Platelet Count and Function during Pediatric Extracorporeal Membrane Oxygenation

Katherine Cashen, DO[1] Kathleen Meert, MD[1] Heidi J. Dalton, MD[2,3]

[1] Department of Pediatrics, Division of Critical Care Medicine, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan
[2] Department of Pediatrics, Division of Critical Care Medicine, INOVA Heart and Vascular Institute, Inova Fairfax Medical Institute, Falls Church, Virginia
[3] George Washington University, Washington, Dist. of Columbia

Address for correspondence Katherine Cashen, DO, Department of Pediatrics, Division of Critical Care Medicine, Children's Hospital of Michigan, Wayne State University, Detroit 48201, MI (e-mail: kcashen@med.wayne.edu).

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Abstract

Extracorporeal membrane oxygenation (ECMO) is a form of life support used to treat neonates, children, and adults with cardiorespiratory failure refractory to conventional therapy. This therapy requires the use of anticoagulation to prevent clotting in the extracorporeal circuit, but anticoagulation also increases the risk of bleeding on ECMO. Both bleeding and thrombosis remain significant complications on ECMO and balancing these risks is challenging. Acquired platelet dysfunction is common during ECMO and quantitative and qualitative platelet dysfunction contributes to bleeding risk. Optimal platelet count, function, and transfusion thresholds are not well established during pediatric ECMO. In this review, we provide an overview of hemostatic alterations during ECMO, changes in platelet count and function, platelet monitoring techniques, bleeding risk, and future needs to best optimize patient management and care.

Keywords
- extracorporeal membrane oxygenation
- platelets
- platelet function
- thrombosis
- bleeding

Extracorporeal membrane oxygenation (ECMO) is a form of life support used to treat neonates, children, and adults with cardiorespiratory failure refractory to conventional therapy. The Extracorporeal Life Support Organization (ELSO) most recently reported outcomes from 66,932 ECMO runs in children from 1989 to January 2019. The ELSO registry separates children into two groups: neonates defined as 0 to 28 days and pediatric patients defined as 29 days to 17 years of age. Survival for neonatal respiratory ECMO is highest at 73% followed by pediatric respiratory ECMO at 58%. Among cardiac indications for ECMO, neonates have 42% survival and pediatric patients have 52% survival.[1] Patients who receive ECMO as a resuscitative tool during cardiac arrest have the lowest survival at approximately 42%.

While ECMO is lifesaving, morbidity is common and both bleeding and thrombosis are associated with worse outcome.[2–4] The Bleeding and Thrombosis during ECMO (BATE) study performed by the Collaborative Pediatric Critical Care Research Network found high rates of bleeding and thrombosis during ECMO with 70.2% of children having a bleeding event (defined as a need for transfusion) and 37.5% suffering from a thrombotic event.[4] Bleeding was associated with increased mortality, although thrombosis was not.[4]

The mechanisms of bleeding and thrombosis during extracorporeal support are complex due to the nonendothelial surface of the ECMO circuit, multiple alterations in hemostatic factors, maturational changes that occur, and anticoagulants used. Acquired platelet dysfunction, both quantitative and qualitative, is assumed to contribute to bleeding risk during ECMO, although data in neonates and children confirming this are sparse. Optimal platelet count, functional assays, and transfusion thresholds are not well established during pediatric ECMO. In this review, we first provide an overview of developmental hemostasis and factors affecting coagulation during ECMO. We then focus on changes specific to platelets, bleeding risk, and potential treatments to limit bleeding and thrombosis.

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Developmental Hemostasis

Variations in hemostasis have been well reported in children compared with adults (►**Table 1**). Hemostatic equilibrium is a dynamic process requiring both procoagulant and anticoagulant factors and evolves from fetal to adult life.[5–8] The major differences between neonatal and adult hemostasis are quantitative with different plasma levels of coagulation factors, platelets, and fibrinogen. However, the qualitative function of the hemostatic system in children is similar to adults.[5–8]

During primary hemostasis, activation of platelets leads to aggregation and then platelet plug formation.[8–12] Although the platelet count of term neonates is usually normal or slightly higher than normal, platelet hyporeactivity is well described.[8–14] Neonatal platelets are less reactive to physiological agonists in whole blood compared with adult platelets. However, despite hyporeactive platelets, the bleeding time and platelet closure time (platelet function analyzer or PFA-100 closure time, a measure of overall platelet function which includes functional activity of von Willebrand factor [VWF]) are shortened in neonates and do not normalize until the first month of life.[9–11] Higher levels of VWF and higher percentage of larger VWF multimers likely increase the adhesive activity of platelets in neonates compared with adults despite the overall platelet hyporeactivity per se.[9,11,12] Thus, in healthy neonates, elevated VWF level and functionality seem to balance platelet hyporeactivity and qualitatively normal primary hemostasis is maintained.

Table 1 Developmental hemostasis-coagulation parameters in the neonate compared with adult levels and time to normalize

Parameter	Neonatal period (value)	Time to reach adult levels
Platelets	Normal or increased	~1 y
Von Willebrand factor	Increased	~3 mo
Platelet closure time (PFA-100)	Shortened	~2–4 wk
Factor II, VII, IX, X	Decreased	~1 y (FVII takes longer)
Factor XI, XII, Prekallikrein (PK), high molecular weight kinogen (HMWK)	Decreased	~1 y
Factor V	Normal or decreased	~1 y (up to 16 y)
Factor VIII	Normal or increased	1 mo
Fibrinogen	Decreased or normal (fetal fibrinogen present)	~1 y
Prothrombin time (PT)	Prolonged or normal	~1 y
Activated partial thromboplastin time (aPTT)	Prolonged	~1 y (up to 16 y)
Antithrombin	Decreased	~3 mo
Protein C	Decreased	up to 16 y
Protein S	Decreased	~3 mo
Plasminogen	Decreased	~6 mo

Source: Modified from Andrew et al.[5]

Secondary hemostasis, reflecting the coagulation pathways and ultimately fibrin generation, is also different in neonates compared with older children and adults. Plasma levels of most coagulation proteins are almost half adult levels at birth and prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) in neonates has been reported.[6,7] Decreased protein C and S, antithrombin (AT), decreased thrombin generation, and reduced clot lysis have been reported in neonates.[7,13] In addition, although neonates and children have less fibrinogen than adults, the structure of "fetal fibrinogen" is different from adults but fibrinogen activity is comparable.[14] In critically ill neonates on ECMO, lack of reserve capacity and immaturity of the coagulation system interferes with the fine balance of hemostasis present in healthy neonates, and disequilibrium within the hemostatic system commonly results in bleeding or thrombosis. The effects of critical illness and maturational state on hemostasis are further compounded by alterations due to the ECMO circuit itself, the underlying disease process, comorbidities, or secondary organ dysfunction and anticoagulant medication dosing and monitoring algorithms (►**Table 2**).

Hemostatic Alterations during ECMO

ECMO-induced coagulopathy is a pathologic state of hemostasis seen in children during ECMO as described above and also varies with indication for ECMO. For example, children placed on ECMO for respiratory failure have fewer bleeding complications than children placed on ECMO after undergoing cardiopulmonary bypass[3] or recent surgery. Cardiopulmonary bypass itself is associated with disturbance of the hemostatic system that may lead to reductions in coagulation factor activity, platelet count and function, and fibrinogen concentration.[15] Critically ill children may have a generalized systemic inflammatory response and endothelial dysfunction even without exposure to ECMO. Cross-talk between innate inflammation and coagulation is well described and may lead to unopposed amplification of various coagulation pathways and a hypercoagulable state.[16]

The ECMO circuit represents a nonendothelial surface which consists of a mechanical blood pump, gas exchange devices, heat exchanger, tubing and cannulas. ECMO can be provided in a venoarterial mode (neonatal venoarterial [VA] cannulation shown in ►**Fig. 1**) or in a veno-venous configuration where blood is both drained and returned to the venous circulation. Cannulation can occur via cervical, femoral, or central vessels based on age and indication for ECMO. Activation of the coagulation pathway and inflammatory response pathway

Table 2 Factors contributing to hemostatic alterations during ECMO

Developmental hemostasis	Variations in hemostasis and immaturity of coagulation system
Critical Illness	Underlying alterations in hemostatic system (hematologic diagnoses, sepsis, systemic inflammatory state, crosstalk between immune system and hemostatic system)
ECMO-induced coagulopathy (EIC)	Contact activation from the nonbiologic surface of the ECMO circuit Inflammation Thrombin generation Endothelial dysfunction Platelet dysfunction (activation, consumption, thrombus formation) Acquired factor XIII deficiency Acquired von Willebrand deficiency
Anticoagulation	Unfractionated heparin has significant variation in patient response Heparin-induced thrombocytopenia Direct thrombin inhibitors
Complications	Hemolysis Bleeding Thrombosis Organ dysfunction
Transfusion	Thresholds variable
Medications affecting platelet function	Milrinone, nitric oxide, histamine-2 receptor antagonists, etc.
Additional considerations	Continuous veno venous hemodiafiltration, plasmapheresis, postoperative surgical patients, exposure to cardiopulmonary bypass

Abbreviation: ECMO, extracorporeal membrane oxygenation.

occurs from exposure of blood to the ECMO circuit. Platelet activation, increased activation of factor XII, decreased kallikrein inhibitory capacity, increased thrombin-AT formation, and increased tissue factor and decreased VWF have all been reported.[17–22] Our current understanding of the blood–biomaterial interaction is that initially fibrinogen binds to the ECMO circuit nonbiologic surface within minutes of contact followed by binding of coagulation factors, cellular adhesion, and activation of platelets and polymorphonuclear (PMNs) cells.[22] Activation of the complement system leads to further activation of platelets and PMNs and increased adhesion and release of cytokines, which contribute to the overall hypercoagulable state.[22–25] Some reports suggest distinct periods of activation in neonates, with contact activation and complement activation predominant in the first 24 hours after ECMO exposure followed by a second period of activation characterized by clotting and fibrinolytic activity without activation of the complement system observed 72 hours after ECMO initiation.[26] The cell-based model of coagulation proposes that subendothelial tissue factor exposure and binding and activation of circulating Factor VII then cause downstream thrombin generation. This process is separate from the contact activation described above.[22] To add even more complexity to the hemostatic alterations from the ECMO circuit itself, turbulent flow and shear stress from the pump, tubing, and cannula contribute to cellular damage, hemolysis, thrombosis, acquired VWF syndrome, and ongoing platelet activation.

Finally, anticoagulation, which is necessary to prevent thrombosis during ECMO, contributes to altered hemostasis. Unfractionated heparin (UFH) remains the most commonly used anticoagulation agent during ECMO.[27] UFH potentiates the anticoagulant effect of AT by forming a UFH–AT complex that inactivates free thrombin and prevents thrombin generation in addition to weakly inhibiting factor Xa.[28] Although UFH is reversible and low cost, there is significant variation in patient response to fixed dosing in part due to heparin binding proteins and reliance on AT levels which are lower in critically ill children and neonates and UFH's inability to inhibit factor Xa bound to platelets.[29–31] Data suggest that AT levels decrease over time in children on ECMO. Studies on replacement of AT, however, have noted mixed results, with some finding increased bleeding or no effect on heparin dosing or outcome.[32,33] Thus this practice remains controversial and confusing.[34–36] ELSO acknowledges this uncertainty and suggests considering replacing AT in infants and children with escalating UFH requirements and confirmed low AT activity.[37] Centers that routinely replace AT target levels ranging from >50 to 80% and some centers empirically treat suspected or confirmed low AT activity levels with frozen plasma.[37]

Another disadvantage of UFH is the risk of development of heparin-induced thrombocytopenia (HIT), an immune mediated adverse drug reaction caused by antibodies to complexes of platelet factor 4 and heparin. A recent systematic review on HIT in children found seroconversion in 0 to 1.7% of neonates and in 1.3 to 52% of pediatric patients studied, although no case of confirmed HIT was noted in neonates.[38] The variability in HIT observed is likely due to differences in definitions, how HIT is established both clinically and via laboratory testing and clinicians' willingness to change from heparin to another anticoagulant.

Platelet Count and Function during ECMO

Platelets play a critical role in hemostasis. Changes in platelet count on initiation of ECMO have been well described. The nonbiologic surface of the ECMO circuit induces platelet adhesion and activation and hemodilution from the ECMO circuit priming volume also contribute to the decrease in platelet count noted on ECMO initiation.[22] Acquired thrombocytopenia and platelet dysfunction are multifactorial in ECMO patients, with critical illness, extracorporeal circuit effects, medication effects (milrinone, nitric oxide, histamine-2-receptor blockers, etc.), and complications during ECMO which consume platelets or alter function of all contributing factors.

Platelet Count

Thrombocytopenia, often defined as a platelet count $<150\times10^9$/L, is common among children with critical illness. The incidence of thrombocytopenia is 18 to 35% in neonatal intensive care units (NICU) patients, with even lower counts occurring in preterm neonates.[39,40] Up to 30% (15–30%) of children in pediatric intensive care unit have

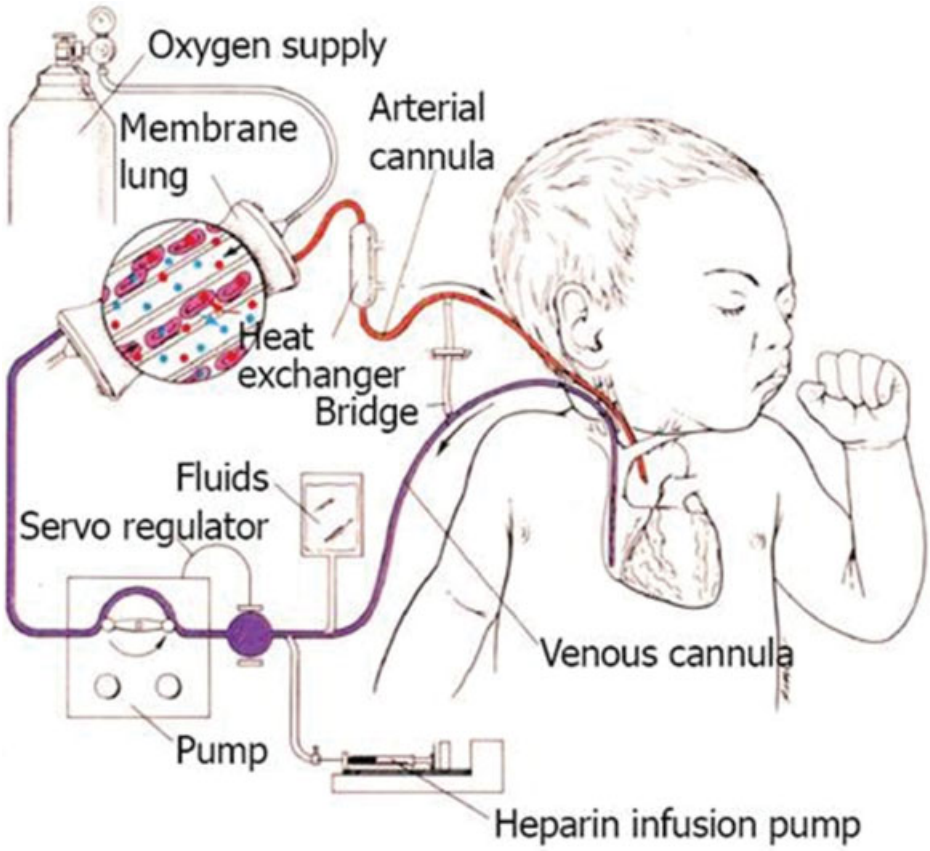

Fig. 1 Venoarterial ECMO circuit. Venous blood drains from the patient's internal jugular vein/right atrium and is pumped through the oxygenator. The oxygenated, warmed blood then passes back into the carotid artery of the baby. There are multiple infusion and access ports as well as pressure and flow monitors. (Reproduced with permission of Maslach-Hubbard A, Bratton SL. Extracorporeal membrane oxygenation for pediatric respiratory failure: History, development and current status. World J Crit Care Med 2013;2(4):29–39 Creative Common Attribution Non Commercial (CC BY-NC 4.0) http://creativecommons.org/licenses/by-nc/4.0/.) ECMO, extracorporeal membrane oxygenation.

thrombocytopenia.[41–43] Many disease processes such as sepsis and immune reactions associated with the need for ECMO cause bone marrow suppression and thrombocytopenia. Perhaps the largest declines are noted in neonates on initiation of ECMO. In one report of neonatal respiratory ECMO patients, Cheung et al describe a decrease in mean platelet count by $47 \pm 7\%$ from pre-ECMO levels of $132 \pm 17 \times 10^9$/L within 1 hour of ECMO initiation.[44] Another report of neonatal respiratory ECMO patients found a mean decrease of 26% from baseline platelet count 15 minutes after initiation of ECMO with an additional mean decrease of 16% by the end of 1 hour.[45] In a large multicenter study, 154 neonates had a decline in median platelet count from pre-ECMO level of 174 (interquartile range 128, 235) $\times 10^9$/L to 77 (47, 114) $\times 10^9$/L at 1.6 hours after ECMO initiation. Pediatric patients also had a decline in median platelet count from a pre-ECMO level of 167 (96, 253) $\times 10^9$/L to 104 (58, 162) $\times 10^9$/L at 1.5 hours after ECMO initiation.[46] Thus, neonates had a larger decline in platelet count than pediatric patients. In this study, the unadjusted association of average daily platelet count with mortality had a roughly linear association with the log odds of mortality up to a platelet count of approximately 115×10^9/L. At levels above this threshold, there was little evidence of any change in the risk of mortality with increasing platelet count. On multivariable analyses, average daily platelet count was not independently associated with mortality; however, platelet transfusion volume was associated with mortality.[46] Thus, the optimal platelet count during ECMO is still unclear. While most programs maintain a transfusion trigger for platelets of 100×10^9/L in uncomplicated pediatric ECMO, there is actually little evidence that this is required.[25,46] One novel approach to prevent the immediate decline in platelet count on initiation is to consider adding platelets to the priming mixture. Anecdotal evidence has found this mitigates the rapid drop in platelet count noted in neonates and results in less platelet transfusion in the first 24 hours but this practice has not been subjected to rigorous study.

Platelet Function

Platelet activation occurs due to multiple mechanisms during ECMO. The nonendothelial ECMO circuit activates platelets and amplifies thrombin generation on the surface of platelets. Platelet activation also occurs due to elevated shear flow from the circuit which causes platelet receptor shedding of key adhesion glycoproteins, GPIbα and GPVI, and an associated loss of high molecular weight VWF multimers.[47] Platelet adhesion to a fibrin-covered surface is impaired when the platelet glycoprotein (GP)Ibα–GPVI complex is unable to bind to VWF. Acquired von Willebrand syndrome occurs during

ECMO due to a loss of high molecular weight VWF multimers from shear stress which disrupts VWF multimers and is associated with increased bleeding complications.[48,49] Shear stress also causes platelets to produce small circulating fragments of platelet plasma membranes called platelet microparticles which contribute to thrombus formation.[50] Platelet microparticles are increased in neonatal ECMO systems in vivo but no study to date has demonstrated that they contribute to a prothrombotic state in vitro.[51] Platelet consumption is ongoing during ECMO with adhesion to the surface of the circuit and formation of microthrombi. Thus, platelet activation and consumption of key platelet adhesion glycoproteins may contribute to both bleeding and thrombotic risk during ECMO.

Platelet adhesion, activation, and aggregation measures have been reported utilizing different methods[52] but the majority of these studies have been in adult ECMO patients. Adult studies demonstrate impaired platelet aggregation[53–56] and reduced levels of adhesion receptors for collagen and VWF on circulating platelets during ECMO.[47] In contrast to the previous studies, Chung et al reported a decrease in platelet activation markers (β-thromboglobulin and platelet factor 4) during the first 72 hours of ECMO.[57] These markers of platelet activation are secreted from α granules and may initially represent platelet activation but, over time, levels may decrease due to ongoing release and exhaustion of this mechanism of platelet activation. An alternative mechanism to explain decreasing levels of β-thromboglobulin and platelet factor 4 is that these markers may bind to heparin coated surfaces of the ECMO circuit.[57,58]

Platelet function measurement is challenging in neonatal and pediatric patients due to the relatively large volume of blood required for testing and limitations in interpretation for in vivo significance.[58] Current studies in neonatal and pediatric ECMO patients have utilized aggregometry, thromboelastography (TEG; Haemonetics), and rotational thromboelastometry (ROTEM; TEM International). A study utilizing platelet function analysis (PFA-100/200) is also currently underway. Decreased platelet aggregation has been reported using different agonists including adenosine diphosphate (ADP), ristocetin, collagen, and epinephrine.[44,45,52–54] In one report, using whole blood platelet lumiaggregometry, platelet aggregation at 15 minutes after starting ECMO showed a 46% mean decrease in the response to collagen from baseline, and a significantly reduced response to ristocetin and to adenosine 5′-diphosphate.[45] Platelet adenosine triphosphate release was also significantly reduced. Platelet transfusion failed to correct observed platelet aggregation abnormalities.[45] Repeated testing found that platelet aggregation did not normalize until 8 hours after ECMO discontinuation. In another study, Cheung et al reported significant inhibition in collagen-induced platelet aggregation using whole blood platelet-ionized lumiaggregometry during the first 24 hours of ECMO which did not return to pre-ECMO baseline until 24 hours after ECMO discontinuation.[44] This study also showed that time-dependent platelet activation was found in the absence of endothelial activation (defined as normal plasma concentration of soluble E-selectin and nitric oxide metabolites) and that matrix metalloproteinase-2 may play a role in the development of platelet dysfunction on ECMO.[44] These studies suggest that impaired platelet aggregation persists for the duration of ECMO and continues after discontinuation of ECMO for some hours prior to resolution.

In a combined neonatal and pediatric ECMO population, Saini et al used TEG with platelet mapping (TEG-PM) to show 75% of patients had severe qualitative platelet dysfunction. Despite severe qualitative platelet dysfunction on TEG-PM, maximum amplitude, *R* time, *K* time, and lysis at 30 minutes on kaolin-activated heparinase TEG were low normal or normal. The authors suggest that a compensatory supranormal fibrinogen response via glycoprotein IIb/IIIa-mediated platelet aggregation and thrombin response via protease-activated receptor-mediated platelet aggregation to overcome decrease in ADP- and arachidonic acid-mediated platelet aggregation may explain these findings and that TEG-PM was a better predictor of severe bleeding and mortality.[59] Thus, although limitations in methods exist, few studies using platelet function testing in children demonstrate prolonged platelet dysfunction during ECMO.

Platelet Monitoring

Different combinations of laboratory monitoring have been proposed to measure anticoagulation during neonatal and pediatric ECMO. Platelet function testing is challenging in neonatal and pediatric patients due to the relatively large volume of blood needed, lack of standardization and established norms, special expertise required to interpret results, cost, and challenges of applying these results to real patients.[52] For many of these reasons, most programs use platelet count alone to monitor platelets.[27] TEG-based protocols and TEG-PM have also been utilized in the pediatric population but data are sparse. To date there is a paucity of evidence to devise and standardize platelet monitoring protocols and additional studies in critically ill children to determine the ideal test for platelet function are needed.

Platelet Transfusion Threshold

The optimal threshold for transfusion of platelets during ECMO is unknown. Thresholds vary by center, location of ECMO care, and by clinical scenario. Both neonatal and pediatric data suggest that prophylactic platelet transfusion for nonbleeding children is common, associated with severity of illness, and platelet transfusion is associated with increased mortality.[41–43,46] In a study including 511 children on ECMO, 97% received at least one platelet transfusion during ECMO.[46] Neonatal age, VA ECMO, location of ECMO care in the NICU, specific acute diagnoses including congenital diaphragmatic hernia, and persistent pulmonary hypertension of the newborn, and a diagnosis of congenital anomaly or chromosomal defect were associated with increased average daily platelet transfusion volume.

Most centers reported a target platelet count around 100×10^9/L in the neonatal ICU and slightly lower target in the pediatric and cardiac ICU. Average daily platelet transfusion volume was an independent predictor of bleeding,

thrombosis, and mortality but average daily platelet count was not.[46] These findings suggest that platelet transfusion practice should not be focused on platelet count alone but on the clinical status of the child including risk factors for bleeding, underlying diagnosis, age, ECMO modality, complications during ECMO, and platelet activity. A recent randomized trial in premature neonates with severe thrombocytopenia compared a restrictive platelet transfusion strategy to a more liberal strategy and found neonates in the liberal group had a higher rate of death or major bleeding.[60] Studies evaluating optimal transfusion threshold and restrictive transfusion strategies are needed to guide therapy in children on ECMO.

Complications and Management

Complications during ECMO including hemolysis, bleeding, and thrombosis also contribute to changes in platelet count and function. Rates of hemolysis, bleeding, and thrombosis vary significantly by ECMO center.[3] Hemolysis is a common complication during neonatal and pediatric ECMO but monitoring for hemolysis is variable between centers and the level at which it is considered "significant" is poorly standardized. In the BATE study mentioned earlier, 33% of all patients were reported as having some degree of hemolysis (defined as a plasma free hemoglobin level $>$ 50 mg/dL); yet, only three of eight centers routinely measured plasma free hemoglobin. In centers where plasma free hemoglobin was regularly monitored, 57% of patients had hemolysis.[3] When only centers who routinely monitored for hemolysis were analyzed, hemolysis existed in all but 1.9% of the patients, and moderate to severe hemolysis (plasma free Hb $>$ 50 mg/dL) was noted in 67% of children. Hemolysis was associated with renal failure and use of continuous renal replacement therapy.[61] Hemolysis causes high levels of bilirubin and plasma free hemoglobin which may lead to hemoglobin-mediated nitric oxide scavenging and reduced plasma nitric oxide resulting in thrombocytopenia.[62] Hemolysis has been associated with increased thrombosis during ECMO and laboratory evidence suggests that plasma free hemoglobin interacts with VWF and this interaction augments platelet adhesion and microthrombi formation at high shear stress.[63]

Bleeding events occur commonly during ECMO in children and are associated with increased mortality.[3,4] Bleeding occurred in 70% of the BATE cohort and intracranial hemorrhage occurred in 16%.[4] Predictors of bleeding include cardiac or extracorporeal cardiopulmonary resuscitation, direct transition from cardiopulmonary bypass to ECMO, age, and higher organ failure index.[4] Management of bleeding depends on the site of bleeding and primary cause. Local site bleeding may be treated with direct pressure or may require surgical intervention. Initial management may include decreasing anticoagulant dose and transfusion of blood products based on deficiencies identified on laboratory analysis. Target levels for fibrinogen or platelets may be adjusted in the setting of bleeding, although there is little data that this is effective. Centers often increase the goal platelet count to even higher levels in instances of bleeding or high-risk patients[64] such as repaired congenital diaphragmatic hernia, but there is even less evidence that this practice prevents bleeding.

For bleeding patients with adequate fibrinogen and platelet counts, acquired VWF deficiency and FXIII deficiency should be considered. With catastrophic life-threatening bleeding, UFH may be held for hours or even days, although the risk of circuit thrombosis is high especially at low flow rates. Antifibrinolytic agents like aminocaproic acid and tranexamic acid have been used to manage surgical site bleeding in pediatric patients and are commonly employed in postoperative congenital diaphragmatic hernia repair patients.[65] Recombinant activated factor VII (rVIIa) and prothrombin complex concentrate (PCC) have been used to treat severe refractory bleeding on ECMO. rVIIa forms complexes with tissue factor and binds to platelet surfaces to generate thrombin. In one center, rVIIa was used at a mean dose of 98 μg/kg for refractory hemorrhage in pediatric patients on ECMO with limited efficacy.[66] Another study in children after cardiac surgery undergoing ECMO with intractable hemorrhage utilized median doses of rVIIa of 90 μg/kg of rVIIa and found no difference in mortality, bleeding reduction, or transfusion requirements.[67] The goals of decreased bleeding and reduced need for transfusion must be balanced with risk of fatal thrombosis and such agents should be used only after factor replenishment has been performed. Having an emergent backup ECMO circuit is mandatory when holding anticoagulation or giving agents such as rVIIa and doses used in reports are variable.

Thrombotic events occurred in 37.5% of the patients in the BATE study. Of these, 31.1% involved circuit thrombosis and 12.8% were patient related.[4] Thrombus formation may occur during periods of low ECMO flow, at sites of stasis or turbulent flow, and during periods of inadequate anticoagulation. Others have noted that connector sites within the ECMO circuit serve as initiators of thrombus, and eliminating as many connection sites is advisable. With the change from roller pump to centrifugal systems in many centers, circuit length and need for many connectors have been reduced. Whether this will affect thrombotic risk in the future is unclear, as some studies find improved outcomes, less bleeding, and transfusion with centrifugal setups while others find more hemolysis and worse outcomes with centrifugal pump ECMO, especially in neonates. If thrombus formation has occurred then changing circuit components may be required. If inadequate anticoagulation with UFH or development of HIT occurs then an alternate anticoagulant should be considered.

Direct thrombin inhibitors (DTIs) have been used in children during ECMO and are becoming the first-line anticoagulant in some centers. Of the DTIs, bivalirudin has been utilized the most in the pediatric population but isolated reports of argatroban and lepirudin have also been published. Pediatric reports of bivalirudin have used either no bolus or a small bolus of 0.5 mg/kg loading dose followed by an infusion of 0.05 to 0.15 mg/kg/h targeting aPTT 1.5 to 2 times baseline.[68,69] Others report a lower bolus 0.05 to 0.5 mg/kg loading dose followed by an infusion rate of 0.03 to 0.3 mg/kg/h and targeting aPTT 1.5 to 2.5 times baseline.[65]

Ranucci et al used bivalirudin in postcardiotomy pediatric ECMO patients and reported less total blood loss and decreased transfusion needs.[68] Safety and dosing concerns, as well as lack of reversibility, are limitations to the use of DTIs. However, their short half-life, lack of the need for cofactors such as AT to exert effects, and increasingly described use in adults make DTI use in pediatrics more attractive. Caution is urged, however, until additional systematic study can evaluate more specifically their safety and efficacy in children. Use of other medications, such as antiplatelet agents, is infrequent but some pediatric centers are employing these agents.[27] Experience from adult and pediatric ventricular assist device patients has noted the favorable impact of aspirin and other agents on thrombosis prevention and would be of interest to study in the ECMO population.

Attempts to mitigate bleeding and thrombotic effects have led to modifications in circuitry and surface coatings. Circuit modifications to reduce redundant length, decrease areas of stasis, limit connector sites and areas of turbulence have evolved and led to slightly different configurations of the circuit. Modifications to the surface of the circuit and oxygenator have also been made to decrease thrombotic risk. Heparin-coated circuits were developed and are utilized by 59% of ECMO center respondents in a multicenter survey of ELSO centers.[27] Heparin-coated circuits are associated with reduced platelet, leukocyte, and coagulation activation and decreased thrombin generation,[70] although whether they truly reduce need for anticoagulation or improve outcome is debatable. Almost every manufacturer today has some type of surface coating on ECMO circuits. Nitric oxide embedded surfaces or bound substances which release nitric oxide are also under development and are associated with local antiplatelet properties preventing platelet adhesion.[71] The efficacy of surface coatings during prolonged ECMO runs is unknown and research to develop fluid-repellent surfaces and endothelialization of ECMO surfaces to inhibit thrombogenesis is ongoing. Newer generations of membrane lungs made with polymethylpentene or polypropylene hollow fibers which are hydrophobic and provide excellent gas exchange at low resistance to blood flow have also become universal in ECMO circuits. These membranes have limited or no plasma leakage across fibers, which was a major detriment in earlier devices, and their low resistance allows use of centrifugal pumps to provide adequate blood flow. Blending of nitric oxide into the gas phase has been described as a means to limit platelet adhesion and activation, although this practice has not resulted in a published study of efficacy.[71]

One final aspect of platelet effects and bleeding and thrombosis is the impact of genetic variability. Genetic testing and variation in clotting and bleeding risk have been well reported in multiple disease processes. Small endogenous nucleotides, microRNAs, have been identified as post-transcriptional regulators of gene expression and may play a role in hemostatic function.[72] MicroRNAs have been reported as biomarkers for cardioembolic disease, stroke, and deep vein thrombosis and current studies are focused on use as biomarkers for platelet activation.[72–74] They are more stable to collect and analyze than messenger RNA, can be frozen and thawed for analysis and do not require tissue for DNA evaluation. How microRNA and genetic variability effect patients on ECMO has not been evaluated, but may have the ability to answer the perplexing questions as to why some patients with similar disease processes, ages, anticoagulant regimens, similar testing results and similar ECMO equipment bleed while others clot. The ability to harness observed microRNA expression may help tailor medication regimens for optimal anticoagulation, help predict who is at greatest risk for bleeding or thrombosis and guide care in the future. The large blood volume to obtain microRNA is a hindrance to this work in children.

Conclusion

Acquired platelet dysfunction is common during ECMO in children, but rigorous study of this condition is limited. Both qualitative and quantitative platelet dysfunction has been reported and contributes to bleeding risk. Balancing the risk of bleeding and thrombosis is challenging during ECMO due to the complex mechanisms involved, developmental hemostasis, multiple alterations in hemostasis seen in these patients, and lack of high quality evidence. Optimal platelet count, function, transfusion threshold, and ideal monitoring strategies are not well established. Treatment of bleeding includes surgical hemostasis, surgical site compression, transfusion of blood products based on laboratory values, decreased anticoagulant dosing, identification and treatment of VWF deficiency and FXIII deficiency, antifibrinolytic agents and, rarely, treatment with rVIIa and PCC. Treatment of thrombosis is focused on preventing subtherapeutic anticoagulation, low flow ECMO, or stasis in the circuit. The changing role of DTI not only for HIT but also as a primary anticoagulant in children requires more study. Antiplatelet agents may play a role in preventing thrombosis but inadequate data exist to recommend their use during ECMO in children. Finally, circuit modifications and treatment of ECMO surfaces with heparin-like substances or molecules such as nitric oxide are promising.

As with all areas of hemostasis and extracorporeal circulatory devices, lack of universally agreed upon definitions of bleeding and thrombosis remain major impediments to study evaluation. Lack of similar end points, standardization of equipment, monitoring techniques, and other factors make extrapolation of single center reports to the ECMO field at large difficult. Multiple groups are working to correct these deficiencies and it is hoped that at least bleeding and thrombosis definitions will be developed which are agreed upon by all. Well-designed multicenter trials with clearly defined end points are sorely needed. Observational studies to correlate details of circuitry, anticoagulation, laboratory monitoring, and transfusion thresholds with bleeding and thrombotic complications should be accomplished to identify "best practices." Data obtained from "best practice" reports can then be tested further until refinement of the optimal protocol for laboratory monitoring and anticoagulation management is

obtained and can then be studied in a systematic randomized controlled trial. Alternatively, technology advancement or genetic assay may allow for elimination of the need for anticoagulation at all. As the field of extracorporeal life support continues to advance, elimination of bleeding and thrombosis represents a critical need.

Conflict of Interest
H.J.D. reports personal fees from Innovative ECMO Concepts LLC, outside the submitted work. K.M. reports grants from NIH, outside work submitted work.

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