Neonatal Hemostasis Disorders: Bleeding and Thrombosis

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Abstract: Neonatal bleeding or thrombosis are serious problems and life threatening events. The diagnostic approach needs to consider the theory of the "developmental hemostasis" for interpreting blood tests. The causes, diagnosis and management of the main neonatal hemostatic abnormalities will be reviewed.

Keywords: Bleeding, Fresh frozen plasma, Hemostasis, Heparin, Newborn, Thromboelastography, Thrombosis.

INTRODUCTION

Hemostasis disorders are relatively rare in term and healthy newborns. Conversely, bleeding or thrombosis are frequent events in the critically ill or premature infant. In these cases, the study of hemostasis and the interpretation of laboratory tests are essential steps for a proper diagnosis and adequate treatment.

This review article aims to:

1. Describe the developmental physiology of the hemostatic system and emphasize the need of using appropriate age-specific reference ranges

2. Discuss the role of standard laboratory tests investigating neonatal hemostasis and introduce the most recent studies on thromboelastography (TEG)

3. Attempt to provide clinical guidance for diagnosis and treatment of the main hemostasis disorders in the newborn by grading the quality of evidence and strength of recommendations. The criteria for assigning the grade are summarized in Figure **1** [1].

THE DEVELOPMENT OF THE HEMOSTATIC SYSTEM

The term "developmental hemostasis" was coined by Maureen Andrews to describe the age-related physiological changes of the hemostatic system occurring during childhood [2].

In fact, the hemostatic system is subject to an age-dependent dynamic evolution process. At birth, plasma concentrations of vitamin K-dependent coagulation factors and contact factors are reduced compared to those in adults [3]. During the first 6 months of life, the concentration of these coagulation factors gradually increases until it reaches values close to the adult ones. The change of these coagulation proteins results in a corresponding change of the coagulation tests, such as prothrombin time (PT) and activated partial thromboplastin time (APTT). Otherwise, the plasma concentrations of fibrinogen, FV, FVIII, FXIII and von Willebrand factor are not reduced at birth and their levels are similar to adult values. As for the fibrinolytic system, the plasma concentration of plasminogen is reduced at birth, while the tissue plasminogen activator and the plasminogen activator inhibitor are increased. In the natural inhibitor system, at birth the plasma concentration of antithrombin is reduced and reaches adult levels at about 6 months of life. The plasma concentration of protein C is also very low at birth, and remains low during the first 6-12 months of life. The total amount of protein S is lower than the adult one; however, the overall activity is similar because protein S is present only in its free active form. This variation of pro-coagulant and anti-coagulant proteins, which is observed in both full-term and preterm infants, defines a dynamic equilibrium of the hemostatic system that, in normal conditions, functions regularly.

REFERENCE RANGES OF COAGULATION TESTS IN THE NEWBORN

Considering changes of coagulation proteins during infancy, age-specific reference ranges of laboratory coagulation tests are needed to make a proper diagnosis of coagulopathy in the newborn [4-6]. Furthermore, it is important to remember that standard tests of coagulation such as PT and APTT may also vary according to the type of method and reagents used [7].

In the newborn, the diagnosis of coagulopathy is defined by PT and APTT values above the upper limit or by fibrinogen values below the lower limit compared to the specific reference ranges for gestational age and postnatal age (Figure **2**).

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Figure 1: Criteria for assessing grade of evidence and strength of recommendations.

Quality of Evidence	Type of Clinical Study	Consistency of Results
A= High	Randomized trial without important limitations	Considerable confidence in the estimate of effect
B= Moderate	Randomized trial with important limitations or exceptionally observational studies with strong evidence	Further research likely to have impact on the confidence in estimate, may change estimate
C= Low	Observational studies or case series	Further research is very likely to have impact on confidence, likely to change the estimate

Strength of Recommendations	Balance between Benefits and Harms
1= Strong	Certainty of imbalance
2= Weak	Uncertainty of imbalance

The grading scheme classifies the quality of evidence as high (grade A), moderate (grade B), or low (grade C) according to the study design and the consistency of results. The strength of recommendations was further classified as either strong (1) or weak (2) according to the balance between desirable and undesirable outcomes [1].

Figure 2: PT, APTT and fibrinogen values in healthy premature and term neonates, at birth (**A**) and during the first 3 months of life (**B**).

(**A**)

Gestational Age at Birth (week)	PT, Upper Limit* (sec)	APTT, Upper Limit* (sec)	Fibrinogen, Lower Limit* (mg/dL)
< 28 [4]	>21	>64 sec.	<71
28-34 [4]	>21	>57 sec.	<87
30-36 [5]	>16	>79 sec.	<150
≥ 37 [6]	>16	>55 sec.	<167

(**B**)

Gestational Age at Birth (week)	PT, Upper Limit* (sec)	APTT, Upper Limit* (sec)	Fibrinogen, Lower Limit* (mg/dL)
30-36 [5]			
and post-natal age of			
5 days	>15	>74	<160
30 days	>14	>62	<150
90 days	>15	>51	<150
≥ 37 [6]			
and post-natal age of			
5 days	>15	>60	<162
30 days	>14	>55	<162
90 days	>14	>50	<150

*The upper limit of PT and APTT, and the lower limit of fibrinogen are defined for values outside the 95% confidence limits of the age-related reference ranges; sec: second.

For an accurate diagnosis of neonatal thrombosis, it is necessary to check the values of the main natural anticoagulants (natural inhibitor system): protein C, protein S and antithrombin [8]. Also in this case the interpretation of the results must be made using age-specific reference values (Grade 2C) (Figure **3**).

DIAGNOSTIC APPROACH TO THE BLEEDING NEWBORN

The diagnostic setting of the newborn with bleeding due to possible coagulopathy requires some clinical considerations. The most important of these is probably the clinical context in which the bleeding occurs.

Figure 3: Reference values for inhibitors of coagulation in healthy premature and term neonates, at birth (**A**) and during the first three months of life (**B**).

(**A**)

Gestational Age at Birth (week)	Protein C, mg/dL mean (CI 95%)	Free Protein S, mg/dL mean (CI 95%)	Antithrombin, mg/dL mean (CI 95%)
24-29* [8]	10.4 (8–13)	27.9 (19–40)	30.0 (20–39)
30-36 [5]	28 (12-44)	26 (14-38)	38 (14-62)
≥ 37 [6]	35 (17–53)	36 (12–60)	63 (39–87)

(**B**)

Gestational Age at Birth (week)	Protein C, mg/dL mean (CI 95%)	Free Protein S, mg/dL mean (CI 95%)	Antithrombin, mg/dL mean (CI 95%)
30-36 [5]			
and post-natal age of			
5 days	31 (11–51)	37 (13–61)	56 (30–82)
30 days	37 (15–59)	56 (22–90)	59 (37–81)
90 days	45 (23–67)	76 (40–112)	83 (45–121)
≥ 37 [6]			
and post-natal age of			
5 days	42 (20–64)	50 (22–78)	67 (41–93)
30 days	43 (21–65)	63 (33–93)	78 (48–108)
90 days	54 (28–80)	86 (54–118)	97 (73–121)

CI: confidence interval: 95% confidence limits for age-specific reference values; * Values obtained from fetal blood.

Bleeding in an otherwise healthy infant suggests inherited coagulopathy or immune-mediated thrombocytopenia. In contrast, bleeding in a critically premature infant is more likely to be caused by consumption-acquired coagulopathy such as disseminated intravascular coagulation (DIC). A positive family history of bleeding disorders or the presence of a previously affected child can be an important diagnostic sign. Complications at the time of delivery can involve the hemostatic system and lead to activation of coagulation and consequent DIC. Finally, some drugs taken by the mother during pregnancy, which interact with the metabolism of vitamin K, can cause significant coagulopathy in the newborn.

Initial screening tests usually include a complete blood count and standard coagulation tests: PT, APTT, and Fibrinogen. The results of these tests can lead to a specific diagnosis of coagulopathy to be confirmed by testing the single coagulation factors (Grade 1C) (Figure **4**) [9].

DIAGNOSTIC APPROACH TO THE NEWBORN WITH THROMBOSIS

The diagnosis of thrombosis in the newborn is based both on the evaluation of clinical setting and on confirmation through instrumental investigations. The clinical symptoms of thrombosis are highly variable and depend mainly on the side and extension of the thrombus. Today Doppler ultrasound and angio-magnetic resonance, with and without contrast medium, are the most appropriate instrumental investigations for the diagnostic confirmation of vascular thrombosis.

Thrombocytopenia is the most sensitive indicator of thrombosis of micro- and macro-circulation. Therefore, persistent reduced levels of platelets and/or fibrinogen must always lead to suspect a thrombosis event. In case of suspected thrombosis, the laboratory tests to be performed for the initial evaluation of hemostasis are Complete blood count, PT, APTT and fibrinogen. In the newborn, the increase level of D-Dimer has a poor predictive value; therefore, its dosage is not recommended (Grade 2C).

Once the diagnosis of thrombosis has been ascertained, it may be useful to investigate any conditions of hereditary or acquired thrombophilia according to the following recommendations:

1) A hereditary thrombophilia should always be sought in the event of spontaneous neonatal

Figure 4: Laboratory investigation of neonatal coagulation disorders [9].

Condition	PT	APTT	Fibrinogen	Platelets
Inherited disorders				
Hemophilia A	N	↑	N	N
Hemophilia B	N	↑	N	N
vWD (type III)	N	↑	N	N/↓
FVII	↑	N	N	N
FX	↑	↑	N	N
Fibrinogen	N/↑	N/↑	↓	N
FXIII	N	N	N	N
Acquired disorders				
DIC	↑	↑	↓	↓
Vitamin K deficiency	↑	N/↑	N	N
Liver disease	↑	↑	N/↓	N/↓

N: normal; F: Fattor; vWD: von Willebrand disease.

thrombosis, ischemic skin lesions or a clinical picture of purpura fulminans (Grade 1C);

2) Since the detection of hereditary thrombophilia in the newborn with thrombosis does not affect the treatment except in cases of protein C, protein S or antithrombin deficiencies, these are the only investigations recommended as urgent, together with the search for maternal antiphospholipid antibodies (Grade 1C);

3) Other hereditary thrombophilia investigations can be performed after 3-6 months of life (Grade 2C) [10].

HEMOSTASIS ASSESSMENT BY THROMBOELASTOGRAPHY (TEG)

Due to the complexity of the coagulation process, standard laboratory tests are unable to globally measure all the individual elements involved in the hemostatic function. Consequently, first line coagulation tests must be interpreted with caution. As occurring in adults, also in newborns one of the main limitations of the standard coagulation tests is their poor predictive power for the risk of bleeding [4, 11]. TEG is a laboratory instrument that analyzes the viscoelastic properties of the clot by exploring the entire hemostatic process, from the initial formation of the clot to the polymerization of fibrin. This analysis provides comprehensive information on the interaction between plasma coagulation proteins, platelets and blood cells during clot formation, and more closely reflects what occurs *in vivo* than standard tests do. Experience on the use of TEG in neonates is still limited to a few studies; however, its use seems promising in the diagnosis and treatment of acquired coagulopathies [12]. In a recent retrospective study, Motta *et al* evaluated TEG parameters in healthy preterm neonates and organized the obtained data into reference intervals. These results showed similar TEG parameters between early-preterm (< 32 weeks' gestation) and moderate-/late preterm (32 to < 37 weeks' gestation) neonates, suggesting a normal hemostatic function regardless of gestational age at birth [13]. Also in Washington, Sewell *et al* established normative ranges for citrated-modified and heparinase-modified TEG parameters in term neonates [14]. In an observational study, Ghirardello *et al* performed TEG duplicate measurements for blood samples taken from very low birth weight neonates and found an acceptable level of agreement between duplicates, confirming the reliability of TEG to assess hemostasis in the neonatal population [15].

USE OF FRESH FROZEN PLASMA (FFP), CRYOPRECIPITATE AND PLATELET TRANSFUSION IN NEONATAL BLEEDING

Today, due to the availability of recombinant or highly purified virus-inactivated plasma- derived concentrates the use of FFP and cryoprecipitate is no longer considered the first-choice treatment for inherited coagulopathies. However, FFP may be used when the treatment is urgently required, and the diagnosis of inherited clotting factor deficiency needs to be confirmed (Grade 1C). Moreover, FFP use is recommended for the treatment of single inherited clotting factor deficiency without safer replacement product available (Grade 1C). Currently this applies only to factor V [16]. Cryoprecipitate transfusion is used

for the treatment of factor XIII deficiency and for the treatment of hemorrhages due to congenital fibrinogen deficiency or dysfibrinogenemia (Grade 1C) [17].

DIC is an ordinary problem in sick neonates and is usually associated to sepsis, respiratory distress syndrome, acidosis, necrotizing enterocolitis, birth asphyxia. The treatment of the underlying condition along with the restoration of hemostasis with transfusions of platelets, FFP and/or cryoprecipitate needs to be prompt and effective. The use of FFP, at a dose of 10-20 mL/kg, is recommended for an active bleeding associated with coagulopathy defined on the basis of age-specific reference values (Grade 1C) (Figure **2**) [18]. If the bleeding is secondary to vitamin K deficiency, it is necessary to administer both intravenous vitamin K and FFP transfusion (Grade 1C). In absence of bleeding, prophylactic use of FFP is only indicated prior to an invasive surgical procedure in presence of coagulopathy (Grade 2C). The use of cryoprecipitate, at a dose of 5-10 mL/kg, is indicated in case of active bleeding during DIC if the fibrinogen value falls below 100 mL/kg (Grade 2C) [4].

An Italian study, involving 17 NICUs, found that FFP administration was a quite frequent intervention, with 8% of admitted neonates receiving one or more FFP transfusions. This study reported a high proportion of FFP transfusions non-compliant with current guidelines, and FFP was administered prophylactically to 63% of transfused neonates, without any evidence of bleeding [18].

Clinical studies do not support prophylactic FFP administration for preventing intracranial hemorrhage in preterm infants (Grade 1A), for treating hypovolemic hypotension (Grade 1B), for improving the immune function during sepsis (Grade 1C), or for preventing hemorrhagic/thrombotic complications in neonates with hypoxic-ischemic syndrome treated with systemic hypothermia (Grade 2C).

If there is an active bleeding platelet transfusion (10-15 ml／kg) may be necessary to maintain a platelet count of > 50 x 10^9/L (Grade 1C). [19] Platelet transfusions are also commonly administered to preterm neonates with a low platelet count to reduce the risk of bleeding [20]. Nowadays, this practice has been changing. The PlaNet-2 trial assessed different platelet count thresholds in preterm neonates with very low platelet counts (< 50 x 10^9/L). It demonstrated that giving platelets transfusions at high-threshold (< 50 x 10^9／L) versus low-threshold (< 25 x 10^9/L) to prevent bleeding increases the risk of death or major bleeding (Grade 1A) [21].

TREATMENT OF THE NEONATAL THROMBOSIS

Treatment of thrombosis during the neonatal period remains controversial mainly due to the lack of adequate clinical trials. Furthermore, the use of antithrombotic drugs must also be considered in relation to the peculiarities of the newborn's hemostatic system, the different metabolism of anticoagulant drugs and the limited knowledge of the bleeding risks associated with the treatment. Supportive therapy, such as hydration and correction of any acidosis, is indicated in all cases of thrombosis (Grade 1C). It is necessary to correct a concomitant coagulopathy or thrombocytopenia with values platelets below 50 x 10^9/L particularly in critically sick infants or in infants affected by cerebral thrombosis, especially if

Figure 5: Recommended doses for anticoagulant/fibrinolytic therapy in neonates [24].

UFH (continuous i.v.)	**Loading dose (U/Kg)**	**Maintenance dose (U/Kg)**
GA < 28 weeks	50	15 U/Kg/h
GA 28-36 weeks	50	20 U/Kg/h
GA >37 weeks	100	25 U/Kg/h
LMWH (s.c.) Enoxaparin	**Loading dose (U/Kg)**	**Maintenance dose**
GA < 28 weeks	NA	1 mg/Kg 12 hourly
GA 28-36 weeks	NA	1.5 mg/Kg 12 hourly
GA > 37 weeks	NA	2 mg/kg 12 hourly
rt-PA (continuous i.v.)	**Loading dose (U/Kg)**	**Maintenance dose**
	NA	0.1-0.3 mg /Kg/h

UFH: unfractioned heparin; LMWH: low molecular weight heparin; rt-PA: recombinant tissue plasminogen activator; GA: gestational age, i.v.: intravenously; s.c.: subcutaneously; NA: not applicable.

associated to a hemorrhagic component (Grade 1C) [11].

Unfractionated heparin (UFH) and low molecular weight heparin (LMWH) are the first-choice drugs for anticoagulant therapy in the neonatal period (Figure **5**). [22] In neonates, the optimal length of anticoagulant therapy is not yet well defined, and a short treatment period, 15 to 30 days, associated to monitoring of thrombus using ultrasound imaging is generally recommended (Grade 2C). In relation to the low risk of recurrence and the physiological differences of the neonatal hemostatic system compared to the adult one, secondary long-term prophylaxis is usually not necessary, except in case of cardio-embolic etiology with persistence of heart disease or in case of persistence of the triggering cause (*e.g.* catheter in situ), until its removal (Grade 2C). Thrombolytic therapy should be considered for extensive thrombosis with organ dysfunction or with risk of loss of a limb (Grade 1C). The tissue plasminogen activator, produced by recombinant technique (rt-PA), due to its short half-life (about 5 minutes) is currently the drug of choice for thrombolytic treatment in the newborn (Grade 2 C) (Figure **5**) [23-24].

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