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Thrombocytopenia in the NICU: new insights into causative mechanisms and treatments

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A B S T R A C T

Thrombocytopenia affects 20-35% of neonates admitted to the NICU. Over the last decade, our understanding of the mechanisms underlying severe neonatal thrombocytopenia increased due to the development of non-invasive measures of thrombopoiesis suitable for use in neonates, including serum thrombopoietin (Tpo) concentrations, circulating megakaryocyte progenitors, and reticulated platelet percentages. The use of these tests in combination led to the recognition of different mechanisms underlying neonatal thrombocytopenia. In contrast to the progress made in understanding the pathophysiology of neonatal thrombocytopenia, therapeutic advances have been few. For most thrombocytopenic neonates, platelet transfusions remain the only therapy. However, there is a lack of scientific evidence to guide neonatal transfusion decisions, and studies to generate evidence-based guidelines for neonatal transfusions are needed.

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The evaluation and management of thrombocytopenic neonates is a frequent challenge for neonatologists, since 22-35% of infants admitted to the neonatal intensive care unit (NICU) are affected by thrombocytopenia at some point during their hospital stay.[1,2] Despite this high prevalence, little is known about the mechanisms underlying most cases of neonatal thrombocytopenia (i.e. decreased platelet production, increased consumption, hypersplenism, or a combination of processes). Fortunately, in a large percentage of affected neonates the thrombocytopenia is mild and/or short lived, and no intervention is required. However, in approximately 25% of cases the thrombocytopenia is significant (platelet count $<50x10^9$/L), and intervention – usually with platelet transfusions – is considered.

Platelet production in neonates

The process of platelet production can be schematically represented as consisting of four main steps (Figure 1). The first is the thrombopoietic stimulus that drives the production of megakaryocytes and ultimately platelets. Although a number of cytokines (i.e. stem cell factor, IL-3, IL-6, IL-11, and GM-CSF) contribute to this process, thrombopoietin (Tpo) is the most potent known stimulator of platelet production.[3] Tpo mostly acts by promoting the proliferation of megakaryocyte progenitors (the cells that multiply and give rise to megakaryocytes), and the maturation of the megakaryocytes. This process is characterized by a progressive increase in nuclear ploidy and cytoplasmic maturity that leads to the generation of large polyploid (8N-64N) megakaryocytes. Through a poorly understood process, these mature megakaryocytes then generate and release new platelets into the circulation.

Although the process of platelet production follows the same steps in neonates and adults, there are important developmental differences that need to be taken into consideration when evaluating neonates with platelet disorders (Table 1). For example, plasma Tpo concentrations are higher in normal neonates than in healthy adults, but neonates with thrombocytopenia have in general lower Tpo concentrations than adults with similar degrees and mechanisms of thrombocytopenia.[4,5] Megakaryocyte progenitors of neonates have a higher proliferative potential than those of adults, giving rise to larger megakaryocyte colonies when cultured *in vitro,*[4,6,7] and are more sensitive to Tpo *in vitro* and *in vivo* than adult progenitors.[6,8] Neonatal megakaryocytes, in contrast, are smaller and of lower ploidy than adult megakaryocytes.[9-11] Since smaller megakaryocytes produce less platelets than larger megakaryocytes,[12] it has been postulated that neonates maintain their normal platelet counts on the basis of the increased proliferative rate of their progenitors.

Table 1. Differences between neonatal and adult thrombopoiesis.

	Adults	*Neonates*
Tpo concentrations	Very high in hyporegenerative thrombocytopenia	Not as high in thrombocytopenic neonates as in thrombocytopenic adults
Megakaryocyte progenitors	Sparse in the blood	Abundant in the blood
	Give rise to small colonies	Give rise to large colonies
	Less sensitive to Tpo	More sensitive to Tpo
Megakaryocytes	Large	Small
	High ploidy levels	Low ploidy levels
Effects of rTpo	Stimulates megakaryocyte proliferation	Stimulates megakaryocyte proliferation
	Stimulates megakaryocyte maturation	May inhibit megakaryocyte maturation

Tpo: thrombopoietin.

Mechanisms underlying neonatal thrombocytopenia

An important question that has remained unanswered has been whether (and how) these developmental differences impact the ability of neonates to respond to thrombocytopenia. Specifically, it is unknown if neonates can increase the number and/or size of their megakaryocytes, as adult patients with platelet consumptive disorders do.[13,14] Finding the answer to this question has been challenging, mostly due to the limited availability of bone marrow specimens from living neonates, the rarity of megakaryocytes in the marrow (<1% of nucleated cells), and the inability to accurately differentiate small megakaryocytes from cells of other lineages. Findings from a study in our laboratory evaluating megakaryocytes in bone marrow biopsies from neonates and adults with and without thrombocytopenia suggested that thrombocytopenic neonates sometimes increase their megakaryocyte number, but do not increase their megakaryocytes size.[15] It remains unclear, however, whether these findings represent a true developmental limitation of neonates compared to adults, or rather reflect differences in the disease processes underlying the thrombocytopenia in the different age groups.

Since bone marrow studies remain technically difficult in neonates, significant efforts have been aimed at developing blood tests to evaluate platelet production that would be suitable for use in neonates. Traditionally, platelet-associated IgG (PAIgG) was used as a test to indicate immune-mediated platelet destruction. However, the significance of PAIgG in neonates is unclear, since platelets from healthy neonates have higher levels of IgG than platelets from healthy adults,[16] and elevated PAIgG levels are also found in 15% of non-thrombocytopenic neonates.[1]

More recently, however, a new generation of indirect tests of thrombopoiesis suitable for use in neonates was developed, including plasma or serum Tpo concentrations,[4,5,17,18] circulating megakaryocyte progenitors,[19–21] and reticulated platelet percentages (RP%).[22–25] The use of these tests, particularly in combination, showed promise in defining the mechanisms underlying thrombocytopenia in neonates, and evidenced that neonatal thrombocytopenia – just like thrombocytopenia in adults – can be the result of a variety of mechanisms. Specifically, we recently described the case of a neonate with congenital HIV infection, who presented with elevated plasma Tpo concentrations and increased numbers of circulating megakaryocyte progenitors and marrow megakaryocytes, but with a decreased reticulated platelet percentage.[26] This combination suggested a dissociation between megakaryocyte mass (or platelet producing substrate) and actual platelet production, a pattern known as *ineffective platelet production,* which had been previously described in adult patients with HIV-related thrombocytopenia. The same panel of tests was applied to the evaluation of two neonates with congenital amegakaryocytic thrombocytopenia and proximal radio-ulnar synostosis, a syndrome characterized by synostosis of the radial and ulnar bones (which clinically manifests itself by decreased ability to rotate the forearm) and severe amegakaryocytic thrombocytopenia. These infants displayed high serum Tpo concentrations and very low to absent circulating megakaryocyte progenitors, marrow megakaryocytes, and reticulated platelet percentages, a pattern consistent with amegakaryocytic thrombocytopenia.[27]

Although the use of these tests in combination undoubtedly offers the most complete information, some of them have also been used independently to assess the mechanisms underlying different varieties of thrombocytopenia. Murray *et al.* quantified circulating megakaryocyte progenitors in preterm neonates with early thrombocytopenia secondary to maternal pre-eclampsia or intrauterine growth restriction. These investigators found that the concentration of circulat-

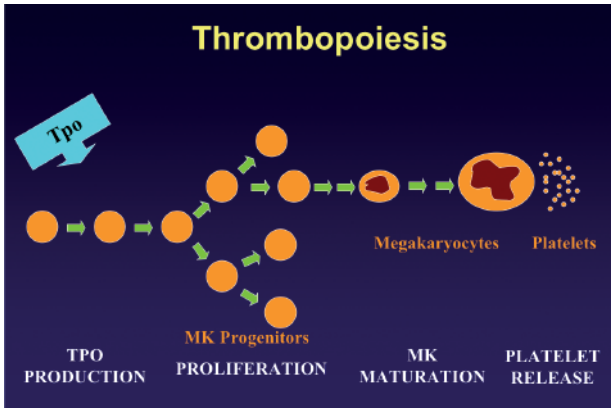

Figure 1. Schematic representation of the process of thrombopoiesis. ***Adapted from Sola MC. Fetal megakaryocytopoiesis. In: Christensen RD, ed. Hematologic problems of the neonate. Philadelphia: W.B. Saunders, 2000 p. 43-59, with permission. Tpo, thrombopoietin; Mk, megakaryocyte.***

ing progenitors in those neonates was significantly lower than in non-thrombocytopenic preterm neonates, and increased as the thrombocytopenia resolved.[21] Based on these observations they concluded that decreased platelet production underlies this variety of thrombocytopenia.

Serum Tpo concentrations have also been used by many investigators as a marker of thrombopoiesis.[21,28,29] However, the interpretation of serum Tpo concentrations in thrombocytopenic neonates is difficult in the absence of other measures of thrombopoiesis, because Tpo levels are a reflection of both the level of Tpo production and the availability of Tpo receptors (located on progenitor cells, megakaryocytes, and platelets). Thus, elevated Tpo concentrations during thrombocytopenia can be caused by up-regulated gene expression (i.e. during infections),[30] or by a decrease in the megakaryocyte mass (such as in aplastic anemia or amegakaryocytic thrombocytopenia).[31] Therefore, the mechanistic significance of Tpo concentrations can only be established by the concomitant use of other measures of thrombopoiesis.

Although Tpo concentrations and circulating megakaryocyte progenitors have proven useful in the mechanistic evaluation of thrombocytopenic neonates, practical issues make them unlikely to ever become routine clinical tests. A test that could potentially become available to clinicians, however, is the reticulated platelet percentage (RP%). RPs are newly released platelets (<24 hours old), which contain residual RNA[32] and can therefore be detected and quantified in the blood. In adults and children, the reticulated platelet percentage (RP%) has been evaluated as a way of classifying thrombocytopenia kinetically, similar to the way the reticulocyte percentage is used to evaluate anemia. Unfortunately, however, the methodology to measure RP% has not been standardized, and this has accounted for great variability in the values reported for healthy adults and children. Several studies have measured RP% in non-thrombocytopenic term and preterm neonates, and (similar to adult studies) the methods used and RP% values varied significantly.[22-25]

Therapeutic advances in neonatal thrombocytopenia

Thrombopoietic factors

Recombinant IL-11 (rhIL-11) is the only thrombopoietic growth factor approved in the USA for the prevention of severe chemotherapy-induced thrombocytopenia. Reports of experimental benefits for NEC and sepsis in animal models have made the potential use of this cytokine in neonates appealing.[33,34] However, significant side effects (such as fluid retention and atrial arrythmias), coupled with reports of lack of efficacy in certain varieties of thrombocytopenia (i.e. refractory ITP),[35] have raised questions about its safety and potential efficacy in neonates. These issues have never been investigated in NICU patients, and its use in the neonatal period should therefore be restricted to well-controlled clinical trials.

The cloning of Tpo in 1996 led to a flurry of studies that quickly progressed from bench research to clinical trials. However, subjects treated with a truncated form of recombinant Tpo (PEG-rHuMGDG) developed neutralizing antibodies against endogenous Tpo, which resulted in severe thrombocytopenia and aplastic anemia.[36] Ultimately, these complications led to the discontinuation of clinical trials involving Tpo. As an alternative, much interest has recently been devoted to the development of thrombopoietin-mimetic molecules. These are small molecules that have no sequence homology to Tpo, but bind to the Tpo receptor and have biologically comparable effects. Among the significant number of thrombopoietin receptor agonists that have been described, AMG-531 (Amgen, Inc.)[37] has shown the most promising results in early clinical trials involving adult patients with refractory ITP.

No *in vitro* or *in vivo* studies evaluating the potential use of these compounds in neonates have been reported. However, a number of in vitro studies have demonstrated that there are important quantitative and qualitative differences between neonates and adults in the response to Tpo, which will have to be taken into consideration if and when Tpo-mimetics become a therapeutic alternative for neonatal thrombocytopenia. Specifically, we previously reported that megakaryocyte progenitors obtained from the bone marrow of thrombocytopenic and non-thrombocytopenic neonates were more sensitive to Tpo than pro-

genitors obtained from adults.[6] More recently, we observed that neonatal and adult megakaryocytes have significant biological differences in their response to this cytokine: while Tpo is a potent stimulator of maturation on adult megakaryocytes, it actually inhibits this process on neonatal megakaryocytes.[38] These different responses might underlie the observation that neonatal megakaryocytes do not seem to increase their size in response to thrombocytopenia.

Platelet transfusions

In approximately 75% of all neonates with thrombocytopenia, the thrombocytopenia is transient and/or mild, and does not prompt intervention. However, in 20-25% of the cases (2-9% of NICU admissions), one or more platelet transfusions are ordered in an attempt to treat or decrease the risk of hemorrhage. In the case of neonates with active hemorrhage, most experts agree that platelet transfusions should be administered for platelet counts <100x10^9/L. However, the great majority of transfusions are administered to non-bleeding neonates, and there is significant variability in neonatal transfusion practices among institutions and among individual neonatologists.

Several recent single-center studies have evidenced the variability that exists worldwide in the use of platelet transfusions to treat thrombocytopenic neonates.[39-41] In these studies, platelet transfusions were administered to 2% (Mexico), 3% (United Kingdom), and 9% (USA) of all neonates admitted to the NICU. In addition, a recent study (limited to VLBW in the first week of life) reported a 10-fold difference in platelet transfusion usage among 10 NICUs in the USA, which could not be accounted for by severity of illness or incidence of thrombocytopenia.[42]

While multiple factors likely contribute to this variability, to a large extent it reflects the lack of solid evidence to guide neonatal platelet transfusion decisions. The only controlled randomized trial on this subject was limited to very-low birth weight infants in the first week of life, and excluded those with platelet counts <50x10^9/L. In that study, Andrew *et al.* [43] randomized 152 thrombocytopenic premature infants to either a treatment group (which received platelet transfusions for any platelet count <150x10^9/L), or to a control group (platelet transfusions only for clinical indications or for a platelet count <50x10^9/L). These investigators found no significant differences in frequency or severity of intracranial hemorrhages between the two groups, and concluded that non-bleeding premature infants with platelet counts >50 x10^9/L should not receive prophylactic platelet transfusions. Since all neonates with platelet counts <50 x10^9/L were transfused, however, this study did not address whether lower platelet counts could be safely tolerated. To answer this question, Murray *et al.*[41] performed a retrospective review of platelet transfusions among neonates with platelet counts <50x10^9/L admitted to their NICU (n=53 over 3 years). Overall, they transfused 51% of these neonates: those with a platelet count <30x10^9/L, and those with a platelet count between 30 and 50x10^9/L who had a previous intracranial hemorrhage or were clinically unstable. They did not observe any major hemorrhage in this group, regardless of whether platelet transfusions were given or withheld. Thus, they concluded that a prophylactic platelet transfusion trigger of <30x10^9/L probably represents a safe practice for clinically stable NICU patients.

In the absence of any other studies to provide evidence-based practice parameters, numerous experts and consensus groups have published guidelines for the administration of platelet transfusions to neonates. Two tendencies are evident from these recommendations: 1) Over the last decade there has been a trend toward accepting lower platelet counts in neonates, particularly if they are clinically stable and not bleeding; and 2) this tendency has been particularly evident in the United Kingdom, as demonstrated by the most recent recommendations from the British Committee for Standards in Haematology Transfusion Task Force,[44] which are more restrictive than the Guidelines from the Pediatric Hemotherapy Committee of the American Association of Blood Banks.[45]

In summary, platelet transfusions constitute the only therapy currently available for most thrombocytopenic neonates. However, there is a striking lack of scientific evidence to guide the use of this valuable resource, and particularly to decide at what platelet count the risk of bleeding increases to a degree that a platelet transfusion is justified. Since other therapeutic alternatives are not likely to be available for routine clinical use in the near future, there is a pressing need to conduct well designed randomized controlled trials aimed at generating the scientific evidence needed to guide platelet transfusion practices in neonates.

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