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Chapter

Platelets in the Newborn

Ijeoma Esiaba, Iman Mousselli, Giulia M. Faison, Danilyn M. Angeles and Danilo S. Boskovic

Abstract

Platelets were first described in the mid-nineteenth century. Since then, their roles were identified in hemostasis and thrombosis, inflammation, leukocyte interactions, angiogenesis, and cancer growth. But there is little information about such platelet functions in the newborn. Several studies highlighted some platelet differences between newborns and adults. Yet, in spite of these differences, healthy newborns appear to be adequately protected. A number of factors, however, were reported to negatively affect neonatal platelets. These include maternal hypertensive disorders or infections, neonatal asphyxia or respiratory distress, therapies such as ampicillin or indomethacin, and treatment modalities such as ventilators, nitric oxide, or extracorporeal membrane oxygenation (ECMO). Their effects on newborn platelets are usually transitory, lasting from several hours to a few days or weeks. If these effects are well characterized, they could serve as reporters for diagnosis and monitoring during therapy. Careful studies of neonatal platelets are needed to improve the understanding of basic physiology and pathophysiology in this cohort and to identify possible targets for intervention and therapy.

Keywords: platelet function, hemostasis, prematurity, platelet transfusion, newborn, sepsis, ECMO

1. Introduction

Platelets are small discoid cellular particles, produced by megakaryocytes, and best known for their role in thrombus or platelet plug formation. Since their initial description in the mid-nineteenth century, further details have emerged about their structure and function. More recently, their roles in processes as wide ranging as tissue repair and wound healing, angiogenesis, tumor killing, tumor growth and metastasis, inflammation, and host defense have come to light [1, 2]. Platelets perform these varied functions and diverse interactions because of several receptors and ligands on their surface, and a store of over 300 proteins within their cytoplasm and granules. With newer technological advances, more platelet functions were discovered and the mechanisms for some of them are now clearer.

Platelets mediate primary hemostasis, a dynamic process involving several reactions resulting in thrombus formation. Initially, platelets aggregate to form a platelet plug at the site of injury [3, 4]. In secondary hemostasis, thrombin is generated after a cascade of enzymatic reactions. The generated thrombin subsequently cleaves fibrinogen to fibrin [5]. Fibrin spontaneously polymerizes forming a fibrous network which stabilizes the platelet plug [6]. The process of tertiary hemostasis, or fibrinolysis, restricts clot formation to the site of injury, dissolves clots after the

damaged endothelium has been repaired, and prevents the formation of pathologic thrombi [7, 8]. These reactions are tightly regulated to minimize the risk of either bleeding or thrombosis. Components of the hemostatic system are usually preformed and circulate in their respective inactive forms. Apart from their role in hemostasis, some of these factors also play a role in other physiological processes such as embryonic development, angiogenesis, or immunity [9].

Most of the information about platelets is based on studies conducted in adults or in animal models. Despite recognized roles of platelets in processes as wide ranging as inflammation and angiogenesis, information about these roles of neonatal platelets is limited. However, clinical observations suggest that there likely are some functional differences between neonatal and adult platelets. Newborns are at greater risk of contracting infections and may not cope adequately with inflammatory stresses [10]. New blood vessels are formed to meet the demands of rapidly growing tissues. The roles of platelets in these processes and reactions in the newborn are not yet well described. Additionally, certain prematurity-related morbidities such as intraventricular hemorrhage, retinopathy of prematurity, and necrotizing enterocolitis are associated with bleeding and inflammation [11, 12]. Platelets or their functional deficits are believed to be involved in these disorders. Studying platelet function in the newborn is difficult, but emerging methodological approaches requiring small volumes of newborn's blood are making such studies feasible. Following a general description of platelet structure and functions, this review will highlight documented differences in the newborn.

2. Platelet structure and functions

Platelets mediate primary hemostasis and play roles in procoagulant and fibrinolytic processes [13]. They are nonnucleated fragments derived from bone marrow megakaryocytes. The adult human produces about 10^{11} platelets daily, rising by more than 20-fold during increased need [14]. In the fetus and neonate, platelets are produced largely in the liver and spleen [15, 16]. Thrombopoietin maintains platelet homeostasis by regulating thrombopoiesis [17, 18]. The resting platelet is disk shaped with a diameter of about 1.5 μm and a lifespan of 7–10 days [19]. Its surface does not promote coagulation or aggregation. In circulation, the platelets' resting state is further supported by the release of prostacyclin and nitric oxide from endothelial cells, by the expression of CD39 (an ADPase) on the endothelial surface, and by the inability of normal plasma vWF to bind spontaneously to the platelet surface [20].

Platelets have a complex internal structure with a series of organelles. Lacking a nucleus, they nevertheless contain some nucleic acid in the form of ribonucleic acid (RNA), which is used for synthesis of new proteins, especially during or after platelet activation [21]. The secretory granules comprise the α-granules and dense or δ-granules. The α-granules contain adhesion molecules important for platelet interactions with other platelets and blood cells, angiogenic and mitogenic factors, plasma proteins, and several factors relevant for coagulation and fibrinolysis [22]. The dense- or δ-granules contain non-protein molecules such as adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium and serotonin [22]. These play central roles in amplification of platelet activation and aggregation and in modulation of vascular endothelium and leukocyte functions. Within lysosomes are membrane proteins and acid hydrolases that digest the material in platelet aggregates through hydrolytic degradation [23]. Over 300 proteins are secreted from these granules during activation [24]. In the unstimulated platelets, the granule contents remain internalized. When stimulated, however, platelets release such contents through an open canalicular system [25].

A number of factors and agonists can stimulate platelets. These include shear stress during blood flow, agonists such as thrombin, collagen, or ADP, and recognition of and interaction with viruses, bacteria, or damaged vascular endothelium [26, 27]. Typically, platelet activation is triggered when there is a break in the vascular endothelium. The activated platelets first adhere to the damaged endothelium by binding to von Willebrand factor (vWF) through its surface membrane glycoprotein Ib (GPIb). Further interactions of platelet glycoprotein VI (GPVI) with fibrillary collagen and platelet β1 integrin with laminin, collagen, and fibronectin maintain platelet adhesion to exposed extracellular matrix [8].

Following activation, platelet membrane phospholipid distribution changes to include exposure of phosphatidylserine on the outer surface, thus promoting the condensation of vitamin K-dependent coagulation factors on this surface, and inducing the activation of the procoagulant cascade [28]. Additionally, a rearrangement of the cytoskeleton leads to a change in platelet structure [29] from the resting discoid form, via an intermediate spherical shape, to a fully activated amoeboid form with numerous extending pseudopodia able to interact with some nearby surfaces [29]. Meanwhile, the contents of α- and δ-granules are released into the immediate environment, further amplifying the original activation signal [23]. As a result, in response to a number of biological mediators, the activated platelets adhere to each other, to leukocytes and endothelial cells, and to components of the sub-endothelial matrix [30].

During extension of platelet plug formation, activated platelets accumulate on top of the initial monolayer of platelets bound to collagen of the sub-endothelium [3]. Expressed receptors on each platelet allow binding of agonists such as ADP, thrombin, and thromboxane A_2, which are released from activated platelets [31]. Consequently, more platelets are recruited to the site of injury, thereby consolidating the initial hemostatic plug. Binding of fibrin to aggregated platelets through activated receptor glycoprotein GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$) helps to further stabilize the hemostatic plug [32].

Several proteins are released during platelet aggregation at a damaged blood vessel surface [22]. Some are believed to be responsible for repair of damaged blood vessels and development of new ones. Although precise mechanisms are not well understood, it was suggested that platelets are necessary for formation of new blood vessels [33]. Supporting this are observations of reduced retinal neovascularization in a mouse hypoxia-induced retinal angiogenesis model due to thrombocytopenia or in response to treatment with inhibitors of platelet aggregation [33]. In this context, platelet granules are believed to contain pro- and anti-angiogenic compounds [34, 35]. Similarly, platelet interactions with cancer cells appear to play a role in the development of metastases and tumor angiogenesis [36]. While cerebrovascular remodeling is known to occur in the newborn in the first few postnatal weeks [37], the roles of platelets in this developmental process are not yet well described.

Platelets may also play a role in newborn's inflammatory processes and host defense. Neonates are generally believed to be at least partially immunologically incompetent and susceptible to a variety of infections. In this context, it is of interest that platelets have toll-like receptors (TLRs) that directly recognize and interact with a number of microorganisms or their products. Platelets may be responsible for killing microbes directly by phagocytosis, by release of microbicidal agents, or as sentinels communicating information about microbial encounters to cells of the innate immune system [15]. Bacterial infections, found in preterm newborns admitted to the neonatal intensive care unit (NICU), appear similar to infections found in adults with severe neutropenia [38]. This suggests reduced neutrophil functions in these babies. Neonates rely heavily on innate immunity for protection because their adaptive immunity is not yet fully developed [39]. Neutrophils are usually the first cells to be recruited to infection sites. They kill pathogens by various mechanisms

including (a) direct phagocytosis and chemical killing by degranulation and (b) by formation of neutrophil extracellular traps (NETs) [40]. Recent studies showed that platelet interactions with neutrophils are important for optimal neutrophil functions [41–44]. One such aspect of neutrophil function involves their chemotaxis and extravasation to sites of infection. Platelets were observed to act as "pathfinders" guiding neutrophils to infection sites, and platelet inhibition resulted in poor neutrophil chemotaxis [45, 46]. Interaction of platelets with leukocytes may induce inflammation. Understanding the role and mechanisms involved in platelet-leukocyte interactions in the newborn, particularly those born prematurely, could lead to development of more rational approaches to morbidities common to this group.

3. Newborn platelets

Thrombopoietin, a protein regulator of platelet synthesis and homeostasis [18], was detected in the fetal liver as early as the sixth week of gestation [18]. In turn, megakaryocytes, the precursor cells that form and release platelets into circulation, were detected in the liver and circulation at the eighth week [19]. The megakaryocyte numbers were observed to be, at least in part, inversely correlated to gestational age, so that healthy preterm newborns characteristically have higher levels, while levels in healthy full term newborns and adults are similar [47, 48]. Neonatal megakaryocyte progenitor cells are more sensitive and have higher proliferative potential in response to thrombopoietin compared to adult cells, and this sensitivity is even greater in preterm newborns [49]. However, neonatal megakaryocytes, tending to be smaller and with a lower ploidy than adult cells, produce fewer platelets per megakaryocyte [48, 50, 51]. Newborn and adult platelets are ultra-structurally similar [52, 53] and contain comparable membrane receptor glycoproteins (GPs) [54] and thromboxane receptors [55]. However, newborn platelets tend to include more immature forms, with the ability to form fewer pseudopods, fewer developed microtubular structures, and fewer α-granules [53]. Additionally, neonatal platelets have fewer adrenergic receptors [56]. Although they store comparably adult levels of ADP, ATP and serotonin in their dense granules, the overall dense granule release during platelet activation is lower in the newborn [57].

Platelet count is dependent on gestational age, increasing during fetal life, but usually reaches the expected adult range of 150,000 to 450,000/μL [58] from about 22 weeks of gestation [59]. The percentage of reticulated platelets, an indication of newly produced platelets, is higher in the newborn circulation [60], while the mean platelet volume (MPV), a measure of platelet size, tends to be comparable to adults.

Platelet adhesion to, and coverage of, sub-endothelial extracellular matrix is higher in the newborns than in adults [61, 62]. This is in spite of comparable collagen binding or platelet aggregation [61]. The enhanced neonatal platelet adhesion is believed to be mediated by the neonatal plasma von Willebrand factor (vWF) [61], which was reported to include unusually large multimers [63, 64]. Nevertheless, compared to full-term newborns, platelet adhesion tended to be lower in earlier gestational age neonates [62]. In part, these observations could help to explain how hemostatic function is usually maintained in full-term newborns, despite decreased intrinsic platelet activation, and why the preterm neonates are progressively decompensated the earlier their gestational age.

The phospholipid content and baseline exposure of platelet surface phosphatidylserine is comparable in adults and newborns [65, 66]. However, more platelet microparticles are generated and more phosphatidylserine molecules are exposed in the term and preterm platelets when thrombin or calcium ionophores were used as activators [67]. Microparticles or exposed phosphatidylserine is expected to induce a procoagulant state. Yet, the procoagulant activity, especially in the preterm newborn,

is generally lower despite the higher levels of generated microparticles and exposed phosphatidylserine [67]. Supplementing neonatal plasma with coagulation factors improves its procoagulant activity so that it becomes comparable to adults. This implies that newborn platelets can often present adequate procoagulant surface, but the apparent poor activity may in part be due to a deficiency of humoral factors [68].

P-selectin expression, as an index of α-granule secretion, was reported lower in newborn platelets compared to adults, especially in the <30 week gestation group [69, 70]. Neonatal dense granule secretion, measured by secreted serotonin, was similar to that in adults when inositol triphosphate, 1-oleoyl-2-acetyl-glycerol, or thrombin was used as an agonist. Collagen-mediated stimulation, however, resulted in lower serotonin secretion in cord blood, although the number of dense granules in adults and neonates was found to be similar [57, 68]. GPIIb/IIIa receptors are expressed early during gestation. Yet, the fraction of active GPIIb/IIIa in neonatal cord and peripheral blood is lower compared to adults [69].

During the first few days of life, platelet activation appears to be less effective, as indicated by flow cytometric studies [69]. However, these activation profiles approach the adult patterns between the tenth and the fourteenth day of life [71]. Proposed explanations for this observed hypo-responsiveness include: relative deficiencies of phospholipid metabolism including thromboxane production, differential regulation of GPIIb/IIIa activation, impaired mobilization of calcium and intracellular signaling, impaired granule secretion, and lower aggregation [72]. These could result from lower intrinsic signal transduction in neonatal platelets [72]. Such effects are further enhanced by lower expression of protease-activated receptor-1 (PAR-1) and PAR-4 [73, 74], which mediate thrombin-dependent platelet activation.

Various components of the hemostatic system in the fetus and neonate are qualitatively and quantitatively different from those in adults [8]. Such differences could be explained either by lower synthesis, higher clearance, or higher consumption [75, 76]. Although the hemostatic system is sometimes thought to be incomplete at birth [72], it nevertheless appears to be adequate for the majority of healthy full-term newborns.

4. Perinatal factors affecting platelet function in the newborn

Acquired platelet dysfunctions are common during the neonatal period especially in preterm newborns. These disorders are usually secondary to perinatal or neonatal conditions such as maternal and neonatal state of health, presence of infections, medications given to mother or to newborn, or interventions for the newborn (**Tables 1** and **2**). Platelet count and function are usually restored several hours or days after the triggering condition is removed.

4.1 Prenatal and maternal factors associated with neonatal platelet dysfunction

There are several maternal factors that can impact neonatal platelet function especially during the days preceding delivery. These include maternal hypertensive disorders and prenatal use of aspirin, magnesium sulfate, or antibiotics.

Hypertensive disorders of pregnancy are associated with platelet dysfunctions in the newborn. Pregnancy-induced hypertension (PIH) is a risk factor for early onset thrombocytopenia in the newborn [77]. This is especially true for babies born prior to 36 weeks of gestation [78, 79]. Neonatal platelet counts tend to be inversely correlated to maternal blood pressure [79]. These platelets also exhibited lower adhesion properties [80]. Babies with low birth weight, meconium aspiration, or infections are also at greater risk for thrombocytopenia [81, 82]. Flow cytometric analyses of platelets from premature newborns from preeclamptic mothers demonstrated lower expression of

Factor	Effect on newborn platelet
Hypertension in mother	• Reduced platelet adhesion and surface coverage [80] • Low platelet count [78, 79] • Decreased secretion and expression of CD62P, CD63, and CD36 [83] • Increased expression of CD62P and CD63 [84] • Low platelet and megakaryocyte counts [84]
Magnesium sulfate (prenatal)	• Reduced ADP-mediated platelet aggregation [97]
Low dose aspirin (prenatal)	• Reduced platelet aggregation [95] • No change in platelet count and aggregation [92, 93] • Reduced thromboxane B2 production [94]
Indomethacin	• Prolonged bleeding time and gastrointestinal hemorrhage [104, 105] • Reduced prostacyclin and prostaglandin levels [104] • Abnormal platelet aggregation up to 4 days after medication [106]
Ibuprofen	• Prolonged PFA-100 closure time [108]

Table 1.
Maternal factors affecting platelets in the newborn.

Neonatal factor	Effect on newborn platelet
Nitric oxide	• Abnormal thromboelastogram values [109] • Prolonged prothrombin time [109] • Prolonged bleeding time [111]
Therapeutic hypothermia	• Abnormal thromboelastogram values, associated with bleeding [199] • Reduced platelet count [123, 200] • Prolonged bleeding time and PFA 100 closure time [201]
Asphyxia and RDS	• Reduced platelet count [113, 118, 120] • High MPV and PDW [113, 117] • Increased thrombopoietin level [118] • Increased thromboxane level [120]
Mechanical ventilation	• Reduced platelet count [121]
Extracorporeal membrane oxygenation (ECMO)	• Reduced platelet count [125, 126] • Reduced platelet activation [126]

RDS, respiratory distress syndrome; MPV, mean platelet volume; PDW, platelet distribution width.

Table 2.
Neonatal factors affecting platelets in the newborn.

CD62P (P-selectin), CD63 (platelet activation marker), or CD36 (platelet glycoprotein IV (GPIV)) after thrombin stimulation, compared to full-term neonates [83]. However, when compared to other similar preterms, the expression of platelet CD62P and CD63 was relatively higher in newborns from preeclamptic mothers [84]. Additionally, this cohort was also characterized by lower platelet and megakaryocyte counts [84], implying possible disturbances in platelet production [85]. Infants born to hypertensive mothers tend to be hypoxic [86]. Animal model studies suggest that hypoxia tends to favor erythropoiesis over megakaryopoiesis, leading to lower platelet counts [87].

Low dose aspirin (LDA), about 60–100 mg, is sometimes given to pregnant women, who are at risk of developing a hypertensive disorder, or whose fetus has

intrauterine growth restriction [88, 89]. Aspirin inhibits cyclooxygenase, which catalyzes the initial steps in conversion of arachidonic acid to prostaglandins and thromboxanes [90]. Thromboxane A2 (TxA2) serves to amplify the signal during platelet activation [91]. Nevertheless, while some reports suggest that prenatal aspirin does not alter cord blood platelet count and aggregation [92] or thromboxane B2 (TxB2) inhibition [93], others challenge this with clear TxB2 differences in newborns of mothers exposed to LDA even several days after the medication was stopped [94]. This apparent contradiction may be resolved by taking into account the actual timing of LDA treatment prior to delivery. It was noted that newborn platelet dysfunction, including reduced collagen-stimulated platelet aggregation, is generally observable if the mother had aspirin within a week of delivery [95]. In this context, aspirin also increases the risk for mucocutaneous bleeding in the newborn, especially if the mother took it within five days of delivery [72, 95].

While aspirin is perhaps the best described with respect to its potential to alter platelet functions, it is not the only drug to do so. Indomethacin, given to the mother as a tocolytic, increased the risk of subsequent neonatal intraventricular hemorrhage (IVH) [96], presumably by affecting cerebral blood flow and by altering platelet and neutrophil functions. Similarly, platelets in newborns, from mothers receiving tocolytic magnesium sulfate, tended to be less effective in forming aggregates in response to ADP-mediated activation, but not so in response to collagen stimulation [97].

4.2 Postnatal and neonatal factors associated with platelet dysfunction in the newborn

Neonatal infections tend to lead to platelet consumption. This is implied by the observed upregulation of thrombopoietin and elevated megakaryocyte progenitor cells in septic newborns [98]. Reduced platelet adhesion was also reported in such neonates [99]. However, enhanced granule secretion and aggregate formation in response to agonists during experimental conditions [100] suggest that these circulating platelets may already be to some extent primed and not in their resting state. Furthermore, neonates born following chorioamnionitis had significantly higher levels of soluble P-selectin and higher CD40L (CD40 ligand, able to bind CD40 protein on antigen presenting cells) on their platelets [101].

Antibiotics are often used to treat infections and sepsis, and some of them could alter hemostatic responses. Prolonged template bleeding and PFA-100 closure times were correlated with duration and dosage of neonatal ampicillin treatment [102, 103].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are known to affect platelet function in adults. Yet, they are sometimes given to newborns as a treatment for patent ductus arteriosus (PDA). Indomethacin is associated with prolonged bleeding time and gastrointestinal bleeding in preterm newborns [104]. These effects tend to last up to 48 h [105], but normal platelet values are restored about the tenth day [106]. Nevertheless, in preterm newborns with intracranial hemorrhage, indomethacin administration for treatment of PDA did not extend the hemorrhage [107]. In contrast, ibuprofen treatment is associated with prolonged PFA-100 closure time, but not with altered bleeding time [108].

Inhaled nitric oxide (NO) is used as a selective pulmonary vasodilator to treat hypoxemic respiratory failure or pulmonary hypertension in newborns (≥34 week gestation) [109]. Inhaled NO prolongs bleeding time in adults [110]. Persistent pulmonary hypertension and treatment with inhaled NO were reported to alter neonatal platelet thromboelastogram (TEG) values [109]. Bleeding time was also prolonged in such babies [111]. These clinical tests, however, returned to normal after about 24 h of stopping therapy. It is of interest that newborns receiving NO therapy did not

experience increased risk of intracranial hemorrhage [72]. Nitric oxide is known to inhibit platelet adhesion and aggregation by inhibiting GPIIb/IIIa activation [110]. A reduction in expressed P-selectin and activated GPIIb/IIIa on collagen-stimulated platelets was reported after NO treatment in adults and newborns [112].

Term newborns who were small for gestational age tended to have lower platelet counts but higher mean platelet volumes (MPVs) [113, 114]. A similar pattern was also observed in asphyxiated term newborns [113]. MPV is a measure of average platelet size [115] and may serve as a marker of platelet production, consumption, or severity of some disorder of bone marrow, thrombosis, or infection [116, 117]. For example, MPV was elevated in preterm newborns with respiratory distress [117], suggesting potential issues in platelet production, consumption, or both. Asphyxia is associated with upregulation of thrombopoietin concentration, which in turn is negatively correlated to platelet count up to the 7th day of life [118]. Thrombocytopenia, however, when associated with perinatal asphyxia, does not tend to resolve until about the 19th to 21st day of life [119]. Increased thromboxane levels in asphyxiated newborns [120] suggest platelet activation, and possibly consumption, as an explanation for the observed thrombocytopenia. Hypoxia leads to preferential upregulation of erythropoiesis over megakaryopoiesis [87, 114], consistent with elevated thrombopoietin observed [118].

Mechanical ventilation is generally used to resuscitate hypoxic or asphyxiated newborns. It was reported that mechanical ventilation led to reduced platelet counts in newborns with respiratory distress, or in rabbit models, regardless of the oxygen concentration used [121]. However, using newborn piglet models of hypoxia, it was found that various platelet indices were affected particularly by high oxygen level used [122]. Resuscitation with 100% oxygen led to enhanced collagen-stimulated platelet aggregation, while using 18–21% oxygen did not do so [122].

To prevent permanent brain damage following perinatal asphyxia, the newborns are sometimes treated with therapeutic hypothermia. The hypothermia treatment, in turn, led to decreased platelet counts, but had an overall protective effect by reducing risk of cerebral hemorrhage [123] and restoring other hemostatic parameters [124]. Similarly, extracorporeal membrane oxygenation (ECMO) is used to rescue term newborns with persistent pulmonary hypertension, asphyxia, or congenital diaphragmatic hernia [125]. Platelet counts and rates of activation were reduced during ECMO therapy, and were not fully restored with transfusion, until several hours post-ECMO [125, 126].

5. Platelets and sepsis

Sepsis is a complex syndrome characterized by disordered immune, endocrine, and metabolic responses to infection [127]. The exaggerated responses can lead to multi-organ failure (MOF), shock, and death [127]. Sepsis is generally considered if a documented or suspected infection is present with at least one additional finding (e.g., fever/hypothermia, elevated heart rate, and leukocytosis/leukopenia). In contrast to infection, however, sepsis is defined by additional evidence of organ dysfunction and a dysregulated host immune response [127], its key features. Notably, interactions between the innate immune system and the hemostatic system, including platelets and coagulation factors, were identified as principal steps in the pathogenesis of sepsis. Progressive thrombocytopenia and coagulopathy are strong negative prognostic findings in severe sepsis and have recently been included in the updated definition of the disease [127]. Platelets are able to release cytokines, recruit leukocytes, interact with bacteria and the endothelium, and contribute to formation of microthrombi [128]. These processes are adaptive and protective in the context of

Biomarker	Association	References
Thrombocytopenia	Mortality	[133]
Impaired platelet function	MOF, mortality	[132, 133]
Impaired platelet aggregation	ALI	[133]
P-selectin	MOF	[133]
Platelet-neutrophil aggregates	Sepsis progression	[132, 133]
Immature platelet fraction	MOF	[133]

MOF, multi-organ failure; ALI, acute lung injury; TPO, thrombopoietin.

Table 3.
Platelet-related biomarkers of sepsis severity in human studies.

a localized infection, but may become dysregulated and "maladaptive" during sepsis, contributing to organ damage [129]. A low platelet count is a well-known biomarker for disease severity. More recently, attention has been focused on the active role of platelets in the pathogenesis of multi-organ failure.

The correlation between thrombocytopenia and sepsis is well documented [130]. Platelet count below <50,000/μL is a strong negative prognostic marker in patients with sepsis and is thought to result from platelet activation and consumption [131, 132]. A number of platelet function markers were proposed as biomarkers for sepsis correlating with its severity [133] (**Table 3**).

Moreover, platelets interact with neutrophils in the formation of NETs (neutrophil extracellular traps) resulting in the trapping and killing of pathogens [133]. They also play a central role in driving and modulating host inflammatory and immune responses, influencing directly the function of endothelial cells, neutrophils, and lymphocytes [134]. Platelets are the most numerous blood cells with immune function, able to interact with bacteria in several ways: (1) direct interaction between platelet glycoproteins and bacterial surface proteins, as occurs between GPIb and *S. sanguis* SrpA [134]; (2) indirect interactions, such as the interaction of platelet $\alpha_{IIb}\beta_3$ with fibrinogen to which the clumping factors of *S. aureus* bind [134]; and (3) upon activation, platelets release a series of factors which can modulate the immune response or have direct microbicidal effects. For example, released thrombin-induced platelet microbicidal protein (tPMP-1) can directly lyse bacteria, like *S. aureus* [134].

5.1 Conclusions

In addition to hemostasis, platelets actively participate in the innate immune defense system. Participating in the recognition of pathogens, signal transduction, or the release of cytokines/chemokines, they reveal a functional similarity with leucocytes in sepsis and septic shock. There is abundant evidence that platelets can influence key host responses to sepsis. Further studies are needed to address the effects of platelet transfusion or inhibition toward sepsis prevention and treatment particularly in the newborn.

6. Platelets in neonates with extracorporeal membrane oxygenation

Extracorporeal membrane oxygenators are used to provide gas exchange in severe respiratory failure employing venovenous (VV) circuits or, increasingly, if associated with concurrent cardiac failure, veno-arterial (VA) circuits, while

waiting for organ recovery to occur. Support by cardiopulmonary bypass (CPB) systems decreased the morbidity and mortality of children, especially those who require surgery for life-threatening anatomical heart defects [135, 136]. ECMO contributed to decreased mortality for children with severe cardiac or respiratory failure [137, 138]. While annually thousands of neonates are helped by ECMO support, thromboembolic complications also frequently occur [139]. Nevertheless, for many neonatal patients, survival is made possible only because of ECMO support [136]. ECMO is used to treat a variety of conditions in neonatal patients, including respiratory and cardiac failure as a result of persistent pulmonary hypertension (PPHN), congenital diaphragmatic hernia (CDH), meconium aspiration syndrome (MAS), respiratory distress syndrome (RDS), pneumonia, severe air-leak syndromes, or sepsis [140].

Both bleeding and clotting complications can occur during ECMO support, often coexist in the same patient, and are associated with significant morbidity and mortality [141]. Moreover, patients requiring ECMO are critically ill, thus making it difficult to distinguish the relative contributions of the underlying pathology from that of the ECMO circuit as such. Rates of reported ECMO-associated venous thromboembolism (VTE) in general population, ranging from 18 to 85% in various centers, may be at least partly dependent on anticoagulation regimens [141]. Severe hemorrhage is reported in nearly 40% and intracranial hemorrhage in 16–21% of patients [142, 143]. At the same time, there is broad variation in practice, without clear consensus, on the administration and monitoring of anticoagulation during ECMO, or the management of ECMO-related hemorrhage and VTE [144].

Activation of the coagulation system is initiated by the exposure of blood to foreign synthetic surfaces and by shear stresses of the circuit, especially from device pumps. The shift to a pro-coagulant state appears to be mediated primarily by thrombin, while an excessive fibrinolytic tendency is mediated by plasmin, resulting in a consumption of clotting factors, impaired platelet function, thrombocytopenia, and fibrinolysis [145]. Initial fibrinogen deposition and subsequent activation of coagulation and complement factors allow platelets and leukocytes to adhere to oxygenator surfaces further enhancing thrombin generation. Such changes contribute to higher rates of thrombosis in these patients [145]. Meanwhile, several of a series of processes contribute to higher bleeding rates. (a) Primary hemostasis is impaired because of platelet dysfunction and loss of key adhesive molecules. (b) Shear stress causes the development of an acquired von Willebrand defect. (c) Widespread fibrin deposits on surfaces trigger an enhanced fibrinolytic response. (d) Administration of systemic anticoagulation, required to maintain circuit patency, raises bleeding risks [146].

Balancing the relative risks of bleeding and thrombosis can be difficult. Factors related to patient's illness, the extracorporeal support, and the interplay between pro-inflammatory and anti-inflammatory processes vary among patients.

6.1 Platelet dysfunction during ECMO

If the ECMO circuit is primed only with crystalloid or RBCs and plasma, then dilutional coagulopathy and dilutional thrombocytopenia develop as the ECMO is initiated. Dilutional coagulopathy is generally not severe, but will complement the systemic anticoagulation. Dilutional thrombocytopenia, however, may further aggravate any preexisting thrombocytopenia or platelet dysfunction, frequently present in premature neonates. Accurate assessment of platelet function under these circumstances can be difficult, further complicating evaluation of patient's

bleeding or thrombotic potential. Impairment of platelets can occur as early as 15 min after starting ECMO and last until it is discontinued [125].

Platelets adhere to the protein-coated monolayer of the ECMO circuit surfaces and interact with activated components of the coagulation and complement systems [147]. Elevated shear flow from the ECMO circuit causes some platelet receptor shedding. Of particular interest are the losses of key platelet adhesion glycoproteins GPI and GPVI, and the associated reduction of high molecular weight vWF multimers. GPI serves as a receptor for vWF and GPVI as a receptor for collagen [126]. Adhesive proteins, vWF, and fibrinogen assist platelets to bind to damaged vessel wall surface and to other platelets [148]. As platelet thrombus is being formed, the prothrombinase enzyme complex assembles on the activated platelet surfaces to produce thrombin. In turn, thrombin cleaves fibrinogen to form fibrin, which spontaneously polymerizes to form the fibrin meshwork, which further strengthens the thrombus [149]. Consequently, shedding of GPI and loss of high molecular weight vWF lead to dysfunctional platelet responses to vascular injury. This persists despite platelet transfusion and throughout the period of ECMO use [126]. Subsequently, lower levels of platelet aggregation are observed by light aggregometry using various agonists including ADP, ristocetin, collagen, or epinephrine [150]. Such decreased potential for platelet aggregation may lead to increased bleeding risk particularly when combined with the effects of anticoagulants or antiplatelet agents. Flow cytometry of blood, from those receiving ECMO support, showed severely reduced membrane-bound P-selectin (CD62P) and CD63, both of which modulate platelet spreading [151].

Despite reduced aggregation and lower expression of key platelet adhesion and structural molecules, there is a time-dependent platelet activation marked by increased levels of circulating matrix metalloproteinase-2 (MMP-2) and soluble P-selectin [126]. This is not associated with significant activation of the endothelium [126], but may be due to the release of platelet granules [152]. Furthermore, this time-dependent platelet activation is also accompanied by platelet receptor shedding and the release of platelet microparticles (PMPs) [153]. These are small cell-derived particles, typically 0.1–1 μm in size, that are produced from activated platelets in situations of shear stress [154]. While these PMPs can present a prothrombotic surface, it is not clear whether they are a major contributor to the prothrombotic phenotype or to the pathogenesis of ECMO-associated coagulopathy [153].

6.2 Platelet counts during ECMO

Thrombocytopenia is common in critically ill patients. A constant shear force, caused by the ECMO pump, is implicated in acquired platelet dysfunctions. Appropriate anticoagulation is difficult to achieve during ECMO since severe thrombocytopenia of <50,000/μL may be present even prior to ECMO. This situation increases the practice of platelet transfusions [155]. Minimal target platelet counts vary from 25,000 to 100,000/μL between hospitals. However, if bleeding occurs or is expected, then target platelet counts are increased to 150,000/μL or higher, particularly if platelet dysfunction is suspected. As in any setting of thrombocytopenia, it is important to try and identify the cause and treat appropriately [156, 157]. Bleeding in critically ill patients with a platelet count of 30,000/μL is believed to be associated with additional disturbances of hemostasis [158]. Platelet transfusion is recommended in bleeding patients with either primary or secondary platelet abnormalities regardless of platelet counts [158]. Required thresholds for prophylactic platelet transfusions, however, are

generally at platelet levels above 20,000/μL, given the requirements of invasive procedures and potential bleeding risk [158]. Many centers describe targeting a platelet count of >100,000/μL during an ECMO [159]. Research to support this practice, however, is lacking and urgently needed.

6.3 Anticoagulation during ECMO

Unfractionated heparin is the most widely used anticoagulant during ECMO [160]. Heparin levels tend to be monitored primarily indirectly by activated clotting time (ACT) [159]. While there are a number of devices that promise to describe certain characteristics of platelet function, it is not yet clear to what extent the data produced by them actually reflect the physiological platelet interactions and roles. Viscoelastic tests using rotational thromboelastometry (ROTEM) assess whole blood coagulation, and thus provide information on the dynamics of clot development, stabilization, and dissolution. Several reports suggest that ROTEM-guided coagulation management could reduce bleeding episodes in ECMO patients [160, 161]. Similarly, whole blood platelet aggregometry using the Multiplate (Roche Diagnostics, Munich, Germany) demonstrated decreased platelet aggregation in ECMO patients [161].

Other intravenous anticoagulants, such as bivalirudin and argatroban, are used increasingly, particularly if heparin-induced thrombocytopenia (HIT), heparin resistance, or allergy is suspected [162, 163]. At present, there is no clear consensus on administration and monitoring of anticoagulation during ECMO or on management of ECMO-related hemorrhage and VTE [164]. The current aim of anticoagulation is to reduce thrombin generation. This, however, increases the risk of hemorrhage. The ideal therapeutic agent, which would reduce thrombotic risk without increasing the risk of bleeding, remains elusive.

6.4 Heparin-induced thrombocytopenia

Heparin-induced thrombocytopenia (HIT) is an immune-mediated coagulation side effect of heparin therapy characterized by a prothrombotic state mediated by platelets, leukocytes, and antibodies against complexes of platelet factor 4 (PF4) with long chain heparins [165]. Rapid platelet consumption leads to thrombocytopenia. HIT was considered to be very rare in the pediatric population. However, more recent reports indicate that it occurs in children receiving unfractionated heparin therapy with an incidence similar to that seen in adults [166]. The highest incidence of pediatric HIT was found in pediatric intensive care units supporting patients following cardiac surgery [167].

At least 70 cases of reported HIT were documented in pediatric patients [168], with the majority occurring during care following cardiac surgery. HIT in children was reported to occur in all age groups, but with a bimodal distribution. The higher incidences occur a) early in life, between 0–2, and b) during puberty, between 11–17 years of age [168, 169]. The balance between the risk of procoagulant and thromboembolic events on one hand and the risk of severe, sometimes fatal, bleeding on the other hand can be very challenging in ECMO patients with HIT. Pollak et al. reported a case of HIT with evidence of small vessel arterial thrombosis in a 5-day-old newborn receiving ECMO for congenital diaphragmatic hernia. It was assumed that the leading cause of death in this patient was massive disseminated intravascular coagulation. In this case, however, it is more likely that repeated platelet transfusions proved fatal and, retrospectively speaking, should have been avoided [170]. Although HIT is a recognizable and treatable complication, its relative infrequency increases the risk for delayed diagnosis leading to significant morbidity.

Diagnostic studies for HIT tend to be unreliable. Therefore, early intervention using alternative anticoagulants is a crucial step when HIT is suspected. This can hopefully lead to improved outcomes in these patients. Treatment of confirmed or suspected HIT in patients on ECMO includes removing unfractionated heparin, and possibly the entire ECMO circuit. Certain modern ECMO circuit components are heparin bonded in an effort to reduce immune reactivity to foreign surfaces [171–173]. If platelet recovery does not occur after withdrawal of heparin, it is possible that ongoing exposure to heparin bonding may be a factor [173]. Options for alternative anticoagulation if HIT is suspected include direct thrombin inhibitors (argatroban and bivalirudin) as well as short heparinoids (fondaparinux and danaparoid) [162, 174].

6.5 Conclusions

The predominant challenge for the clinician caring for a patient on ECMO is making an informed assessment of bleeding and clotting risks. The goal is to minimize bleeding and transfusion requirements while avoiding formation of micro or macro thrombi either in the circuit or within the patient's cardiovascular system [175]. Assessment of the patient's hemostasis includes consideration of the pathophysiology, type and severity of organ failure, and extent of tissue trauma during cannulation. A holistic approach to hemostatic management is needed to balance all these factors. ROTEM and whole blood platelet aggregometry provide rapid information on whole blood coagulation, and may be helpful in providing blood product support, factor replacement, anti-coagulation therapy and anti-fibrinolytics. Further research using ROTEM and whole blood platelet aggregometry in ECMO patients is needed to demonstrate efficacy in support of real-time hemostatic management in this cohort.

7. Platelet transfusions in neonates

Transfusion of blood products in neonates is not an uncommon practice in neonatal intensive care units. Extremely premature neonates (<28 week gestation) or extremely low birth weight (ELBW) infants (<1000 g) receive at least one packed red blood cell (pRBC) transfusion per hospital admission [176]. Platelet transfusions are also quite prevalent in ELBW infants, occurring in up to 90% of those weighing less than 750 g [177]. Unfortunately, while platelet transfusions for thrombocytopenia may be helpful, they are not without risk. Further, because the guidelines for transfusion thresholds in neonates are not based on a well-developed body of evidence, there is considerable variability in circumstances for such transfusions, and in particular clinical scenarios triggering them.

Thrombocytopenia is defined by a platelet count of less than 150,000/μL, and is further sub-classified as mild (100,000–149,000/μL), moderate (50,000–99,000/μL), or severe (<50,000/μL) [178]. The reference values for very low birth weight (VLBW) babies (<1500 g), however, remain controversial [179]. Thrombocytopenia affects 18–35% of all neonates in the neonatal intensive care unit [180, 181] and up to 73% of ELBW infants [182]. While clinical significance of platelet counts between 100,000 and 150,000/μL is debatable, it is well known that platelet counts below 20,000/μL are associated with increased risk of hemorrhage, at least in the adult population [183]. The relationship between thrombocytopenia and hemorrhage, however, remains one of the associations. It is not clear that neonatal thrombocytopenia directly causes hemorrhagic events [184].

Thrombocytopenia may be described by time of onset, where early onset (occurring in the first 72 h of life) is distinguished from late onset (occurring after the first 72 h of life) [179, 184]. In the premature population, the early onset thrombocytopenia is most often mild to moderate, develops gradually, and tends to be related to causes of chronic fetal hypoxia, as seen with intra-uterine growth restriction (IUGR), pregnancy-induced hypertension (PIH), hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome, or preeclampsia [185]. In term neonates, however, platelet destruction tends to be antibody-mediated [184]. In contrast to early onset, late onset thrombocytopenia tends to be more severe, more acute, and most frequently associated with infections (NEC, sepsis, and viral infections) [184].

7.1 Indications for platelet transfusion

Due to limited understanding of neonatal platelet functions, transfusion practice in the newborn is generally extrapolated from what is recognized as beneficial within the pediatric and adult populations. However, neonates are vulnerable to particular illnesses with varying underlying disease processes. Moreover, they tend to have developmental differences in regulation of primary hemostasis [178]. Nevertheless, platelet transfusions are typically given in two distinct clinical scenarios: (a) acutely, as a life-saving procedure and (b) prophylactically, under the presumption that they diminish the risk for hemorrhage. Surprisingly, an overwhelming majority of neonatal transfusions are done prophylactically, accounting for 98% of platelet transfusions [59]. Yet, it is not clear that this practice is beneficial. Prophylactic platelet transfusion in clinically stable neonates with no active bleeding remains controversial at best [186], consistent with the wide range of national and international clinical practices by neonatologists [177]. In this context, the severity of thrombocytopenia does not correlate with increased risk of intraventricular hemorrhage (IVH), and platelet transfusion for mild to moderate thrombocytopenia does not appear to prevent or reduce the incidence of intracranial hemorrhage [183, 187].

Although adequate quantities of platelets are necessary for hemostasis, increased risk of hemorrhage appears to be dependent on factors other than thrombocytopenia alone. Additional relevant platelet parameters include functional competency, immature platelet fraction, and developmental differences in neonatal thrombopoiesis [49, 188, 189]. Underlying clinical conditions associated with increased risk of hemorrhage include: preterm premature rupture of membranes, low birth weight, sepsis, shock, pulmonary hypertension, respiratory distress, NEC, and premature gestational age [182, 190–192]. Thus, recommendations to transfuse platelets should not be solely based on thrombocytopenia, but also on the presence of such other contributory factors [178, 190]. In spite of such considerations, it appears that management of thrombocytopenia in the newborn still lacks adequately rigorous scientific basis [179].

7.2 Clinical guidelines for platelet transfusion in neonates

In the United States, there are currently no national guidelines for neonatal platelet transfusion and only two published randomized controlled trials assessing prophylactic transfusions [183, 193]. Most countries recommend therapeutic transfusion in actively bleeding neonates when platelets fall below 50,000/μL. However, there is no agreement regarding prophylactic transfusions when platelets are anywhere between 20,000 and 90,000/μL [184, 194, 195]. A wide range of thrombocytopenia thresholds are employed, tending to be markedly higher in the United States, between 50,000 and 149,000/μL [187, 196, 197]. Nonetheless, such trends are based on clinical experience and judgment, rather than on reliable and consistent data.

7.3 Adverse outcomes of platelet transfusions

A growing number of adverse effects of platelet transfusions are being documented, including but not limited to increased risk of infection, transfusion-related injuries in various organs, alloimmunization, hemolytic reactions, febrile reactions, allergic reactions, anaphylaxis, and NEC [177, 185]. Randomized controlled trials comparing thresholds for platelet transfusion in thrombocytopenic neonates concluded that the frequency of IVH is not reduced by more aggressive thresholds [183]. Additionally, platelet transfusions, themselves, are implicated in increased mortality, linking the number of transfusions with death rate [198]. This was further supported by a recent large, multicenter, randomized clinical trial suggesting that significant hemorrhage and death could be prevented by lowering thrombocytopenia transfusion thresholds from 50,000 to 25,000/μL [193].

8. Conclusions

Platelets are best known for their role in hemostasis. But beyond forming a platelet plug, they are also important in several processes such as recognition and elimination of invading microorganisms, inflammation and interaction with leukocytes, wound healing and tissue repair, angiogenesis, and even tumor growth. These are emerging areas of investigation and there is little to no information on the roles of platelets in such processes in the newborn. Although current understanding suggests that newborn platelets may be somewhat different from adult platelets, they nonetheless protect the healthy newborn adequately. Certain perinatal factors were identified to affect platelet counts and function, but the platelet dysfunctions induced by them are acquired and transitory in nature. Premature neonates are likely at greatest risk for reduced platelet counts and functions, and by extension, at greatest risk of hemorrhage, particularly if prematurity is in combination with antenatal infections or postnatal respiratory disorders. There is, however, still a lot that is not known about platelets in the newborn. Such information is critical to improving the standard of care, intervention, and therapy.

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Conflict of interest

The authors declare that they have no conflict of interest.

Author details

Ijeoma Esiaba[1,2], Iman Mousselli[3], Giulia M. Faison[4], Danilyn M. Angeles[3] and Danilo S. Boskovic[1,5]*

1 Department of Earth and Biological Sciences, School of Medicine, Loma Linda University, Loma Linda, CA, United States

2 Department of Biochemistry, School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria

3 Division of Physiology, Department of Basic Sciences, School of Medicine, Loma Linda University, Loma Linda, CA, United States

4 Division of Neonatology, Department of Pediatrics, School of Medicine, Loma Linda University, Loma Linda, CA, United States

5 Division of Biochemistry, Department of Basic Sciences, School of Medicine, Loma Linda University, Loma Linda, CA, United States

*Address all correspondence to: dboskovic@llu.edu

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