PART 13 Coagulation Parameters in Pediatric Populations

Pierre Toulon

TOPICS COVERED

- ✓ Developmental Hemostasis
- ✓ Coagulation Parameters in Neonates and Children versus Adults

The pediatric hemostatic balance, which is different from that in adults, is an evolving process as the hemostatic system changes and matures from fetal to adult life, particularly during the early months of life. Understanding the concept of developmental hemostasis, which is now universally accepted, is critical to ensure optimal diagnosis and treatment of hemorrhagic and thrombotic diseases in children.

Developmental Hemostasis: Hemostasis is a complex mechanism involving both procoagulant and anticoagulant factors. It ultimately enables the blood to remain liquid when circulating in intact vessels. It also avoids both excessive bleeding by promoting clot formation after endothelial injury and excessive clotting by limiting clot formation to the site of injury. The hemostatic equilibrium mainly depends on many parameters including platelets as well as clotting factors and inhibitors, even though endothelial and blood cells play a significant role. Children are not just miniature adults, at least for hemostasis, as the pediatric hemostatic balance is different from that in adults. Moreover, it is an evolving process, as shown by Andrew M. et al (1987), more than 30 years ago both in pre-term and in full-term infants. These authors demonstrated that the hemostatic system changes and matures throughout the time from fetal life to adulthood, mainly during the early months of life, and promoted the concept of developmental hemostasis. Coagulation factors from maternal origin are unable to cross the placental barrier because of their size. The synthesis of clotting factors by the fetus starts early (e.g. during the fifth week of gestation for fibrinogen), and blood becomes clottable after eleven weeks of gestation. Fetal reference ranges for coagulation parameters were studied in different gestational age groups and the median plasma levels were between 10% and 30% of adult values, depending on the evaluated parameter, in fetuses aged 19 to 23 weeks, progressively increasing to levels between 10% and 50% between 30 and 38 weeks of gestation. The initial findings of Andrew M, et al (1987) were confirmed by several studies evaluating different pediatric populations in various technical conditions (i.e. reagents/analyzers combinations). The selection criteria of the subjects were relatively homogeneous among the studies, however, some had slightly different inclusion/exclusion criteria and age grouping. The main difference between studies was the number of evaluated subjects in each age group, which ranged from 10 to more than 500 individuals. The sampling process, which is a key point to take into account, as drawing blood from young infants or neonates could be more problematic than in adults, was comparable in the different studies, with blood collected by venipuncture into tubes containing 3.2% citrate (1 vol./9 vol.) through 18- to 24-gauge needles, depending on the age of the patients. Most of these studies focused mainly on activity assays for most parameters involved in the coagulation system, whereas one study evaluated antigen concentrations of various analytes. All these studies showed that, at birth, the plasma levels of most coagulation proteins were around half of those measured in adults, except for FVIII:C and VWF, which are elevated, the pre-term infants having lower levels than full-term infants. Adult values were reached between a few months of age and up to more than 16 years for specific parameters such as coagulation FVII or protein C, as shown in Table 39. Whereas the global trend is consistent across the studies, differences in absolute values are likely due to differences in the reagents and/or the instruments used to measure these parameters, particularly global coagulation tests such as PT or APTT. Accordingly,

it is recommended by the Subcommittee of the Scientific and Standardization Committee of the ISTH that each laboratory define its age-dependent reference ranges by using its own technical condition. To comply with the Clinical and Laboratory Standards Institute (CLSI) C28A3 guideline, reference ranges must be established by testing at least 30 different individuals, in each age group. Obtaining enough plasma to perform numerous tests from a high number of "apparently" healthy children, raises logistical issues that would be far beyond the capabilities of many laboratories. To circumvent that difficulty, it is common practice to refer to data from the literature, taking into account identical technical conditions**,** even though the pre-analytical process, and particularly blood collection, could be different from that used in a given institution. The technical conditions (i.e. combination of reagents and instruments) used in the main publications are reported in Table 40. Primary hemostasis was less studied. However, the platelet count is usually normal or elevated at birth, reaching adult values within 1 year after transient increases. Despite hyporeactive platelets, particularly in the neonatal period, the bleeding time and the platelet closure time (PFA-100®) were found to be shortened in newborns, suggesting an increased hemostatic potential. Normalization occurred before the end of the first month of life. Significantly higher levels of VWF were reported in newborns, which then decreased reaching adult values after 1 year of life, at a time when appears the significant increase in plasma levels in non-O blood groups versus O blood groups.

Conclusions

Understanding of the concept of developmental hemostasis, which is now universally accepted, is critical to ensure optimal prevention, diagnosis, and treatment of hemorrhagic and thrombotic diseases in children. Therefore, it is mandatory for the laboratory to use age-specific reference ranges for coagulation parameters. It seems impossible to ask every laboratory to establish its own references intervals for every coagulation parameter in its own technical conditions by testing at least 120 healthy individuals in each age-group, as it is recommended by the CLSI Guideline EP 28-A3C. Therefore, the best option for a laboratory would be to translate the findings of the literature to local reference ranges for neonates and children, by taking into account their specific technical environment. In that respect, data are already available for combinations of reagents and analyzers from current manufacturers. In the case of newcomers, specific, and preferably multicenter, studies would have to be carried out in order to establish the specific pediatric reference ranges using these new reagents/analyzers combinations.

Table 39. Coagulation parameters in neonates and children versus adults: Summary of test results and potential effect on hemostasis (adapted from Toulon et al, 2016)

Component	Parameter	Neonatal period (mean value)*	Normalization	Impact on hemostasis
Primary hemostasis	Platelets VWF Platelet closure time (PFA-100®)	Normal or increased Increased (153%)* Shortened	1 Y (after transient increases) 3 Mo 2–4 W	Enhanced primary hemostasis
Coagulation	FII, FVII, FIX, FX, FXI, FXII, PK, HMWK FV FVIII Fibrinogen PT aPTT	Decreased (40–66%)* Decreased (37–54%)* Normal or decreased (70%)* Normal or increased (100%)* Normal** Prolonged or normal Prolonged	1 Y (up to 16 Y for FVII) 1 Y 1 Y (up to 16 Y) 1 Mo 1 Y 1 Y 1 Y (up to 16 Y)	Decreased coagulation potential
Natural coagulation inhibitors	Antithrombin Protein C Protein S	Decreased (63%) Decreased (35%) Decreased (36%)*	3 Mo 16 Y 3 Mo	Decreased regulatory/ inhibitory potential
Fibrinolysis	Plasminogen alpha 2 antiplasmin tPA D-dimer	Decreased (36%)* Normal or decreased (85%)* Increased Increased	6 Mo 6 Mo 1 W 16 Y	Increased fibrinolytic activity

*In percentage (%) of adult values, from Andrew M, et al. (1987); **Fetal fibrinogen may be present; Mo: month; W: week; Y: year

Table 40. Technical conditions (instrument and reagents brands) used in the main studies reporting usual values of coagulation parameters in pediatric populations (*indicates instrument and/or reagents no longer commercially available)

Authors	Instruments	Reagents
Andrew et al (1987, 1988)	ACL (Werfen)*	Various*
Flanders et al (2005, 2006)	STA-R (Stago) BCS (Siemens)	Mainly Stago
Monagle et al (2006, 2011)	STA Compact (Stago)	Stago
Apple et al (2012)	BCS (Siemens) CA-1500 (Sysmex)	Siemens
Attard et al (2013)	Microplate reader	Stago
Toulon et al (2016)	ACL TOP 500/700 (Werfen)	Siemens

References

Andrew M. Developmental hemostasis: Relevance to hemostatic problems during childhood. *Semin Thromb Hemost* 1995; 21(4): 341-356.

Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, Powers P. Development of the human coagulation system in the full-term infant. *Blood* 1987; 70(1): 165-172.

Andrew M, Paes B, Milner R, Johnston M, Mitchell L, Tollefsen DM, Castle V, Powers P. Development of the human coagulation system in the healthy premature infant. *Blood* 1988; 72(5): 1651-1657.

Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. *Blood* 1992; 80(8): 1998-2005.

Appel IM, Grimminck B, Geerts J, Stigter R, Cnossen MH, Beishuizen A. Age dependency of coagulation parameters during childhood and puberty. *J Thromb Haemost* 2012; 10(11): 2254-2263.

Attard C, van der Straaten T, Karlaftis V, Monagle P, Ignjatovic V. Developmental hemostasis: Age-specific differences in the levels of hemostatic proteins. *J Thromb Haemost* 2013; 11(10): 1850-1854.

Flanders MM, Crist RA, Roberts WL, Rodgers GM. Pediatric reference intervals for seven common coagulation assays. *Clin Chem* 2005; 51(9): 1738-1742.

Flanders MM, Phansalkar AR, Crist RA, Roberts WL, Rodgers GM. Pediatric reference intervals for uncommon bleeding and thrombotic disorders. *J Pediatr* 2006; 149(2): 275-277.

Horowitz GL, Altaie S, Boyd JC, Ceriotti F, Garg U, Horn P, Pasce A, Sine HE, Zakowski J. CLSI Document EP28-A3C. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition. Clinical and Laboratory Standards Institute, Wayne, PA, USA. 2010; Vol.28, n°30.

Ignjatovic V, Kenet G, Monagle P; Perinatal and Paediatric Haemostasis Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Developmental hemostasis: Recommendations for laboratories reporting pediatric samples. *J Thromb Haemost* 2012; 10(2): 298-300.

Lippi G, Franchini M, Montagnana M, Guidi GC. Coagulation testing in pediatric patients: The young are not just miniature adults. *Semin Thromb Hemost* 2007; 33(8): 816-820.

Monagle P, Barnes C, Ignjatovic V, Furmedge J, Newall F, Chan A et al. Developmental haemostasis. Impact for clinical haemostasis laboratories. *Thromb Haemost* 2006; 95(2): 362-372.

Monagle P, Massicotte P. Developmental haemostasis: Secondary haemostasis. *Semin Fetal Neonatal Med* 2011; 16(6): 294-300.

Roschitz B1, Sudi K, Köstenberger M, Muntean W. Shorter PFA-100 closure times in neonates than in adults: Role of red cells, white cells, platelets and von Willebrand factor. *Acta Paediatr* 2001; 90(6): 664-670.

Toulon P. Developmental hemostasis: Laboratory and clinical implications. *Int J Lab Hematol* 2016; 38 Suppl 1: 66-77.
Toulon P, Berruyer M, Brionne-François M, Grand F, Lasne D, Telion C, Arcizet J, Giacomello R, De Pooter N. Age dependency for coagulation parameters in pediatric populations. Results of a multicenter study aimed at defining the age-specific reference ranges. *Thromb Haemost* 2016(1); 116: 9-16.

Williams MD, Chalmers EA, Gibson BE; Haemostasis and Thrombosis Task Force, British Committee for Standards in Haematology. The investigation and management of neonatal haemostasis and thrombosis. *Br J Haematol* 2002; 119(2): 295-309.