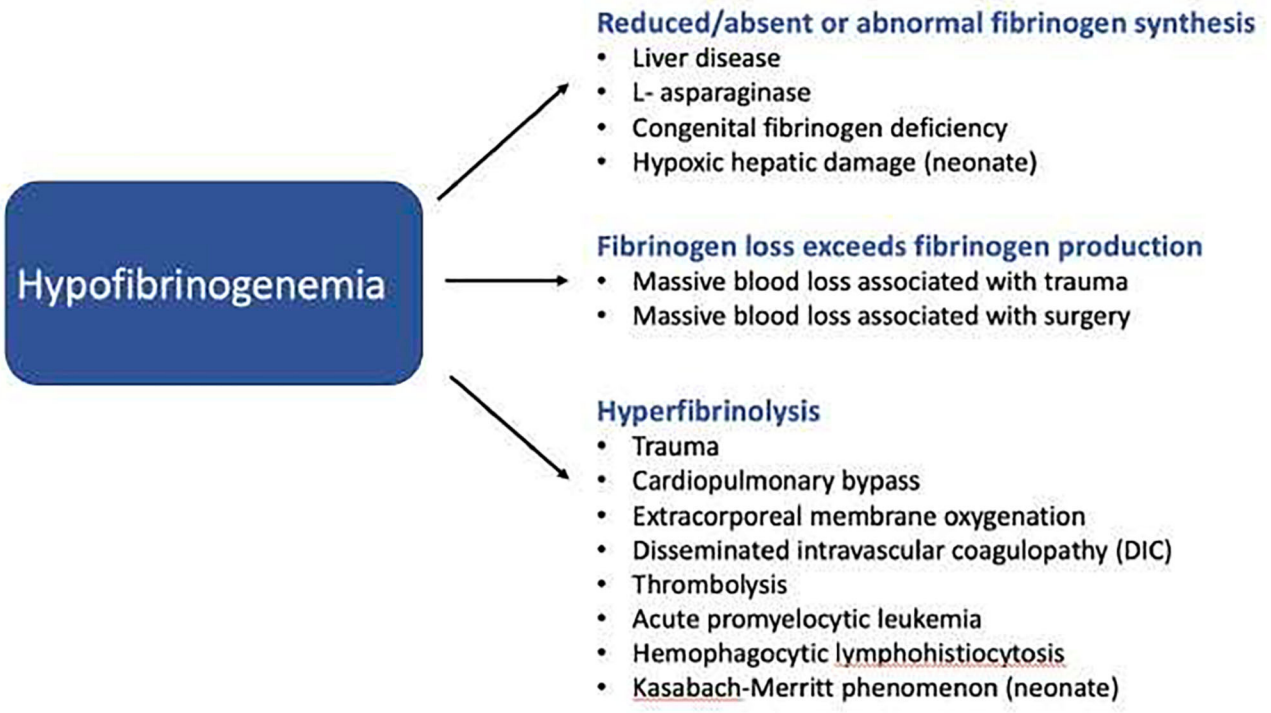

FIGURE 1 | Machanisms by which hypofibrinogenemia may develop in critically ill children.

use of fibrinogen concentrate in perioperative settings and identified 21 RCTs (n = 1,605) where fibrinogen concentrate was given either pre-emptively to prevent bleeding or to treat intraoperative bleeding (28). Two identified studies were performed in children (n = 114) (17, 29). Overall there was considerable variability in the studies with respect to the patient populations, study designs, comparative arms, and outcomes, including bleeding rates. Only seven of the 12 trials that used fibrinogen concentrate to treat clinically relevant bleeding reported decreased bleeding tendency (28). The study authors rationalized that different study designs might explain the inconsistent results. They advocated for better-designed trials that evaluated patients with significant bleeding and hypofibrinogenemia (28).

The second systematic review evaluated the efficacy and safety of cryoprecipitate compared with fibrinogen in bleeding patients and identified one RCT (n = 63) conducted in children undergoing cardiac surgery and three observational studies that included adults. All studies were at high risk of bias and the authors concluded that it was not possible from the current evidence to recommend one fibrinogen replacement product over another (26).

From the systematic reviews and literature search, eight published pediatric RCTs (n = 616) (17, 18, 29–33) evaluating fibrinogen replacement in the surgical setting were identified for the purpose of this review. Of the eight RCTs, five are performed in the cardiac surgery setting (17, 18, 32–34), one in the adolescent scoliosis setting (30), one in infants undergoing craniofacial surgery (31), and the final study included both infants undergoing craniofacial surgery and adolescents undergoing scoliosis surgery (29). The details and results will be discussed further in the respective surgical settings and are presented in **Table 2**.

From the list of ongoing trials identified by the Cochrane systematic review (27), an additional RCT in children (sample size 30) undergoing cardiac surgery has completed recruitment and the results are awaited (35).

Evidence Translated in Guidelines

Whilst the evidence to support fibrinogen supplementation in children is sparse, guidance regarding fibrinogen supplementation has been included in a number of neonatal and pediatric transfusion guidelines (24, 25, 36–38), as well as some adult-centric guidelines (36, 39–44). See **Table 1** for more details. Transfusion guidance provided by national European blood transfusion guidelines differs, since cryoprecipitate is not available and instead, fibrinogen concentrate is used to treat acquired hypofibrinogenemia. There is, however, no consensus European statement or guideline that provides guidance about fibrinogen concentrate indications, thresholds and doses for fibrinogen replacement in neonates and children. Local European guidelines are often difficult to access since they are usually only written in their native language.

The decision to provide fibrinogen replacement to a child, should involve an evaluation a child's condition, the presence of active bleeding, in conjunction with the results of laboratory

TABLE 1 | Guidance for pediatric fibrinogen replacement therapy from international guidelines.

Guideline	Type of guideline and patient population	Transfusion guidance and indications	Dosing information
Cryoprecipitate			
BSH (1)	Neonatal and pediatric specific transfusion guideline	*Recommendations* • Prophylactic cryoprecipitate should not be routinely administered to non-bleeding children with decreased fibrinogen including prior to surgery • Cryoprecipitate should not be used for congenital hypofibrinogenemia unless fibrinogen concentrate is unavailable • Cryoprecipitate may be considered for fibrinogen <100 mg/dL for surgery at risk of significant bleeding or to critical sites • In the setting of DIC—cryoprecipitate may be given if fibrinogen is <100 mg/dL despite FFP, or in conjunction with FFP for very low or rapidly falling fibrinogen • Clinically significant bleeding following CPB and fibrinogen <150 mg/dL, specific component replacement may be warranted Practice Point In patients with critical bleeding—early use of FFP, platelets and cryoprecipitate is recommended in order to reduce coagulopathy and thrombocytopenia Guidance Massive blood loss aim fibrinogen >150 mg/dL	*Cryoprecipitate* 5–10 mL/kg with higher volumes in bleeding patients Massive blood loss *Cryoprecipitate* 10 mL/kg
NBA (2)	Neonatal and pediatric specific PBM guideline	Practice Point • In children, the decision to transfuse cryoprecipitate or fibrinogen concentrate should take into account the potential risks and benefits. The decision should be based not only on laboratory investigations but also on assessment of the patient's clinical condition. Factors that may influence the decision include active bleeding, medications affecting coagulation status, and congenital and acquired bleeding disorders Expert opinion point • Cryoprecipitate should be used to treat active bleeding when the fibrinogen level is <150 mg/dL. • A target level of 200 mg/dL may be appropriate in certain situations (e.g., critical bleeding is occurring or anticipated)	*Cryoprecipitate* 5 mL/kg
Italian neonatal transfusion guideline, 2015 (3)	Neonatal transfusion guideline	Recommended interventions • Term and preterm neonates with hypofibrinogenemia but no bleeding, observation is recommended Recommended interventions • Term and preterm neonates with hypofibrinogenemia and bleeding or about to undergo an invasive procedure, cryoprecipitate is recommended	*Cryoprecipitate* 5–10 mL/kg
SABM (4)	Pediatric PBM Standard in PBM Guideline	Indicators • The decision to transfuse cryoprecipitate should be based on laboratory studies, including point-of-care viscoelastic testing if available, fibrinogen concentration, the patient's clinical status and the etiology of the patient's coagulopathy • Fibrinogen concentrate may alternatively be considered	*Cryoprecipitate* Volume should be calculated based on weight and desired increase in fibrinogen concentration and improvement in coagulations indices
Faraoni, NATA (5)	Pediatric cardiac surgery guideline	Recommendations • The authors suggest FFP should be considered for treating hypofibrinogenemia in bleeding neonates and children **only** when cryoprecipitate or fibrinogen concentrates are not available • In bleeding neonates and children, the authors recommend hypofibrinogenemia diagnosed either by Clauss method (<150 mg/dL) or viscoelastic tests (based on institution-specific algorithm) should be treated either with cryoprecipitate or fibrinogen concentrate	

(Continued)

TABLE 1 | Continued

Guideline	Type of guideline and patient population	Transfusion guidance and indications	Dosing information
NICE guidelines 2015 (6)	Guideline for adults, young people, and children over 1 year	Generic Recommendations • Do not offer cryoprecipitate transfusions to correct the fibrinogen level in patients who: are not bleeding and are not having invasive procedures or surgery with a risk of clinically significant bleeding • Consider cryoprecipitate transfusions for patients with major hemorrhage who have clinically significant bleeding **and** a fibrinogen level <150 mg/dL • Consider prophylactic cryoprecipitate transfusions for patients with a fibrinogen level <100 mg/dL who are having invasive procedures or surgery with a risk of clinically significant bleeding	*Cryoprecipitate* 5–10 mL/kg up to a maximum of 2 pools
Blood Easy 4, Canada, 2016 (10)	Blood Transfusion Guideline	Generic Indications • For bleeding with fibrinogen <100 mg/dL • For massive hemorrhage with fibrinogen <150–200 mg/dL • For acute phase of APML with fibrinogen <150 mg/dL • ICH secondary to treatment with tPA with fibrinogen <200 mg/dL • Treatment of bleeding in patients with vWD or Hemophilia A, **only** when: Factor concentrates are unavailable, remote geographic region and DDAVP is unavailable or ineffective	*Cryoprecipitate* • 1 unit/10 kg body weight to a maximum of 10 units • (~4000 mg fibrinogen)
Fibrinogen concentrate			
German Medical Association, 2014 (7)	Blood Transfusion Guideline with guidance for adults and children.	Recommendations—Acquired fibrinogen deficiency • Fibrinogen can be substituted perioperatively in interventions or lesions with the risk of acute bleeding and confirmed fibrinogen deficiency (massive transfusion, dilution and loss coagulopathy) • Fibrinogen can be substituted in synthesis disorders (liver damage) with fibrinogen deficiency or in hemorrhagic dysfibrinogenemias as prophylaxis and therapy of hemorrhage and confirmed fibrinogen deficiency • Fibrinogen can be substituted as a prophylaxis and therapy of hemorrhage and confirmed fibrinogen deficiency of different origin (e.g., acute leukemia, asparaginase therapy, obstetrical complications, postoperatively) *General Recommendations—Acquired fibrinogen deficiency* • The critical threshold values for the occurrence of spontaneous bleeding are <100 mg/dL (in severe hemorrhage 150 mg/dL) • The fibrinogen level should always be specifically determined. An indirect determination using PT or APTT is not sufficient for any decisions regarding substitution therapy. The detection limit of the laboratory assay must be taken into account • Following administration, fibrinogen levels should be monitored and maintained above the critical threshold value (~100 mg/dL)	*Fibrinogen concentrate* Mean adult dose in acquired fibrinogen deficiency 3,000–5,000 g
European trauma guideline, 2019 (8)	Adult trauma guideline*	Generic Recommendations *Initial coagulation resuscitation* • In the initial management of patients with expected massive hemorrhage, we recommend one of the two following strategies: • FFP or pathogen-inactivated FFP in a FFP:RBC ratio of at least 1:2 as needed. • Fibrinogen concentrate and red cells *Fibrinogen supplementation* We recommend treatment with fibrinogen concentrate or cryoprecipitate if major bleeding is accompanied by hypofibrinogenemia (viscoelastic signs of a functional fibrinogen deficit or a plasma Clauss fibrinogen level ≤150 mg/dL) *Coagulation factor concentrate-based management* • If a CFC-based strategy is used, we recommend treatment with factor concentrates based on standard laboratory coagulation parameters and/or viscoelastic evidence of a functional coagulation factor deficiency • We suggest that monitoring of FXIII be included in coagulation support algorithms and that FXIII be supplemented in bleeding patients with a functional FXIII deficiency	*Fibrinogen concentrate* Adult dose 3,000–4,000 g

(Continued)

TABLE 1 | Continued

Guideline	Type of guideline and patient population	Transfusion guidance and indications	Dosing information
ESA 2017 (9)	Perioperative guideline with pediatric surgery section	No specific recommendation made for fibrinogen replacement in pediatric surgery, as neither the optimal threshold for initiation of fibrinogen replacement nor the dose required to reach the targeted fibrinogen concentration, have been proven by high-quality data	

**Do not provide specific recommendations for children, but state that children who have not been pre-treated with anticoagulant or antiplatelet agents should generally be managed in the same manner as the normal adult patient.*

APML, acute promyelocytic leukemia; APTT, activated partial thromboplastin time; CPB, cardiopulmonary bypass; CFC, clotting factor concentrate; DDAVP, de-amino-D-arginine vasopressin; DIC, Disseminated intravascular coagulopathy; FFP, fresh frozen plasma; ICH, intracranial hemorrhage; FVIII, Factor VIII; FXIII, Factor XIII; PBM, Patient blood management; PCC, Prothrombin complex concentrate; RBC, red blood cells; PT, Prothrombin Time; tPA, tissue plasminogen activator; vWD, von Willebrand disease; vWF, von Willebrand factor.

AABB, American Association of Blood Banks; BSH, British Society of Hematology; ESA, European Society of Anesthesiology; NATA, Network for the Advancement of Patient Blood Management, Hemostasis, and Thrombosis; NBA, National Blood Authority; NICE, National Institute for Health and Care Excellence; SABM, Society for the Advancement of Blood Management.

and hemostatic testing (24, 36). Most guidelines recommend against the transfusion of cryoprecipitate to correct coagulation abnormalities in neonates and children who are not bleeding (24, 25, 38). However, guidance differs between transfusion guidelines with regard to product choice, clinical indications, target fibrinogen levels and doses, reflecting uncertainty, and lack of evidence in these areas. See **Table 1** for further details.

Variability in Practice

It is not surprising, that given the paucity of evidence to support the use of cryoprecipitate and fibrinogen concentration that there is considerable variability in practice and inappropriate use (45, 46).

A single-center retrospective review of 44 critically ill children in the US found that cryoprecipitate was most commonly given in the setting of recent cardiac surgery, DIC, and sepsis (45). More than 60% of transfusions did not meet local institutional indications (45). Many children received cryoprecipitate empirically without a pre-transfusion fibrinogen level and the median dose received was higher than local guidelines suggested (45).

An Australian audit of cryoprecipitate use included 21 cryoprecipitate transfusion episodes for neonates (aged <4 months) and 24 for children (4 months−17 years). A pre-transfusion fibrinogen level and an appropriate indication for transfusion were only present in 57% of neonates and 67% of children (47).

In summary, there are an increasing number of insufficiently powered RCTs that have evaluated fibrinogen supplementation. RCTs are mainly performed in adults, with only eight small published pediatric RCTs. Due to heterogeneity in clinical settings and indications, their results could not be pooled. There is some evidence in systematic reviews that prophylactic fibrinogen concentrate may result in reduced blood loss and reduced transfusion requirements, compared with inactive comparator. There is no evidence to favor cryoprecipitate or fibrinogen concentrate over one another.

Current guidance provided in international and national guidelines for fibrinogen replacement indications, thresholds and dosing are going to be limited by the lack of high quality and well-powered trials. Consensus recommendations or expert opinion statements are likely to be based on the results of a few underpowered trials in specific pediatric patient cohorts.

INDICATIONS FOR FIBRINOGEN REPLACEMENT IN THE CRITICAL CARE SETTING

Reduced/Absent or Abnormal Fibrinogen Synthesis

Congenital Fibrinogen Disorders

Acute presentations of congenital bleeding disorders are uncommon. Congenital fibrinogen disorders (afibrinogenemia and hypofibrinogenemia) are exceedingly rare, estimated incidence of 1–2 per million people (48). They are classified based on antigenic and functional levels of fibrinogen and include quantitative and qualitative defects (49). Afibrinogenemia is characterized by an undetectable fibrinogen level and results in a significant bleeding phenotype, with both spontaneous bleeding (e.g., umbilical cord, muscle, gingival, and intracranial hemorrhage [ICH]), as well as trauma and surgery-associated bleeding (50, 51). ICH is a major cause of death in these patients (51, 52). Bleeding is typically less severe in hypofibrinogenemia and usually, but not always, correlates with the fibrinogen level (49).

Treatment of congenital fibrinogen disorders will depend on the bleeding phenotype, fibrinogen level and family history. Fibrinogen concentrate is the recommended fibrinogen replacement therapy to treat and prevent bleeding in patients with congenital fibrinogen disorders (53). A fibrinogen level of 100 mg/dL is typically targeted for treatment of minor bleeding, with a higher level of 150 mg/dL targeted for major bleeding, such as ICH (54).

Cryoprecipitate (preferably pathogen-reduced, where available) should only be used as an emergency treatment when fibrinogen concentrate is not accessible. In this context only, a dose of 15–20 mL/kg of pathogen-reduced cryoprecipitate is suggested (55).

In summary, congenital fibrinogen disorders are rare. The optimal treatment and prevention of bleeding is fibrinogen concentrate.

TABLE 2 | Randomized controlled trials of fibrinogen replacement in pediatrics.

References	Setting	Population	Intervention	Comparator	Fibrinogen replacement	Outcomes
Cardiac surgery						
Cui et al. (33)	Single center—China Not blinded	Children with cyanotic heart disease undergoing cardiac surgery $N = 40$ (only 31 analyzed)	Fibrinogen concentrate in addition to traditional transfusion, guided by TEG (500–1,000 mg)	Traditional transfusion guided by clinical experience	500–1,000 mg (Fibrinogen concentrate type not specified)	FC and TEG guided transfusion ($n = 17$) vs. Standard transfusion (non-TEG guided) ($n = 14$) ***Closure time (min): 125 (90–162.5) vs. 138.5 (81.3–194**) Total red cell use (U): 0 (0–1.5) vs. 1.5 (0–2.3), $p = 0.109$ Total Plt use (U): 1 (1–1) vs. 1 (0.7–1.9), $p = 0.984$, Total FFP use (mL/kg): 56.6 ± 17.0 vs. 82.5 ± 31.3, $p = 0.006$ FFP ICU first 24 h. (mL/kg): 10.6 ± :6.5 vs. 22.5 ± 13.1 ml, $p = 0.006$ ICU stay (h.): 137 (106.7–161.2) vs. 173.1 (137.7–477.2), $p = 0.009$ Hospital stay (d.): 21 (15.5–30) vs. 32 (24.3–40.3), $p = 0.006$
Galas et al. (17)	Single center–Brazil Outcome assessors blinded	Children (<7 years) undergoing cardiac surgery and diffuse bleeding after CPB and fibrinogen level <100 mg/dL $N = 63$	Fibrinogen concentrate (60 mg/kg)	Cryoprecipitate (10 ml/kg)	Haemocomplettan P ® CSL Behring, Germany	FC ($n = 30$) vs. Cryoprecipitate ($n = 63$) ***Median 48 h. blood loss (ml): 320 (157–750) vs. 410 (215–510), $p = 0.672$** Post-operative red cell transfusion: 25/30 vs. 32/33, $p = 0.094$ Post-operative FFP: 3/30 vs. 8/33, $p = 0.137$ Post-operative Plt: 0/30 vs. 3/33, $p = 0.24$ Post-operative Cryo: 13/30 vs. 14/33, $p = 0.942$ ICU stay (d.): 10 (6–25) vs. 10 (5–24), $p = 0.930$ Hospital stay (d.): 21 (12–32) vs. 20 (10–38), $p = 0.895$ Death: 0 vs. 0
Massoumi et al. (34)	Single-center–Iran Not blinded	Infants (<2 years) undergoing cardiac surgery $N = 90$	Fibrinogen concentrate (70 mg/kg)	Fresh frozen plasma (10 mL/kg)	Haemocomplettan® P, CSL Behring, Germany	FC ($n = 45$) vs. FFP ($n = 45$) **Chest tube drainage (ml/kg/hr) at 3 h.: 4.77 ± 2.05 vs. 6.94 ± 6.05, $p = 0.02$** **Chest tube drainage (ml/kg/hr) at 6 h.: 4.13 ± 1.84 vs. 6.31 ± 6.22, $p = 0.02$** **Chest tube drainage (ml/kg/hr) at 12 h.: 3.40 ± 1.29 vs. 4.52 ± 4.37, $p = 0.04$** **Chest tube drainage (ml/kg/hr) at 24 h.: 1.93 ± 6.63 vs. 2.64 ± 2.18, $p = 0.04$** ICU stay (d.): 3.04 ± 1.79 vs. 3.66 ± 1.80, $p = 0.89$ Total red cells (U): 5(11.1) vs. 9(20), $p = 0.49$ Total Plt (U): 0 (0) vs. 2 (4.44), $p = 0.38$
Downey et al. (18)	Two centers USA Not blinded	Infants (<12 months) undergoing cardiac surgery post CPB protocol $N = 59$	Fibrinogen concentrate (dose to target 300 mg/dL)	Cryoprecipitate (2 units)	RiaSTAP®, CSL Behring, Germany	FC ($n = 30$) vs. Cryoprecipitate ($n = 29$) —ITT analysis ***Total all blood product (U): 4 (3–5) vs. 5.5 (4–7), $p = 0.007$** Total red cells (U): 2 (1–2.8) vs. 2 (1–2), $p = 0.383$ Total FFP (U): 1 (1–1) vs. 1 (0–1), $p = 0.263$ Total Plt (U): 1 (1–1) vs. 1 (1–1), $p = 0.257$ Total Cryo (U): 0 (0–0) vs. 2 (2–2), $p<0.001$

(Continued)

TABLE 2 | Continued

References	Setting	Population	Intervention	Comparator	Fibrinogen replacement	Outcomes
						FC ($n = 29$) vs. Cryoprecipitate ($n = 25$) —PP analysis Chest tube output (mL/kg): 16.1 (12.6–25) vs. 18.1 (10.9–26), $p = 0.671$ ICU stay (d.): 3 (2–7) vs. 4.5 (2–6), $p = 0.487$ Hospital stay (d.): 7 (4–11) vs. 8 (5–19), $p = 0.342$ Death within 30 day: 1 vs. 0
Siemens et al. (32)	Single-centre UK Partially blinded Trial team not blinded, treating clinicians and patients blinded	Infants (2.5–12 kg) undergoing cardiopulmonary bypass surgery $N = 111$ (monitor cohort, $n = 21$; FC, $n = 60$, placebo, $n = 30$)	Fibrinogen concentrate (individualized dose targeted to FIBTEM-MCF of 8–13 mm)	Placebo (0.9% sodium chloride)	RiaSTAP®, CSL Behring, Germany	FC ($n = 60$) vs. Placebo ($n = 30$) ***FibTEM—MCF post dose (mm): 13.0 (3.2) vs. 4.5 (1.7)** ***Fibrinogen level post dose (g/L): 1.7 (0.4) vs. 0.7 (0.2)** 24 h mediastinal drain loss (ml/kg): 11.6 (5.2) vs. 17.1 (14.3), $p = 0.02$ Perioperative red cells, *n* (%): 21 (35%) vs. 12 (40%) Perioperative FFP *n* (%): 3 (5%) vs. 5 (16.7%) Perioperative Plt *n* (%): 8 (13.3%) vs. 5 (16.7%) Perioperative Cryo *n* (%): 5 (8.3%) vs. 2 (6.7%) Thrombosis: 10 vs. 2
Craniosynostosis surgery						
Haas et al. (29)	Single center—Switzerland Staff in PICU blinded	Children (median age 10 months) undergoing craniosynostosis surgery $N = 30$	Early Fibrinogen replacement FIBTEM MCF <13 mm	Conventional Fibrinogen replacement FIBTEM MCF <8 mm	Haemocomplettan P® CSL Behring, Germany (30 mg/kg)[#]	Early ($n = 13$) vs. conventional ($n = 17$) fibrinogen replacement ***Total red cells in 24 h. (ml/kg): 28.2 (21.2–49.9) vs. 55.5(27.5–61.8), $p = 0.03$** Total FFP in 24 h. (mL/kg): 0 vs. 0, $p = 0.97$ Total Plt in 24 h. (mL/kg): 0 vs. 0, $p = 0.43$ Calculated blood loss (%): 90 (78–113) vs. 157 (111–187), $p = 0.02$ ICU stay (d.): 1 (1–1) vs. 1 (1–1), $p = 0.59$ Hospital stay (d.): 9 (9–9) vs. 9 (9–9), $p = 0.54$
Machotta et al. (31)	Single center Netherlands Blinded	Infants and children (5–25 months) undergoing craniosynostosis surgery $N = 114$ (111 in final analysis)	Fibrinogen concentrate (dose to target 300 mg/dL) + infusion 60 mg/kg	Placebo	Haemocomplettan P® CSL Behring, Germany	Fibrinogen concentrate ($n = 56$) vs. placebo($n = 55$) ***Red cells during hospital stay (ml/kg): 29 (24–42) vs. 29 (22–39), $p = 0.36$** ***FPP volume during hospital stay (mL/kg): 8.8 (0–20.5) vs. 0 (0–20), $p = 0.55$** ***Plt volume during hospital stay (mL/kg): 0 (0–0) vs. 0 (0–0), $p = 0.85$** Total post-operative blood loss (mL/kg): 81 (68–103) vs. 78 (64–97), $p = 0.16$ ICU stay (h.): 20.7 (19.7–21.5) vs. 20.6 (19.8–21.3) Hospital stay (d.): 5 (5–5) vs. 5 (5–5)
Scoliosis surgery						
Haas et al. (29)	Single center—Switzerland Staff in PICU blinded	Children (median age 12 years) undergoing scoliosis surgery $N = 19$	Early Fibrinogen replacement FIBTEM MCF <13 mm	Conventional replacement FIBTEM MCF <8 mm	Haemocomplettan P® CSL Behring, Germany (30 mg/kg)[#]	Early ($n = 10$) vs. conventional ($n = 9$) ***Total red cells in 24 h. (mL/kg): 0 (0–15.3) vs. 18.4 (0–23.8), $p = 0.21$** Total FFP in 24 h. (mL/kg): 0 (0–0) vs. 0 (0–0), $p = 1.0$ Total Plt in 24 h. (mL/kg): 0 (0–0) vs. 0 (0–0), $p = 1.0$

(Continued)

TABLE 2 | Continued

References	Setting	Population	Intervention	Comparator	Fibrinogen replacement	Outcomes
						Calculated blood loss (%): 36.5 (14.9–54.3) vs. 51 (38.5–69.2), $p = 0.17$ ICU stay (d): 1 (1–1) vs. 1 (1–1), $p = 0.5$ Hospital stay (d.): 8 (8–10) vs. 8 (6–13), $p = 0.57$
Chen et al. (30)	Single center–China Blinded	Children (12–18 years) undergoing scoliosis surgery, $N = 102$	Fibrinogen concentrate (30 mg/kg to a maximum dose of 2,000 mg)	Placebo	FIBRORAAS, Shanghai RAAS Blood Products Co, Ltd, Shanghai	Fibrinogen concentrate ($n = 51$) vs. placebo ($n = 51$) ***Perioperative blood loss (mL): 885 (755–1,155) vs. 1,035 (818–1,420), $p = 0.041$** Perioperative red cells (U): 0 (0–0) vs. 0 (0–0), $p = 0.34$ Perioperative FFP (mL): 0 (0–0) vs. 0(0–0), $p = 0.29$ Hospital stay (d.): 6 (5–7) vs. 7 (5–8), $p = 0.45$

CPB, Cardiopulmonary bypass; Cryo, Cryoprecipitate; d., days; FC, Fibrinogen concentrate; FFP, fresh frozen plasma; h., hour; ICU, intensive care unit; ITT, intention to treat; MCF, maximum clot firmness; PP, per protocol; Plt, platelets; U, units; UK, United Kingdom; US, United States of America.

** (in bold) Primary outcome of study.*

[#]Note concurrent coagulation management algorithm included administration of intraoperative FXIII concentrate if FXIII <30%, or between 30 and60% during an episode of bleeding requiring transfusion.

Liver Disease

The liver plays a key role in the synthesis of multiple hemostatic proteins. Acquired hypofibrinogenemia may be seen when hepatic damage is severe enough to compromise synthetic liver function (56). Liver dysfunction also results in dysfibrinogenemia, with synthesis of an abnormally functioning fibrinogen (56).

In children with severe liver-disease, our understanding of hemostasis is predominantly derived from the adult literature (10). However, this may not be appropriate, given the etiologies of pediatric liver failure differ considerably compared with those seen in adults (57) and hemostasis is age-dependent.

In adults with cirrhosis, low fibrinogen levels (<60 mg/dL) have been highlighted as a predictor for major bleeding (58). Prophylactic fibrinogen replacement has been evaluated in on a RCT conducted in adults undergoing liver transplantation randomized to prophylactic fibrinogen concentrate (dosed to target Fibrinogen of 290 mg/dL) vs. placebo (59). The study found no difference in the study's primary outcome of red cell transfusion requirements (59).

Low quality recommendations or consensus statements are made regarding fibrinogen supplementation in adult liver disease guidelines. Most advise against prophylactic replacement of coagulation factors and that replacement be limited to instances of active bleeding or high-risk invasive procedures (60–63). Some guidelines specify that in the context of active bleeding, a fibrinogen level of >100 mg/dL be maintained (61, 64).

However, this has not been established in pediatric studies. Children with liver failure rarely develop significant spontaneous bleeding (65) indicating that our understanding of the hemostatic equipoise seen in infants and children with advanced liver disease is limited (10). The role of prophylactically correcting laboratory values in children who are not bleeding or undergoing invasive procedures needs to be justified. However, in the context of active bleeding, children with acquired hypofibrinogenemia secondary to liver disease may benefit from fibrinogen replacement (65).

In summary, the risk of bleeding in children with advanced liver disease and acquired hypofibrinogenemia appears to be less than in adults. Therefore, routine prophylactic fibrinogen supplementation is not advised. There may, however, be a role for correcting hypofibrinogenemia in the context of significant bleeding and advanced liver disease.

L-Asparaginase

L-asparaginase is a key component in pediatric acute lymphoblastic leukemia (ALL) induction chemotherapy protocols (66, 67). As a secondary effect it inhibits the synthesis of hepatic L-asparagine dependent proteins, leading to significant reductions in antithrombin and fibrinogen (68). Hypofibrinogenemia is frequently seen (67), however due to reduced antithrombin levels, thrombotic rather than bleeding complications are encountered (66).

There is a paucity of evidence evaluating fibrinogen replacement in children with acute leukemia and thresholds are largely consensus based and derived from other clinical settings (69). Prophylactic fibrinogen supplementation in this setting is controversial (69–71). Some advocate for therapeutic-only treatment or when there is an increased risk of bleeding due to co-morbidity (70). Others recommend prophylactic cryoprecipitate in all oncology patients with acquired hypofibrinogenemia secondary to medications (72, 73).

In summary, L-asparaginase is frequently used to treat pediatric ALL and commonly causes acquired hypofibrinogenemia. Current evidence does not point towards an increased bleeding tendency.

Therefore, many pediatric ALL protocols do not recommend prophylactic correction with fibrinogen replacement. The role of fibrinogen supplementation in the bleeding child receiving L-asparaginase is unknown.

Fibrinogen Loss Exceeding Fibrinogen Production

Massive blood loss may be encountered in children, in the setting of refractory surgical bleeding and trauma (74). Fibrinogen is the first coagulation factor to fall to a critical level of 100 mg/dL and may be encountered after loss or replacement of 1–1.5 blood volumes (75). Additional factors that may contribute to hypofibrinogenemia developing in pediatric trauma and surgical settings include: lower baseline fibrinogen levels in children, hemodilution from crystalloids, or unbalanced hemostatic resuscitation (11), clotting factor consumption, as well as hyperfibrinolysis.

Pediatric Cardiac Surgery

Neonates and children undergoing major cardiac surgery are at risk of post-operative bleeding and as a result, commonly receive red cell transfusions (76). Cardiopulmonary bypass (CPB) results in hemodilution, clotting factor consumption, platelet dysfunction, and fibrinolysis (77).

We identified five completed RCTs ($n = 295$) specific to the pediatric cardiac surgical setting (17, 18, 32–34). See **Table 2** for further details of these studies.

Two RCTs have compared fibrinogen concentrate with placebo, one as part of a TEG-guided transfusion strategy (33) and the other, as part of a feasibility study evaluating intraoperative ROTEM for screening patients at risk of bleeding (32). A further RCT was identified, comparing fibrinogen concentrate with FFP (34).

Cui et al. randomized 40 children (of which 31 were analyzed) with cyanotic heart disease undergoing cardiac surgery to a TEG-guided transfusion strategy using fibrinogen concentrate (500–1,000 mg) compared to a transfusion guided by clinical experience strategy (without fibrinogen concentrate), with a primary outcome of time to chest wall closure (33). There was no significant difference seen in time to chest wall closure, nor total red cell or platelet usage between study arms. There was, however, statistically lower FFP usage in the fibrinogen concentrate arm, ($p = 0.006$). Unfortunately, the study was not blinded, intention-to-treat principle was not applied, and no information was provided about the median dose, the number or timing of fibrinogen concentrate doses, or the TEG intervention points or any adverse events, limiting its applicability to other settings (33). Use of anti-fibrinolytics was not reported.

Siemens et al. enrolled 111 infants with congenital heart disease undergoing CPB surgery to investigate the feasibility of using intraoperative ROTEM® as a screening tool to predict post-operative bleeding and guide fibrinogen replacement (32). The trial included a monitoring arm of 21 children with a FIBTEM-Maximum Clot Firmness (MCF) >7 mm and an intervention arm (FIBTEM-MCF <6 mm) comprising 60 children randomized to individually-dosed fibrinogen concentrate (target FIBTEM-MCF 8–13 mm) and 30 to placebo (32). Co-primary outcomes were FIBTEM-MCF and fibrinogen levels 5 min post fibrinogen concentrate/placebo administration (32). In addition, the study aimed to review the dosing, safety, and efficacy of fibrinogen concentrate. Whilst FIBTEM-MCF and fibrinogen levels were higher in the fibrinogen concentrate arm, no *p*-values were provided. The study was not powered for efficacy outcomes and the authors did not report *p*-values for any transfusion data. They did however, report significantly lower 24-h mediastinal drain losses in those receiving fibrinogen concentrate ($p = 0.02$). Ten cases of thromboembolism were reported in children who received fibrinogen concentrate compared with two in the placebo arm (32). The study was partially blinded; clinicians and patients were blinded to treatment group. All patients received tranexamic acid.

One RCT was identified where fibrinogen concentrate was compared with FFP (34). Massoumi et al. randomly allocated children undergoing cardiac surgery to receive either FFP (10 ml/kg) or fibrinogen concentrate (70 mg/kg), with a primary outcome of post-operative chest tube drainage. The study reported that chest tube drainage was less in those receiving fibrinogen concentrate ($p = 0.04$). Although not statistically significant due to low numbers, more blood products were given in the FFP-arm. Limitations of the study include the lack of blinding and the method of randomization. Only the first patient was randomized to the fibrinogen treatment arm and subsequent children allocated to FFP or fibrinogen in a 1:1 ratio. Anti-fibrinolytics were not routinely administered.

Two additional RCTs have been conducted comparing cryoprecipitate with fibrinogen concentrate: one in the setting of post-operative bleeding (17) and one as part of a post-CPB protocol (18).

Galas et al. randomized 63 children with diffuse bleeding after CPB and fibrinogen <100 mg/dL to receive 10 mL/kg of cryoprecipitate vs. 60 mg/kg fibrinogen concentrate with a primary outcome of 48-h postoperative blood loss (17). The study found no difference in 48-h blood loss; 320 mL in the fibrinogen concentrate arm vs. 410 mL in those who received cryoprecipitate ($p = 0.672$). There was no statistical difference seen in red cell transfusion rates between study arms; 83.3% of the fibrinogen concentrate arm received a red cell transfusion compared with 97% in the cryoprecipitate arm ($p = 0.094$) (17). Both fibrinogen concentrate, and cryoprecipitate improved fibrinogen levels post intervention. However, there was no difference seen in fibrinogen levels or FIBTEM-MCF between treatment arms and no difference seen for other secondary outcomes (17). Limitations of the study include the lack of blinding of study personnel to treatment allocation. Other considerations are that the cryoprecipitate arm included a higher proportion of neonates and a longer period of cardiopulmonary bypass. Also, more than 40% of patients in both the cryoprecipitate and fibrinogen study groups received additional doses of cryoprecipitate within the first seven post-operative days (17). All patients received ε-aminocaproic acid.

Downey et al. included 59 infants undergoing cardiac surgery randomized to receive two units of cryoprecipitate or fibrinogen concentrate (dosed to target Fibrinogen of 300 mg/dL) as part of a post-CPB hemostasis algorithm with a primary outcome of

intraoperative transfusions (18). There was no difference in the red cell, FFP or platelet components received between treatment groups, but the fibrinogen concentrate arm received 1.79 less total blood products compared with the cryoprecipitate arm (intention to treat analysis), likely reflecting the intervention of two units cryoprecipitate (18). Both fibrinogen concentrate, and cryoprecipitate improved fibrinogen levels post intervention. In the per protocol analysis, immediately after administration of fibrinogen supplementation, the median fibrinogen level in the fibrinogen concentrate arm was higher (314.5 mg/dL [IQR: 296.5–342.5]) than the cryoprecipitate arm (276.5 mg/dL [IQR: 192.2–323.5]) ($p = 0.025$). There was also a narrower interquartile range in the fibrinogen concentrate arm, reflecting less variability in the fibrinogen level achieved. A consideration must be the dosing strategy, since the fibrinogen concentrate arm had their dose calculated based on weight, whilst the cryoprecipitate group were administered a standardized dose. The dose of two units cryoprecipitate was based on the author's previous experience and was expected to achieve a post-infusion fibrinogen level of 345 mg. In the study, this dose did not result in the desired level, in is unclear if the reflects product fibrinogen variability or insufficient dosing for weight. There was no difference seen for any secondary outcomes or adverse events. Limitations of the study are that it was not blinded, it was conducted over two centers and there were differences between the two centers with respect to their cardio-pulmonary bypass protocols, the use of four factor prothrombin complex concentrate and the type of antifibrinolytics used. One center used tranexamic acid and the other ε-aminocaproic acid for neonates and those undergoing a re-do sternotomy (18).

An important RCT from the adult cardiac setting included 735 adults with post-operative bleeding and hypofibrinogenemia randomized to 10 units of cryoprecipitate or 4 g of fibrinogen concentrate, with a primary outcome of 24-h cumulative blood products after CPB (78). No difference was seen in the study's primary outcome and no difference was found for secondary transfusion outcomes, mortality or other relevant clinical outcomes (78). It is important to note that the fibrinogen concentrate used (Fibryga®) has much higher levels of FXIII than other fibrinogen concentrates (79).

These studies do not infer superiority of either cryoprecipitate or fibrinogen concentrate but suggest that fibrinogen concentrate may be an alternate, safe, and effective fibrinogen replacement therapy to cryoprecipitate, in the pediatric cardiac surgical setting.

The importance of fibrinogen supplementation in children undergoing cardiac surgery, with evidence of hypofibrinogenemia has been recognized in clinical guidelines. The Network for the Advancement of Patient Blood Management, Haemostasis, and Thrombosis (NATA) pediatric cardiac surgery guidelines recommend that hypofibrinogenemia diagnosed by either Clauss method (<150 mg/dL) or viscoelastic tests (based on an institution-specific algorithm) should be treated with cryoprecipitate or fibrinogen concentrate (37). See **Table 1**.

Pediatric Surgery

Children undergoing cardiothoracic surgery, liver transplantation, craniofacial and neurosurgical procedures, hepatobiliary surgeries, and tumor resection procedures have a high frequency of transfusion (80) and may need PICU admission (81). Craniosynostosis surgery in particular, is frequently associated with coagulopathy and transfusion. Hypofibrinogenemia has been identified as a major risk factor for bleeding in this setting (82).

Our review identified three trials ($n = 262$) evaluating fibrinogen supplementation in the scoliosis and craniosynostosis surgery settings (29–31). See **Table 2** for further details.

The timing of preemptive fibrinogen concentrate use has been studied in one pediatric RCT combining infants (median age 10 months) undergoing craniosynostosis surgery and adolescents (median age 12 years) undergoing scoliosis repair (29). Haas et al. randomized 30 children to receive 30 mg/kg fibrinogen concentrate at two predefined FIBTEM cut-offs, a MCF of <8 mm (conventional arm) vs. <13 mm (early substitution arm) with a primary outcome of cumulative volume of red cells received in the first 24 h. Red cell transfusion requirements in the craniosynostosis group were significantly lower in the early fibrinogen replacement group (28 vs. 56 mL/kg, $p = 0.03$). In addition, total blood loss was less in the early fibrinogen replacement group (89.7 vs. 156.9%, $p = 0.02$). There was however, no difference found for red cell transfusion requirements or blood loss in the scoliosis cohort. It is important to note that FXIII replacement was included as part of the study protocol and all subjects received tranexamic acid (29). Specific study limitations were that it was only partially blinded, with only PICU staff blinded to intervention arm and the small sample sizes of the two surgical cohorts. The study ceased early due to difficulties with recruitment with only 57 (instead of 60) subjects randomized. In addition, seven of the 26 randomized scoliosis patients did not meet the FIBTEM trigger for fibrinogen supplementation and could not be included in the analysis (29).

To evaluate the effect of prophylactic administration of fibrinogen concentrate in the craniosynostosis surgery setting, Machotta et al. randomized 114 children to receive fibrinogen concentrate vs. placebo, with a primary outcome of transfusion volume during hospital stay (31). Fibrinogen concentrate (dose to target a fibrinogen of 300 mg/dL [median dose 79 mg/kg]), followed by an infusion of 60 mg/kg during the first hour of surgery (31). Of the 111 children analyzed, fibrinogen concentrations were significantly higher in the fibrinogen treatment arm, but there was no significant difference between the study arms with regard to the transfusion volume, perioperative blood loss, or secondary outcomes. Median volume of red cells received by patients was 29 mL/kg in both the fibrinogen concentrate and placebo arms ($p = 0.36$) (31). This was a double-blinded placebo-controlled trial and the authors attempted to limit confounders, by restricting use of perioperative tranexamic acid, heparin, or non-steroidal anti-inflammatory drugs. They did, however, allow the use of hydroxyethyl starch (HES) which can cause falsely reduced fibrinogen levels (83). Limitations of the study were the slow

study recruitment and early termination after 114 out of the planned 120 patients. Three patients were excluded from the final analysis because they received the wrong intervention or no intervention (83).

Chen et al. conducted a blinded RCT, comparing prophylactic fibrinogen concentrate (30 mg/kg) with placebo in 102 adolescents with idiopathic scoliosis undergoing surgery (30). The study's primary outcome was perioperative blood loss, which included intraoperative blood loss and postoperative wound drainage. Fibrinogen concentrate resulted in statistically reduced perioperative blood loss, median reduction of 155 mL (95%CI: 5–320 mL) ($p = 0.04$) compared with the placebo arm. There was no difference found in transfusion rates between study arms. Administration of fibrinogen concentrate resulted in statistically increased fibrinogen levels and MA on the TEG-FF assay. Postoperative fibrinogen levels were inversely correlated with postoperative bleeding ($p < 0.001$) (30). All patients received tranexamic acid and intraoperative cell salvage. The trial stopped prematurely due to a shortage in fibrinogen concentrate, with 102 out of the intended 104 participants recruited (30).

RCTs from both the pediatric cardiac and craniofacial surgery setting suggest that there are different risk factors that influence the need for fibrinogen supplementation. A prospective study looking at predictors of blood loss and red cell transfusion in children undergoing craniosynostosis surgery, found that the type of procedure, the duration of surgery, in addition to post-operative TEG parameters, α-angle, MA, and K-time were all associated with transfusion. Based on these results they developed a TEG-based predictive algorithm for children with critical blood loss (>60 mL/kg) to guide administration of hemostatic products, including fibrinogen concentrate (84).

The 2016 European guideline for the management of severe perioperative bleeding, do not make any recommendation for fibrinogen replacement in pediatric surgery, stating that neither the optimal threshold for initiation of fibrinogen replacement nor the dose required to reach the targeted fibrinogen concentration have been proven by high quality data (41).

In contrast, both the Australian pediatric PBM guidelines and National Institute for Health and Care Excellence (NICE) 2015 guidelines provide guidance around using cryoprecipitate to treat active bleeding in surgical settings when the fibrinogen is <150 mg/dL (24, 39). Neither of these guidelines however, mention FIBTEM or TEG-FF thresholds. See **Table 1**.

In summary, hypofibrinogenemia is recognized as a risk factor for perioperative bleeding, in both cardiac and general pediatric surgery. The highest quality evidence for the use of fibrinogen supplementation in children is found in the pediatric cardiac, scoliosis and craniofacial surgery settings. A number of small RCTs (mean number of participants 77, range [31-111]) have been performed. Results from systematic reviews published and RCTs appear to indicate that fibrinogen concentrate may reduce bleeding and reduce the risk of a patient needing a transfusion, compared with no intervention. Unfortunately, current data cannot be pooled due to heterogeneity. Therefore, we can only conclude, that there may be a role for fibrinogen supplementation in young children undergoing high-risk surgical procedures. No superiority of cryoprecipitate compared with fibrinogen concentrate have been demonstrated. Thrombo-embolic events should be considered in children receiving fibrinogen replacement.

Pediatric Trauma

Accidental injuries are the leading cause of death in children and high rates of blunt trauma and traumatic brain injury (TBI) are seen in children compared with adult trauma patients (12, 85, 86). Acute traumatic coagulopathy is commonly encountered in severely injured children prior to the administration of fluids and transfusion (87–90). Prolonged PTs/elevated INRs in trauma patients, including those with TBI appear associated with increased mortality (12, 87, 91). Whilst hypofibrinogenemia has been reported to be an independent predictor of mortality in adult major trauma patients (92, 93), this has not been consistently shown in pediatric studies (12, 87, 90, 94).

In the adult trauma setting, validation studies in the ROTEM® have shown that the FIBTEM CA5 (clot amplitude at 5 min) (95), FIBTEM-MCF and FIBTEM A10 (clot amplitude at 10 min) (96) may be used to predict patients requiring massive transfusion and be used as triggers for fibrinogen supplementation. In the pediatric trauma setting, viscoelastic testing is increasingly used, but studies are needed to establish intervention thresholds (97).

At this moment, there is no high-quality evidence that treatment of hypofibrinogenemia in pediatric trauma patient results in improved patient outcomes. A single case report discusses the successful use of fibrinogen concentrate in a pediatric trauma patient with severe abdominal and pelvic injuries after blunt trauma, without the need for FFP or platelet transfusion (98). However, in the adult combat setting, a retrospective review of 252 trauma patients in Iraq, found that delivery of a higher fibrinogen to red cell ratio was independently associated with improved survival in patients requiring massive transfusion (99).

Nevertheless, pediatric consensus transfusion guidelines do support fibrinogen replacement during critical bleeding and massive blood loss (24, 25). The British Society of Hematology (BSH) advises to target a fibrinogen of >150 mg/dL in massive blood loss and after >40 mL/kg red cells to consider cryoprecipitate transfusion (25). The Australian guidelines support the use of higher targets of 200 mg/dL during critical bleeding, using cryoprecipitate (24). Recent European trauma guidelines do not provide specific recommendations for children, but state that children in general can be managed the same way as adults (100). They recommend one of two initial resuscitation strategies for massive hemorrhage; either FFP and red cells in a ratio of at least 1:2 or fibrinogen concentrate and red cells (100). If there is major bleeding and hypofibrinogenemia (viscoelastic signs of functional fibrinogen deficit or fibrinogen <150 mg/dL) they recommend fibrinogen supplementation with either fibrinogen concentrate or cryoprecipitate (100). However, if fibrinogen concentrate-based management is used, they suggest that FXIII be included in coagulation support algorithm (100). See **Table 1**.

The Fibrinogen Early in Severe Trauma Study (FEISTY) Junior is an Australian pediatric feasibility RCT comparing the use of early fibrinogen concentrate against cryoprecipitate

in children with severe trauma using a ROTEM® based transfusion algorithm (FIBTEM A5 <10 mm as their threshold for intervention) (101). The study's primary outcomes include: time to administration of fibrinogen replacement, feasibility outcomes, and effect on fibrinogen levels (101).

In summary, coagulopathy including hypofibrinogenemia frequently occurs in pediatric trauma patients. There is limited evidence in the literature supporting or refuting fibrinogen supplementation in the pediatric trauma settings. Both cryoprecipitate and fibrinogen concentrate are widely used to provide fibrinogen supplementation in pediatric trauma patients and form key components in pediatric massive transfusion protocols, with evidence extrapolated from adult trials. More research is needed to identify pediatric categories and triggers in which fibrinogen supplementation is beneficial.

Hyperfibrinolysis

Disseminated Intravascular Coagulopathy

Disseminated intravascular coagulopathy (DIC) is an acquired, life-threatening condition, resulting in systemic coagulation activation and is associated with both bleeding and thrombosis (102). In children, DIC most commonly occurs secondary to sepsis, but may be seen after major trauma, TBI, malignancy, snakebites, and in vascular malformations (14). The diagnosis of DIC is based on the presence of an underlying causative disorder in conjunction with a combination of laboratory features, including hypofibrinogenemia and fibrinolysis (103). Fibrinogen levels as a single parameter are insensitive in predicting DIC, as they may be elevated as an acute phase reactant and therefore serial testing may be important (104).

The most important aspect of DIC management is timely and appropriate treatment of the underlying condition (103). Transfusion should be reserved for children with active bleeding and should not be given based on laboratory parameters alone (14). The International Society of Thrombosis and Hemostasis (ISTH) recommend fibrinogen replacement only in patients with active bleeding and persistently low fibrinogen levels <150 mg/dL despite treatment with FFP (103). The more recent BSH pediatric transfusion guideline recommends cryoprecipitate be given if the fibrinogen is <100 mg/dL despite FFP or in conjunction with FFP for a very low or rapidly falling fibrinogen (25).

In summary, DIC is a clinico-pathological diagnosis. The focus of management of DIC should focus primarily on treating the underlying condition. The role of fibrinogen replacement is subject to debate but may be indicated in children with clinically significant bleeding and severe hypofibrinogenemia. The optimal fibrinogen replacement product is unknown.

Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) is increasingly used in neonatal and pediatric critical settings to provide life-saving cardiopulmonary support (3). Neonates and children make up more than half of all ECMO runs reported to the international ELSO registry (105). ECMO circuits comprise artificial and non-endothelial surfaces that, when presented to patient blood, result in fibrinogen adsorption, contact pathway activation, coagulation activation, thrombin generation and fibrinolysis (13, 106). ECMO provokes a significant hemostatic challenge, and as a result both bleeding and thrombotic complications are commonly reported (3, 107, 108). Bleeding in children on ECMO is significantly associated with an increased risk of death (3, 107, 108).

A prospective observational cohort study of 514 pediatric and neonatal ECMO patients identified major bleeding (defined as blood loss requiring transfusion or ICH) in more than 70% of children (3). Whilst fibrinogen levels were monitored on more than 90% of ECMO days, a low fibrinogen was not identified as a risk factor for bleeding (3). In contrast, a small retrospective study of 32 neonates with persistent pulmonary hypertension requiring ECMO reported that low fibrinogen levels were associated with a higher incidence of ICH (109).

It is advised that fibrinogen levels are regular monitored (108, 110, 111), however no RCTs have been performed in this patient cohort looking at fibrinogen thresholds and supplementation and hence there is no consensus on the desired fibrinogen levels needed (110). There is variable practice with respect to fibrinogen supplementation in this cohort. The ELSO guidelines, advise for transfusions of plasma or cryoprecipitate to maintain fibrinogen levels >150 mgd/L in neonates (112) and 250–300 mg/dL in children (111, 112). Whilst other centers report targeting fibrinogen levels >100–150 mg/dL in neonates (108) and fibrinogen levels >200 mg/dL in children (110).

Use of fibrinogen concentrate to treat and prevent bleeding complications is reported in adults on ECMO (113, 114), but not children.

In summary, major bleeding is commonly seen in pediatric ECMO patients and hypofibrinogenemia may be encountered. Evidence supports regular monitoring, but appropriate triggers for supplementation with either plasma, cryoprecipitate or fibrinogen concentrate are unknown. Considering the high incidence of bleeding in children on ECMO, this area deserves more attention and research.

Thrombolysis in the Pediatric Setting

Venous and arterial thromboembolism are commonly encountered in the pediatric critical care setting (115). Treatment largely comprises targeted anticoagulation with intravenous heparin or low molecular weight heparin (115). Thrombolysis has a role in the treatment of life and organ threatening thrombosis and tPA is the most commonly studied and used (116, 117).

Systemic thrombolysis with tPA results in decreased fibrinogen levels, with many children developing significant hypofibrinogenemia and major bleeding (118). A retrospective study of 79 children treated with systemic tPA for thromboembolism, found that 56 experienced a drop in fibrinogen levels (median decrease 100 mg/dL) and 10 children had fibrinogen levels <100 mg/dL. Overall, bleeding occurred in 54 (68%) children, with 31 (39%) requiring a red cell transfusion. Bleeding appeared to be seen in those who had

the largest reductions in their fibrinogen levels following thrombolysis (118).

Due to substantial bleeding risk, pediatric thrombolysis guidelines advise that fibrinogen be maintained >100 mg/dL during thrombolysis and major bleeding with hypofibrinogenemia be treated with cryoprecipitate (116, 117, 119). Use of fibrinogen concentrate is reported in adult stroke patients with severe hypofibrinogenemia following treatment with tPA (120).

In summary, children receiving systemic tPA are at significant risk of hypofibrinogenemia and bleeding complications. The evidence is too scarce to define a fibrinogen replacement trigger during or shortly after tPA. On the basis of retrospective data, general consensus advises to maintain fibrinogen levels of >100mg/dL

Acute Leukemia

Hypofibrinogenemia may be seen in the context of pediatric leukemia associated DIC and is frequently encountered with acute promyelocytic leukemia (APML). APML cells express pro-coagulants including tissue factor and cancer procoagulant, as well as fibrinolytic proteins (plasminogen activators [t-PA and u-PA] and inhibitors [PAI-1] and their receptors [annexin II]) (121). As a result, APML is typically associated with a severe coagulopathy, consistent with a picture of DIC, excess hyperfibrinolysis, and marked hypofibrinogenemia (122). In children, hemorrhage is the main cause of early mortality (123). APML is a pediatric medical emergency necessitating urgent treatment with all-trans-retinoic acid (ATRA). ATRA induces terminal differentiation of malignant promyeloblasts to mature neutrophils (124). This cellular differentiation results in loss of the procoagulant and fibrinolytic properties of the APML cells, with improvement in bleeding symptoms and coagulopathy (121, 123).

There are no trials evaluating fibrinogen replacement triggers in APML. Major clinical trials advise that fibrinogen levels be monitored and treated with transfusions (124). The Pediatric International Consortium for Childhood APL (ICC- APL- 01) trial advises that fibrinogen levels >150 mg/dL be maintained during the first 10 days of induction therapy or until resolution of any coagulopathy, using FFP (125). The Children's Oncology Group (COG) Study AAML0631 trial protocol advises that the fibrinogen be maintained >100 mg/dL (126). The European LeukemiaNet guidelines suggest that following the diagnosis of APML immediate fibrinogen supplementation (FFP, fibrinogen, and or/cryoprecipitate) be implemented to maintain a fibrinogen concentration >100–150 mg/dL and to continue through induction therapy until the coagulopathy resolves (127). Similar guidance is provided in the Canadian Blood Transfusion Guideline (42).

In summary, APML-associated hypofibrinogenemia carries a high risk of bleeding. The only definitive treatment is urgent treatment with ATRA, but in instances of clinically significant bleeding bridging with fibrinogen supplementation is advised on expert opinion. Most guidelines advise preemptive supplementation when fibrinogen levels fall <100mg/dL, but the optimal product is unknown.

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening inflammatory syndrome with a peak incidence in infancy. It results from a dysregulated immune response that leads to pathological hyperactivation of NK cells, T lymphocytes, and macrophages (128). Hypofibrinogenemia (<150 mg/dL) is one of the eight criteria, included in the 2004 HLH diagnostic criteria, where five out of eight criteria are required for diagnosis (129). Diagnostic workup for HLH in a child will include immune and genetic evaluation to measure proteins affects in familial HLH and viral serologies (EBV and CMV) to identify a common viral trigger for HLH (130).

It is not known, why hypofibrinogenemia develops in HLH, proposed mechanisms include: hyperfibrinolysis secondary to DIC, decreased fibrinogen production secondary to hepatic macrophage infiltration, cytokine storm, and hyperfibrinogenolysis (131). A retrospective review of 117 adults with HLH, reported that fibrinogen levels <200 mg/dL were associated with severe bleeding and independently associated with higher mortality (131). On the contrary, retrospective studies looking at predictors for mortality in children with HLH, did not identify hypofibrinogenemia as being a significant factor (132–134). Acute bleeding in the setting of hypofibrinogenemia and HLH may require fibrinogen supplementation (135). In general though, hypofibrinogenemia will only resolve with definitive HLH-treatment with etoposide and dexamethasone (130). Guidance around triggers for prophylactic fibrinogen supplementation in children was not found.

In summary, hypofibrinogenemia is one of the eight criteria used in making the diagnosis of HLH. Patients with HLH are at risk of bleeding. The only definitive treatment is HLH-treatment with etoposide and dexamethasone. In cases of clinical bleeding and severe hypofibrinogenemia bridging with fibrinogen supplementation may be considered.

NEONATES AND HYPOFIBRINOGENEMIA

There are many age-dependent differences in fibrinogen and fibrinolysis in neonates, compared with older children. Regardless of the relative immaturity in the neonatal hemostatic system, in general neonates appear to have effective and balanced coagulation, and do not tend to bleed spontaneously (136).

In the critical care setting (NICU), neonates have different etiologies for acquired hypofibrinogenemia compared with older children. DIC in the neonate, most commonly occurs in the setting of perinatal asphyxia, but can be due to sepsis and perinatal acquired infections, respiratory distress syndrome and meconium aspiration (14, 137, 138). Severe perinatal asphyxia can cause significant hypoxic brain injury and multi-organ failure, including hepatic damage (139, 140). Hypoxic hepatic damage can lead to reduced production of coagulation factors and hypofibrinogenemia (140).

Rare, but potentially life-threatening causes of acquired hypofibrinogenemia in neonates include purpura fulminans due to congenital deficiency of protein C or protein S (141) and Kasabach-Merritt phenomenon (an acute consumptive coagulopathy specifically associated with two vascular tumors) (142). In Kasabach-Merritt phenomenon, a neonate may present with a rapidly growing tumor, with thrombocytopenia and severe hypofibrinogenemia, due to platelet sequestration, and fibrinogen consumption (142). There is a high risk of bleeding and management involves surgical or medical treatment of the tumor. If fibrinogen levels are <100 mg/dL then FFP or cryoprecipitate is recommended, especially in the presence of bleeding (143).

The Italian neonatal transfusion guidelines provide definitions and guidance for fibrinogen replacement in neonates. They recommend observation rather than treatment in neonates with hypofibrinogenemia and no bleeding. But when active bleeding is present or a neonate is about to undergo an invasive procedure and the fibrinogen level is below the lower limit for gestational age, they recommend 5–10 mL/kg of cryoprecipitate (38).

In summary, neonates tend to have lower fibrinogen levels without an increased bleeding risk. Therefore, most guidelines do not advise to correct asymptomatic hypofibrinogenemia. When an acquired, severe hypofibrinogenemia occurs and there is a high risk of bleeding, fibrinogen supplementation may be considered. The optimal fibrinogen replacement product in neonates is unknown.

CURRENT KNOWLEDGE GAPS, CONTROVERSIES, AND AREAS FOR RESEARCH

Although prophylactic and therapeutic use of cryoprecipitate and fibrinogen concentrate in both congenital and acquired hypofibrinogenemia has been widely adopted in a variety of pediatric critical care situations, there remain many uncertainties and controversies regarding fibrinogen replacement. Similar sentiments are echoed in the current adult literature (144–146).

The evidence base to support fibrinogen replacement in children is sparse or extrapolated from (also sparse) adult studies. We can only emphasize the need for more, well-designed, and sufficiently powered clinical trials in children. Considering the multiple national and international guidelines that exist about the use of fibrinogen in children, and the lack of consensus between guidelines, we see a medical need for international collaboration between guideline-writing groups.

There are number of questions that still need to be answered and may be proposed as areas for future research in neonates, including those preterm, infants, children, and adolescents with regard to clinical indications for fibrinogen supplementation.

Fibrinogen thresholds:

- What is the relationship between fibrinogen and bleeding in neonates and in children?
- What is the relationship between fibrinogen and bleeding in different clinical settings e.g., massive hemorrhage, trauma, CPB, major surgical bleeding, ECMO, liver disease, DIC, and hyperfibrinolysis?
- What are the clinical indications for prophylactic fibrinogen replacement in neonates and children?
- What are the clinical indications for therapeutic fibrinogen replacement in children?
- What is the optimal fibrinogen level for neonates and children?
- undergoing high-risk surgical or invasive procedures?
- presenting with massive blood loss due to severe trauma?
- with other critical care conditions?

Prediction of hypofibrinogenemia-related bleeding and optimal treatment in children:

- How do we predict which neonates and children will bleed during or following surgery, and who are most likely to benefit from fibrinogen supplementation?
- How do we predict which neonates and children are most likely to benefit from fibrinogen supplement following trauma with massive blood loss or TBI?
- How do we predict which neonates and children are most likely to benefit from fibrinogen supplement in the context of sepsis, DIC, leukemia, and vascular tumors?
- Which fibrinogen product is the best choice in the different pediatric critical care clinical settings?

Clinical trials and guidelines:

- How do we increase the quality of clinical trials studying transfusion medicine in neonates and children?
- Can we come to an international consensus when there is a lack of evidence to reduce the variability of indications and triggers seen between national and international guidelines?

CONCLUSIONS

Critically ill children frequently experience bleeding events and hypofibrinogenemia is implicated in adult and pediatric settings as an important risk factor for bleeding. There remains considerable uncertainty in children of all ages around optimal fibrinogen levels and the best fibrinogen replacement strategies. Cryoprecipitate and fibrinogen concentrate are both given to prevent and treat bleeding due to hypofibrinogenemia, in spite of a sparse evidence base. Neonates and children continue to be under-represented or underpowered in clinical trials. Further evidence and RCTs in pediatric transfusion medicine are needed, so that, rather than extrapolate from adult studies or base practice on experience, best and evidence-based practice is delivered.

AUTHOR CONTRIBUTIONS

GC literature review and writing and editing article. EH reviewing content and editing article. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: EH was an author of one of the RCTs discussed in this article.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pediatrics

REVIEW
published: 21 April 2021
doi: 10.3389/fped.2021.617500

Pediatric Fibrinogen PART I—Pitfalls in Fibrinogen Evaluation and Use of Fibrinogen Replacement Products in Children

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Fibrinogen is a key coagulation protein, playing a critical role in hemostasis. It is the first factor to decrease to critical levels during bleeding. Hypofibrinogenemia is an important risk factor for bleeding in clinical settings, including pediatric surgery. Yet, the optimal measurement of fibrinogen levels is subject to debate, as is the critical threshold for intervention. Fibrinogen replacement may be provided by cryoprecipitate and fibrinogen concentrate. Whilst both products contain fibrinogen, they are not equivalent, each has its own advantages and disadvantages, especially for pediatric use. Unfortunately, medical literature to support fibrinogen replacement in children is limited. In this article we review the current diagnostic tools to measure fibrinogen, with respect to their use in the pediatric critical care setting. Secondly, we evaluate the different fibrinogen replacement therapies, focusing on cryoprecipitate and fibrinogen concentrate and examine their individual product characteristics, associated risks and benefits, different dosing strategies and specific pitfalls for use in children. We summarize by highlighting current knowledge gaps and areas for future research.

Keywords: children, intensive care, fibrinogen, hypofibrinogenemia, Clauss, viscoelastic testing, cryoprecipitate, fibrinogen concentrate

OPEN ACCESS

Edited by:
Jan Hau Lee, KK Women's and Children's Hospital, Singapore

Reviewed by:
E. Vincent S. Faustino, School of Medicine Yale University, United States
Joyce Ching Mei Lam, Paediatric Haematology/Oncology Service, KK Women's and Children's Hospital, Singapore

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Specialty section:
This article was submitted to Pediatric Critical Care, a section of the journal Frontiers in Pediatrics

Received: *14 October 2020*
Accepted: *26 February 2021*
Published: *21 April 2021*

Citation:
Huisman EJ and Crighton GL (2021) Pediatric Fibrinogen PART I—Pitfalls in Fibrinogen Evaluation and Use of Fibrinogen Replacement Products in Children. Front. Pediatr. 9:617500. doi: 10.3389/fped.2021.617500

INTRODUCTION

Fibrinogen (Factor I) is a key coagulation protein and plays a critical role in all aspects of normal hemostasis: from platelet aggregation, to clot formation, and fibrinolysis (1, 2). During major surgical bleeding, fibrinogen is the first hemostatic factor to decrease to critical levels (3). Fibrinogen has been identified as an important risk factor for bleeding in children undergoing surgery (4–6).

As an acute phase reactant, fibrinogen plays a pivotal role in tissue repair and maintaining hemostasis during tissue injury and inflammation (7). Levels are often elevated in children in the context of fever, sepsis and critical illness (8–10). However, if hypofibrinogenemia is present in critically ill children with sepsis, it is associated with increased in-hospital mortality (8, 9).

See "Pediatric Fibrinogen PART II—Overview of Indications for Fibrinogen Use in Critically Ill Children", for further information about the clinical indications for fibrinogen supplementation in children.

Fibrinogen Evaluation and Specific Pitfalls in the Pediatric Setting

The gold-standard for fibrinogen quantification is the clot-based Clauss fibrinogen assay. The Clauss-assay is a functional test that measures the time taken for plasma to clot in a high concentration of thrombin, with comparison against a reference plasma calibration curve (11). Patient factors that can impact the accuracy of the test include the presence of heparin and fibrinogen-degradation products (12). Similarly photo-optical clot detection systems for measuring fibrinogen may be affected by lipemia or hyperbilirubinemia (11). Hydroxyethyl starch (HES), a plasma volume expander used in some pediatric perioperative settings (13), can impair fibrin polymerization (13, 14) causing falsely reduced fibrinogen levels (6, 14). Additionally, direct thrombin inhibitors such as bivalirudin and argatroban can inhibit the thrombin used in the Clauss-assay causing falsely reduced levels (15).

Fibrinogen may also be evaluated by viscoelastic testing. The two most commonly used and evaluated in clinical trials in children are thromboelastography (TEG®) and thromboelastometry (ROTEM®). A qualitative indication of fibrinogen is obtained by inhibiting the platelet contribution to clot formation using cytochalasin D for ROTEM-FIBTEM and abciximab for TEG functional fibrinogen (TEG-FF) assay (16).

Whilst both TEG and ROTEM are based on similar methodologies, there are significant differences in operating characteristics, activators used, reporting nomenclature, and reference ranges. Parameters reported by each of the devices are not equivalent or interchangeable (16). One such example, is the measurement of hyperfibrinolysis, the TEG lysis index is measured 30 min after the maximum amplitude (MA), whereas the ROTEM lysis index is measured 30 min after the clotting time (17).

Viscoelastic measures of fibrinogen have benefits over the Clauss assay, since they are point-of-care test with quick turnaround times (16). Since they are performed using whole blood, they provide a global and visual hemostatic evaluation of hemostasis, from clot initiation and kinetics, through to clot degradation and fibrinolysis.

In the pediatric setting the usefulness and uptake of viscoelastic testing remains limited. There is a lack of standardization, with a scarcity of age-dependent reference ranges for the different reagents used with each viscoelastic method (18, 19). Studies are needed to define clear thresholds and targets for treatment in neonates and children (17). The maximum clot strength thresholds (Maximum Clot Firmness [MCF] for ROTEM or MA for TEG) that indicate the need for fibrinogen supplementation are debated. In addition, validated treatment algorithms using viscoelastic testing and fibrinogen supplementation are needed for the different neonatal and pediatric contexts (17).

Viscoelastic testing in infants and neonates may also be limited by the blood sample requirements; ROTEM requires at least 1.8 mL of whole blood and TEG 2.7 mL (20) although newer viscoelastic devices have smaller minimum sample requirements (17). Another limitation is that viscoelastic tests are unable to detect congenital bleeding disorders, such as platelet function disorders or von Willebrand disease (17).

Other techniques for measuring fibrinogen include Prothrombin-time (PT) derived tests, immunohematological assays (11), the dry-hematology method (21), and thrombin generation assays. These tests are not widely used or only available in research settings (22).

The fibrinogen level obtained will vary depending on the fibrinogen assay used (23). In adults, fibrinogen levels using the Clauss assay are typically reported between 150 and 450 mg/dL, but physiological variation is commonly seen. In children, age-related reference ranges are required for fibrinogen quantification, because of the age-dependency (the so called developmental hemostasis) in hemostatic proteins (24). The lowest fibrinogen levels are reported in fetuses and preterm neonates (25–27), and infants (28, 29). Similarly, there are age-dependent differences seen in viscoelastic testing results (19, 30–32). Pediatric age-specific reference ranges have been described for ROTEM parameters across pediatric age groups, including FIBTEM (31, 33) and for the TEG (19, 34).

Children also have qualitative differences in both fibrinogen and fibrinolysis (35). Neonates have a "fetal" form of fibrinogen (35), an altered fibrin network and clot structure (36), with an overall reduction in fibrinolytic activity (37), and often physiologically elevated D-dimers in the first 72 h of life (38).

The sensitivity of each fibrinogen assay refers to the lowest detectable and quantifiable amount of fibrinogen. The lowest reportable fibrinogen level will vary between individual laboratories but will be reported as less than a certain threshold, e.g., <50 mg/dL. In neonates, this can be rather imprecise, because physiological values can be this low.

In summary, fibrinogen reference ranges vary depending on the test, the analyzer and reagents used, in addition to the age of the child, therefore it is important that local, assay-specific and age-specific reference ranges are developed. Utilization of adult reference intervals, particularly in the neonates has the potential to lead to overtreatment. A normal physiological (low) fibrinogen level in a non-bleeding, preterm neonate, for example, does not need correction.

FIBRINOGEN SUPPLEMENTATION

Cryoprecipitate and fibrinogen concentrate both effectively restore fibrinogen levels (39, 40), and are used in children to provide fibrinogen replacement during active bleeding or as prophylaxis to prevent bleeding (41, 42). Internationally there is variability in practice regarding the favored fibrinogen replacement product, reflecting local legislation, licensing and product availability.

Cryoprecipitate

In the United States (US), Canada, United Kingdom (UK), Australia and New Zealand, cryoprecipitate is the main component available for treatment of acquired hypofibrinogenemia since fibrinogen concentrate is only licensed for the treatment of congenital fibrinogen deficiency (43).

Fibrinogen Concentrate

In contrast, many European countries favor fibrinogen concentrate over cryoprecipitate for all therapeutic uses due to its superior pathogen safety profile and cryoprecipitate has been withdrawn due to safety concerns, principally transfusion transmitted infection (TTI) and prions. Fibrinogen concentrate in Europe is licensed for treatment of both congenital and acquired hypofibrinogenemia (43).

Whilst cryoprecipitate and fibrinogen concentrate are both plasma-derived products, there are considerable differences between the two products (see **Table 1**).

Plasma Transfusion

Plasma is the liquid component of blood that contains coagulation factors and coagulation inhibitors. Plasma products available include plasma frozen within 6–8 h of collection (FFP), plasma frozen within 24 h of collection, thawed plasma, liquid plasma and pathogen-inactivated plasma (44–46). Freeze-dried (lyophilized) or spray-dried plasma is largely restricted to military and research settings (47).

In the pediatric setting however, none of these plasma products, are suitable as fibrinogen replacement products since fibrinogen concentrations in plasma are very low and can vary considerably (100–500 mg/dL) (48, 49). Large volumes of plasma are required to replenish a low or falling fibrinogen level (43), placing a child at significant risk of transfusion-associated circulatory overload (TACO).

In summary, cryoprecipitate and fibrinogen concentrate are superior to plasma as fibrinogen replacement products since they are concentrated products. We therefore will focus this review on these two fibrinogen products.

PRODUCT CHARACTERISTICS

Product Characteristics of Cryoprecipitate

Cryoprecipitate is the unpurified, cold-insoluble protein or "cryoglobulin" proportion derived from plasma. Cryoprecipitate may be manufactured as single apheresis units or pooled from multiple donors with varying volume size. It is stored

TABLE 1 | Comparison of Cryoprecipitate and Fibrinogen Concentrate (RiaSTAP ®/Haemocomplettan ® CSL Behring).

	Cryoprecipitate (1–8)	Fibrinogen concentrate (RiaSTAP®/Haemocomplettan ®) (8–11)
Plasma source	Apheresis single donor or pooled from multiple donors Many countries only use male plasma donors to reduce the risk of TRALI.	Pooled from >10,000 plasma donors.
Content	Fibrinogen, vWF, FVIII, FXIII Platelet microparticles Fibronectin Anticoagulant—e.g., citrate-phosphate-dextrose or ACD	Fibrinogen Albumin L-arginine HCL
Presentation	Yellow, frozen cold-insoluble precipitate	White lyophilized powder
Storage	−25°C of below for a maximum of 3 years	2–6°C for up to 5 years
Compatibility	ABO compatibility with recipient's red cells suggested	No compatibility requirements
Reconstitution	Thawed at 37°C	Sterile water for reconstitution at room temperature
Speed of preparation	17–20 min to thaw	5–10 min
Administration	Standard blood administration set with a 170–200 micron filter.	Direct intravenous infusion by separate infusion line
Shelf life	Used within 4 h after thawing at room temperature	Stable for 8 h after reconstitution when stored at room temperature
Fibrinogen content	Variable fibrinogen content depending on fibrinogen content and cryoprecipitate volume Range from 300 to 3,000 mg/dL	Standardized fibrinogen content—1,000 mg/vial and 2,000 mg/vial
Pediatric infusion	10–20 ml/kg/h	Slow IV infusion, not exceeding 100 mg/min
Pathogen-reduction and viral inactivation procedures	Pathogen- reduced plasma—Photoactivation by visible or UV light, solvent-detergent treatment, or the addition of chemicals to the plasma: methylene blue (MB), amotosalen or riboflavin. Results in 65–84% lower fibrinogen content.	$Al(OH)_3$ adsorption/glycine precipitation /$Al(OH)_3$ adsorption Heat treatment at 60°C for 20 h Glycine precipitation
Adverse events	Risk of transfusion-transmitted infection Allergic transfusion reaction and anaphylaxis Febrile non-hemolytic transfusion reactions TRALI Citrate-induced hypocalcemia (increased risk with massive transfusion)	Allergic reactions and anaphylaxis Infusion related adverse events Thromboembolic complications
Surveillance	Hemovigilance	Pharmacovigilance
Costs per gram fibrinogen	∼ AUD $480 per gram Higher costs when cryoprecipitate is produced from pathogen-reduced plasma.	∼AUD $817 per gram

ACD, acid-citrate-dextrose; FVIII, factor VIII; FXIII, factor XIII; HCL, hydrochloride; IV, intravenous; TRALI, transfusion-associated acute lung injury; UV, ultraviolet; vWF, von Willebrand factor.

TABLE 2 | International standard product specifications for cryoprecipitate.

	Fibrinogen (mg) per unit		FVIII (IU) per unit	FXIII (IU) per unit	VWF per unit	Volume
United States (1, 2)	≥ 150[a]		≥ 80	≥ 80	≥ 80	Maximum 15 mL
EDQM Standards 2020 (3)	≥ 140		≥ 70	Not specified	≥ 100	30–40 mL
United Kingdom (4, 5)	Single unit	≥ 140[b,c]	≥ 70[b]	Not specified	Not specified	20–60 mL[d]
	Pool (5 donors)	≥ 700	≥ 350			100–250 mL[e]

[a]average fibrinogen content 250 mg unit, [b]a minimum of 75% of components tested should meet the specified values, [c]NHBTS mean 454 mg, [d]NHBTS mean 40 mL (4), [e]NHBTS mean 221 ml.

as a frozen product and inventoried by blood group. It contains fibrinogen in addition to coagulation factors VIII (FVIII), von Willebrand factor (vWF), factor XIII (FXIII), as well as fibronectin and platelet microparticles (45). Cryoprecipitate was originally used therapeutically in the treatment of hemophilia A, and then von Willebrand disease (vWD), congenital fibrinogen and FXIII deficiency (50). Today cryoprecipitate is predominantly utilized to replace fibrinogen in acquired hypofibrinogenemia.

Internationally, specific product requirements for the manufacture of cryoprecipitate vary between countries with respect to the minimum fibrinogen, FVIII and vWF concentrations (44) (see **Table 2)**. Whilst minimum fibrinogen content per unit is usually specified by standards (e.g., >140 mg/unit) (45, 46), the actual concentration can vary considerably (300–3,000 mg/dL), due to differences in blood donor fibrinogen levels, varying manufacturing processes and different unit volumes (51).

Advantages and disadvantages of cryoprecipitate in comparison with fibrinogen concentrate are described in **Table 1**.

One potential clinical advantage of cryoprecipitate is the contribution of FXIII in the treatment of bleeding, especially in the surgical setting (52–54). *In-vitro* studies have shown that cryoprecipitate reverses fibrinolysis better than fibrinogen concentrate (55). A second consideration is cost: when evaluated per gram of fibrinogen, cryoprecipitate is cheaper (56, 57), even when economic analysis has considered preparation and wastage costs (58). However, this advantage may not be as notable with pathogen-reduced cryoprecipitate, since pathogen-inactivation comes with additional costs.

An important downside of cryoprecipitate is the risk of pathogen transmission, even with appropriate blood donor and donation screening (59). Cryoprecipitate made from pathogen-reduced plasma can reduce this risk (60–62) and this is the preferred product for the treatment of congenital bleeding disorder in resource-limited settings (60, 61). Unfortunately, pathogen-inactivation results in reduced fibrinogen levels (65–84%) of normal plasma (62). To overcome this, it may be possible to increase the number of donors contributing to the plasma pool or increase the transfusion volume (63, 64).

Cryoprecipitate has been implicated in a number of transfusion-related adverse events including allergic reactions, febrile, non-hemolytic transfusion reactions (65, 66) as well as transfusion-associated acute lung injury (TRALI) (67, 68) and TTI (59, 69). There is also evidence that adverse transfusion reactions occur more frequently in children compared with adult (66, 70). Any patient receiving cryoprecipitate must be monitored for a transfusion reaction and any incidents should be reported to the local hemovigilance reporting system. To maintain traceability, the blood compatibility report should be maintained in the child's medical record.

Cryoprecipitate is less suited to the acute pediatric critical care setting since it requires thawing prior to administration, and once thawed, it has a shelf life of 4 h, after which it must be discarded (46). To overcome this, it would be ideal to keep "ready for-use" units of unthawed cryoprecipitate. *In-vitro* studies evaluating thawed pathogen-reduced cryoprecipitate, held at room temperature for 5 days have demonstrated its ability to restore fibrinogen levels and clot strength (FIBTEM) (63). However, in the pediatric setting di (2-ethylhexyl)phthalate (DEHP) toxicity should be considered (71). DEHP is a chemical added to plastics to make them more flexible and is commonly used in blood bags (72). DEHP levels increase in concentration as blood storage duration is increased (71). Toxic and carcinogenic effects are seen in animal studies, but the evidence for toxicity in humans has not been established, but remains a concern in neonates (72).

Product Characteristics of Fibrinogen Concentrate

Fibrinogen concentrate is a purified, virus-inactivated, lyophilized concentrate derived from pooled human plasma. It comes as a powder that can be reconstituted with sterile water (within 5–10 min) to deliver a reliable and standardized fibrinogen content (73, 74). In children with hypofibrinogenemia, fibrinogen concentrate has a rapid onset of action and is effective at increasing fibrinogen levels (39, 40).

There are a number of fibrinogen concentrates available internationally. The one most widely used is Haemocomplettan P®/RiaSTAP® (CSL Behring) (43) which contains between 900 and 1,300 mg of fibrinogen/50 mL (74, 75). Other available fibrinogen concentrates include: Clottafact® (LFB Biomedicaments) (76), FibCLOT® (LFB Biopharmaceuticals Ltd.) (77) and Fibryga®/Octafibrin ® (Octapharma) (78) and locally produced Fibrinogen HT (Japan) and FibroRAAS/FabuLaishi (China) (79). These fibrinogen concentrates are not all equivalent, they differ in: pathogen reduction strategies (80), fibrinogen concentrations, formulations (for example Fibryga® Octapharma contains more FXIII) (81, 82), stability agents and constituents, storage requirements and reconstitution stability data, and finally, in varying pediatric dosing recommendations (43, 74, 76–78, 81, 83, 84) (see **Table 3)** for the full overview.

An overview of the advantages and disadvantages of fibrinogen concentrate compared with cryoprecipitate are

TABLE 3 | Comparison between fibringeon concentrate products.

	RiaSTAP®/Haemocomplettan P® (1–3)	Fibryga ®/Octafibrin ® (4, 5)	Clottafact ®/FibCLOT® (6–9)
Manufacturer	CSL Behring, Germany	Octapharma, Switzerland	LFB Biomedicaments, France
Pathogen reduction	$Al(OH)_3$ adsorption/glycine precipitation /$Al(OH)_3$ adsorption Heat treatment at 60°C for 20 h Glycine precipitation	$Al(OH)_3$ adsorption Solvent-detergent treatment Nanofiltration (20 nm)	Solvent-detergent treatment Nanofiltration (35 nm) Dry heat treatment at 80°C for 20 h
Vial size	1,000 and 2,000 mg	1,000 mg	1,500 mg
Fibrinogen concentration	900–1,300 mg/50 mL 20 mg/ml	1 g/50 mL 20 mg/mL	1.5 g/100 mL 15 mg/ml
Stability agents	Albumin L-arginine HCL Sodium chloride Sodium citrate	Glycine L-arginine HCL Sodium chloride Sodium citrate dihydrate	Glycine Arginine HCL Isoleucine Lysine HCL Sodium citrate dihydrate
FXIII	N/A	200IU FXIII/g of fibrinogen	N/A
Storage	2–5°C Up to 60 months	2–25°C Up to 36 months	3 years
Reconstitution stability data	Use within 8 h	Up to 24 h at +25°C	After reconstitution, use immediately
Pediatric Dosing	Dose (mg/kg body weight) = [Target level (mg/dL)—measured level (mg/dL)]/1.7 (mg/dL per mg/kg)	Dose (mg/kg body weight) = [Target level (mg/dL)—measured level (mg/dL)]/1.8 (mg/dL per mg/kg)	Dose (mg) = [Target level (mg/dL)—baseline level (mg/dL)] × 1/recovery (mg/dL)/(mg/kg) × body weight (kg)
Pediatric dosing when fibrinogen level unknown	RiaSTAP® 70 mg/kg for congenital fibrinogen deficiency Haemocomplettan P® 20–30 mg/kg treatment for active bleeding in children	60 mg/kg for congenital fibrinogen deficiency	When 1/recovery unknown 53 mg/kg for children <40 kg 43 mg/kg for children ≥40 kg
Pediatric infusion rates	Slow IV infusion, not exceeding 100 mg/min	Slow IV infusion, maximum rate of 100 mg/min.	Clinically stable patients—4 ml/min = 60 mg/min Severe acute hemorrhage—20 ml/min = 300 mg/min

HCL, hydrochloride; IV, intravenous.

described in detail in **Table 1**. An advantage of fibrinogen concentrate for use in neonates and critically ill children is its relatively small infusion volume. Doses of fibrinogen concentrate are going to be in the order of 1–3.5 mL/kg compared with 5–10 mL/kg for cryoprecipitate. Fibrinogen may be able to be administered quicker than cryoprecipitate, depending on where it is stored in the hospital, since it only requires refrigeration for storage.

Fibrinogen concentrate also has a superior pathogen safety profile and low rates of adverse events. A pharmaco-surveillance study of 27 years of Haemocomplettan P®/RiaSTAP® (CSL Behring) use with literature review, reported an excellent safety profile (85). Each fibrinogen concentrate administration be monitored for side effects and any reactions or adverse events be reported to the pharmacovigilance system. Each administration and the batch number must be recorded in the patient record to maintain traceability.

A relative disadvantage of use in neonates is that the dose required for treatment is much less than the smallest formulation of fibrinogen concentrate available (1,000 mg), leading to product wastage and higher costs. For example, a dose of 30 mg/kg fibrinogen concentrate to treat hypofibrinogenemia in a 5 kg infant only equals 150 mg.

In summary, cryoprecipitate is a rich source of fibrinogen and includes other coagulation factors important for hemostasis. Cryoprecipitate is widely used in countries where fibrinogen concentrate is not licensed for use in acquired hypofibrinogenemia, and in resource-limited settings as therapy for congenital hypofibrinogenemia, although pathogen-inactivated cryoprecipitate is favored. Disadvantages are the large inter-unit variability of fibrinogen in cryoprecipitate, the increased risk of transfusion reactions and infectious transmission, in addition to the logistical and blood banking requirements.

Plasma-derived fibrinogen concentrates are able to rapidly and effectively restore and maintain serum fibrinogen concentrations. They are used for treatment of congenital and acquired hypofibrinogenemia. They have an excellent safety profile and are amenable to near-patient storage, which may reduce the time to administration. They come with a higher cost and generally do not contain additional hemostatic factors that may be important for general hemostasis.

DOSING OF FIBRINOGEN SUPPLEMENTS

Dosing of Cryoprecipitate

A standard treatment dose of cryoprecipitate in adults is 10 units or two pools (where one pool is made from five units), leading

TABLE 4 | Guidance for dosing of cryoprecipitate and fibrinogen concentrate in children.

Cryoprecipitate dosing in children		
Guidance	**Dosing**	**Dosing considerations**
National Blood Authority of Australia (NBA) (2)	5 ml/kg for acquired hypofibrinogenemia	Consider patient's clinical condition, the presence of active bleeding, medications affecting coagulation status, and congenital and acquired bleeding disorders.
British Society of Hematology (BSH) (3)	5–10 ml/kg of methylene blue cryoprecipitate Higher doses for active bleeding and acquired hypofibrinogenemia	Lower fibrinogen content in methylene-blue cryoprecipitate ~250 mg/unit compared with ~430 mg/unit in a non-treated unit (4).
Italian Neonatal Transfusion Guideline, 2015 (5)	5–10 ml/kg	
Rare bleeding disorder guideline (64)	15–20 ml/kg of pathogen reduced cryoprecipitate for treatment of congenital fibrinogen disorders when fibrinogen concentrate not available	Fibrinogen concentrate is first line treatment for congenital fibrinogen disorders
Society for the Advancement of Blood Management (SABM) (7)	Cryoprecipitate volume should be calculated based on weight and desired increase in fibrinogen concentration and improvement in coagulations indices.	The decision to transfuse should be based on laboratory studies including point of care viscoelastic testing if available, fibrinogen concentration, the patient's clinical status and the etiology of the patient's coagulopathy.
US Food and Drug Administration (FDA) (8)	Dose of 0.1–0.2 units/kg 1–2 units per 10 kg	Unit volumes will vary. Expected fibrinogen rise of 60–100 mg/dL
AABB (9)	Calculated from formula $Dose\ (units) = \frac{[desired\ fibrinogen\ increment\ (\frac{mg}{dL}) x\ plasma\ volume\ (dL)]}{250mg}$ $Plasma\ volume\ (dL)\ in\ children = Total\ Blood\ volume\ (mL)\ x\ \frac{[1-hematocrit\ (\%)]}{100}$	Takes into account the desired fibrinogen increment (mg/dL), average fibrinogen content per unit of 250 mg and plasma volume of the child (9). Plasma and blood volumes vary in children. Blood volumes in children may be estimated using the formula 70 mL/kg, but this will underestimate the blood volume in a neonate and overestimate the blood volume in an adolescent or overweight child (10, 11)
Blood Easy 4, Canada, 2016 (12)	1 unit/10 kg body weight to a maximum of 10 units (~ 4,000 mg fibrinogen)	Each dose should increase the fibrinogen by 50 mg/dL in the bleeding patient.
NICE Guidelines 2015 (13)	5–10 mL/kg up to a maximum of two pools	Reassess the patient's clinical condition, repeat the fibrinogen level measurement, and give further doses if needed.
Fibrinogen concentrate dosing in children		
Pediatric dosing	**Dosing**	**Dosing considerations**
Riastap ® (CSL Behring, Germany) (14)	**Congenital fibrinogen deficiency** $Dose\ \left(\frac{mg}{kg}\right) = [Target\ fibrinogen\ level\ \left(\frac{mg}{dL}\right) - measured\ level\ \left(\frac{mg}{dL}\right)] / 1.7\ (mg/dL\ per\ mg/kg\ body\ weight)$.	70 mg/kg when patient's fibrinogen level is not known
Rare bleeding disorder guideline (6)	**Congenital fibrinogen deficiency** 50–100 mg/kg	Smaller doses repeated every 2–4 days to maintain fibrinogen >100 mg/dL
Fibryga ® Octapharma	**Congenital fibrinogen deficiency** $Dose\ \left(\frac{mg}{kg}\right) = [Target\ fibrinogen\ level\ \left(\frac{mg}{dL}\right) - measured\ level\ \left(\frac{mg}{dL}\right)] / 1.8\ (mg/dL\ per\ mg/kg\ body\ weight$	60 mg/kg when patient's fibrinogen level is not known Monitor patient's fibrinogen level during treatment
Haemocomplettan P ® (CSL Behring, Germany) (15)	**Acquired hypofibrinogenemia** 20–30 mg/kg	

to an increase of 100 mg/dL fibrinogen (86). In children, dosing for both prophylactic and therapeutic cryoprecipitate indications should be calculated at least taking into account the child's weight (87). Most pediatric transfusion guidelines dose cryoprecipitate based on the child's weight as a single variable (88, 89). Many advise doses of 5 and 10 mL/kg (88–91), with exceptions of 20 mL/kg for the treatment of congenital fibrinogen deficiency with pathogen-reduced cryoprecipitate (64) (see **Table 4**).

However, more complex formulas will take into account a child's baseline fibrinogen level, the desired fibrinogen level, the average fibrinogen content of the local cryoprecipitate unit and a child's plasma volume (92) (see **Table 4**).

In general, blood volumes in children may be estimated using the formula 70 mL/kg (93, 94). However, this equation underestimates the blood volume for a neonate, since the estimated blood volume (EBV) decreases with age, from around

90–100 mL/kg in preterm infants to ∼80 mL/kg in term infants (93) and overestimates the blood volume in obese, post-pubertal adolescents (∼60–70 mL/kg). None of these calculations corrects for ethnic differences and social factors such as malnutrition or obesity which may be important (94).

Dosing of cryoprecipitate in children should also consider the fibrinogen content of local products, particularly with pathogen-reduced cryoprecipitate (92, 95, 96).

The half-life of fibrinogen is relatively long (3–4 days) (64), and therefore, usually one dose is sufficient for prophylactic indications, however in the presence of active bleeding with ongoing loss or a poor fibrinogen increment, additional doses may be required.

Dosing of Fibrinogen Concentrate

Fibrinogen concentrate is dosed by most clinical guidelines in mg/kg. But here also, more complex formulas are seen, taking target and measured fibrinogen levels into the equation (see **Table 4**).

For each of the other available fibrinogen concentrates on the market, there are different dosing recommendations for children, and it is therefore recommended to consult the individual product information for specific dosing advice (see **Table 3**).

In summary, the dosing of cryoprecipitate is influenced by both donor and product variables. The dosing of both cryoprecipitate and fibrinogen concentrate in children should be calculated, taking into consideration the baseline fibrinogen, the child's body weight, the presence of active bleeding and ongoing loss. It is advisable, that after fibrinogen supplementation, both the clinical response to treatment and fibrinogen levels are re-evaluated to assess for any additional requirements.

CURRENT KNOWLEDGE GAPS, CONTROVERSIES, AND AREAS FOR RESEARCH

The decision to supplement fibrinogen firstly relies on adequate measurement of fibrinogen. When evaluating fibrinogen levels and function in the critically ill child, it is important to consider the accuracy of the result, the clinical context, the specific reference ranges and each test's limitations.

When fibrinogen replacement is indicated in critically ill children, there remain many uncertainties regarding the best choice of fibrinogen replacement, the optimal dose, in addition to the target or desired fibrinogen level. Each product has its own set of limitations and benefits specific to children.

The following questions, may be proposed as potential areas for research in neonates, including those preterm, infants, children, and adolescents.

In the area of laboratory testing:

- Can we develop small-volume tests for measuring fibrinogen in neonates and critically ill children?
- How do different viscoelastic measures of fibrinogen function compare with clot based Clauss fibrinogen assays in children?

Fibrinogen and bleeding

- How do age-dependent coagulation differences influence bleeding in neonates and children?
- In particular, what effect does the presence of fetal fibrinogen and altered fibrin clot structure have on fibrinogen function in preterm and term neonates?

Fibrinogen replacement products

- What is the optimal fibrinogen replacement component in children?
- What role does the additional FXIII, vWF, and FVIII in cryoprecipitate play in treating and preventing bleeding in children with a low fibrinogen?
- How do fibrinogen concentrate products that contain FXIII compare with those that do not?
- What is the optimal formula to dose fibrinogen in children?
- What is the optimal dose and timing in children of cryoprecipitate and fibrinogen concentrate
  - for the prevention of bleeding?
  - for the treatment of active bleeding?

Adverse events:

- How do we capture and report adverse events related to fibrinogen supplementation in children?

CONCLUSIONS

Hypofibrinogenemia is increasingly recognized as an important risk factor for bleeding and there has been an increasing focus on the fibrinogen supplementation by clinicians and clinical guidelines. Yet, there are many unknowns. The decision to supplement fibrinogen firstly relies on adequate measurement of fibrinogen and there are many pitfalls around the optimal fibrinogen measurement in children. Cryoprecipitate and fibrinogen concentrate both effectively restore fibrinogen levels, but each product has its own set of advantages and constraints specific to use in children. Fibrinogen concentrate is an attractive alternative to cryoprecipitate, offering a superior safety profile, with apparent efficacy, but not every fibrinogen concentrate product is equivalent. Further randomized controlled evidence is required to support decision-making regarding fibrinogen supplementation in children, including those who are critically ill.

AUTHOR CONTRIBUTIONS

GC literature review, writing, and editing article. EH reviewing content and editing article. All authors contributed to the article and approved the submitted version.

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