Developmental hemostasis: consequences for the laboratory

Heleen van Ommen

Pediatric Hematology

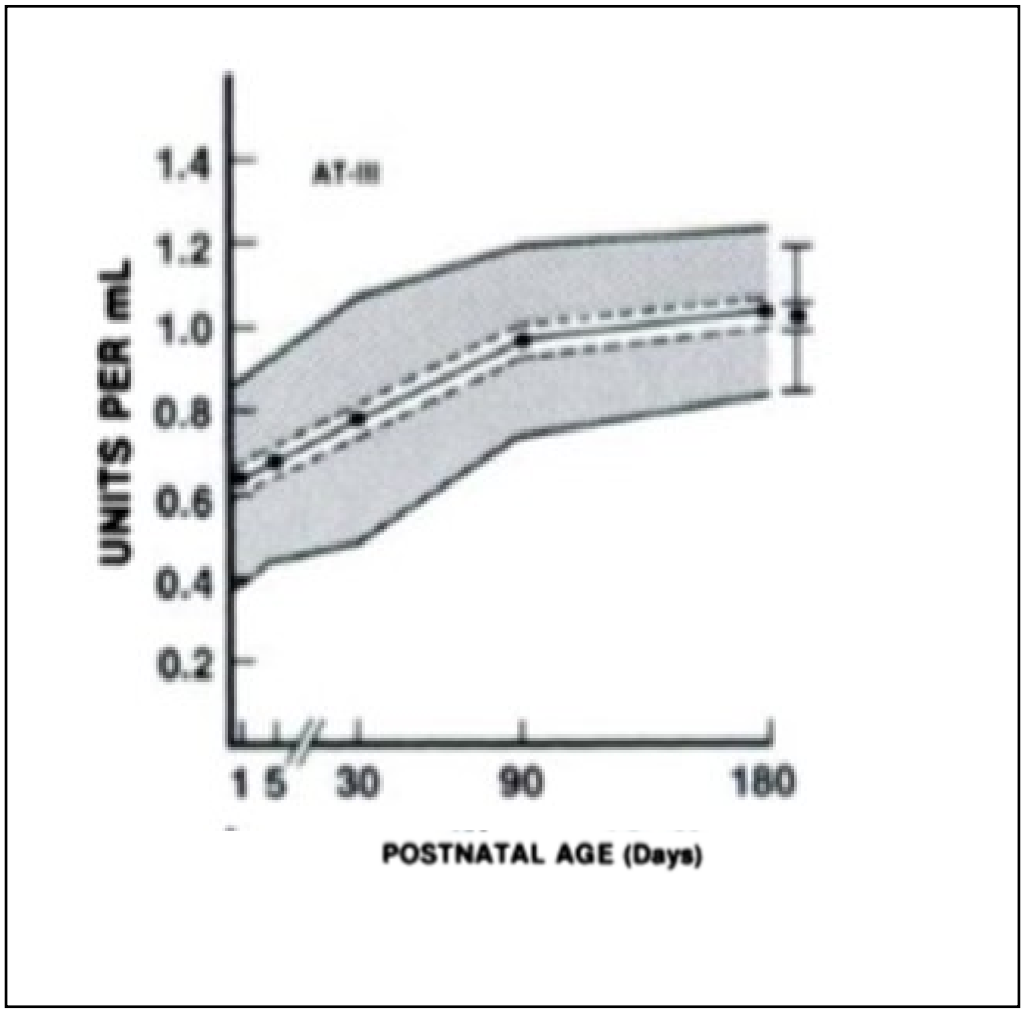
Sophia Children´s Hospital ErasmusMC

Development of the Human Coagulation System in the Full-Term Infant

By Maureen Andrew, Bosco Paes, Ruth Milner, Marilyn Johnston, Lesley Mitchell, Douglas M. Tollefsen, and Peter Powers

The investigation of many hemostatic defects in the newborn is limited by the lack of normal reference values. This study was designed to determine the postnatal development of the human coagulation system in the healthy full-term infant. Consecutive mothers of healthy full-term infants born at St Joseph's Hospital in the city of Hamilton were approached for consent. One hundred eighteen full-term infants (37 to 42 weeks' gestational age) were entered into the study. Demographic information and a 2-mL blood sample were obtained in the postnatal period on days 1, 5, 30, 90, and 180. Between 40 and 79 full-term infants were studied on each day for each of the coagulation tests. Plasma was fractionated and stored at −70°C for batch assaying of the following tests: prothrombin time, activated partial thromboplastin time, thrombin clotting time, and factor assays (biologic): fibrinogen, II, V, VII, VIII, IX, X, XI, XII, and high–molecular weight kininogen. Factor XIII subunits A and S, von Willebrand factor, and the inhibitors antithrombin III, α_2-antiplasmin, α_2-macroglobulin, α_1-antitrypsin, C1 esterase inhibitor, protein C, and protein S were measured immunologically. Plasminogen, prekallikrein, and heparin cofactor II were measured by using chromogenic substrates. The large number of infants studied at each time point allowed us to determine the following: (*a*) the range of normal for each test at five time points in the postnatal pe
vary with the postnatal ag
coagulation factors show
maturation; and (*d*) that ne
most components by 6 m
large cohort of infants stud
tal period allowed us to det
of the human coagulation s

Developmental hemostasis



Other functions of coagulation proteins: anti-angiogenic function of antithrombin

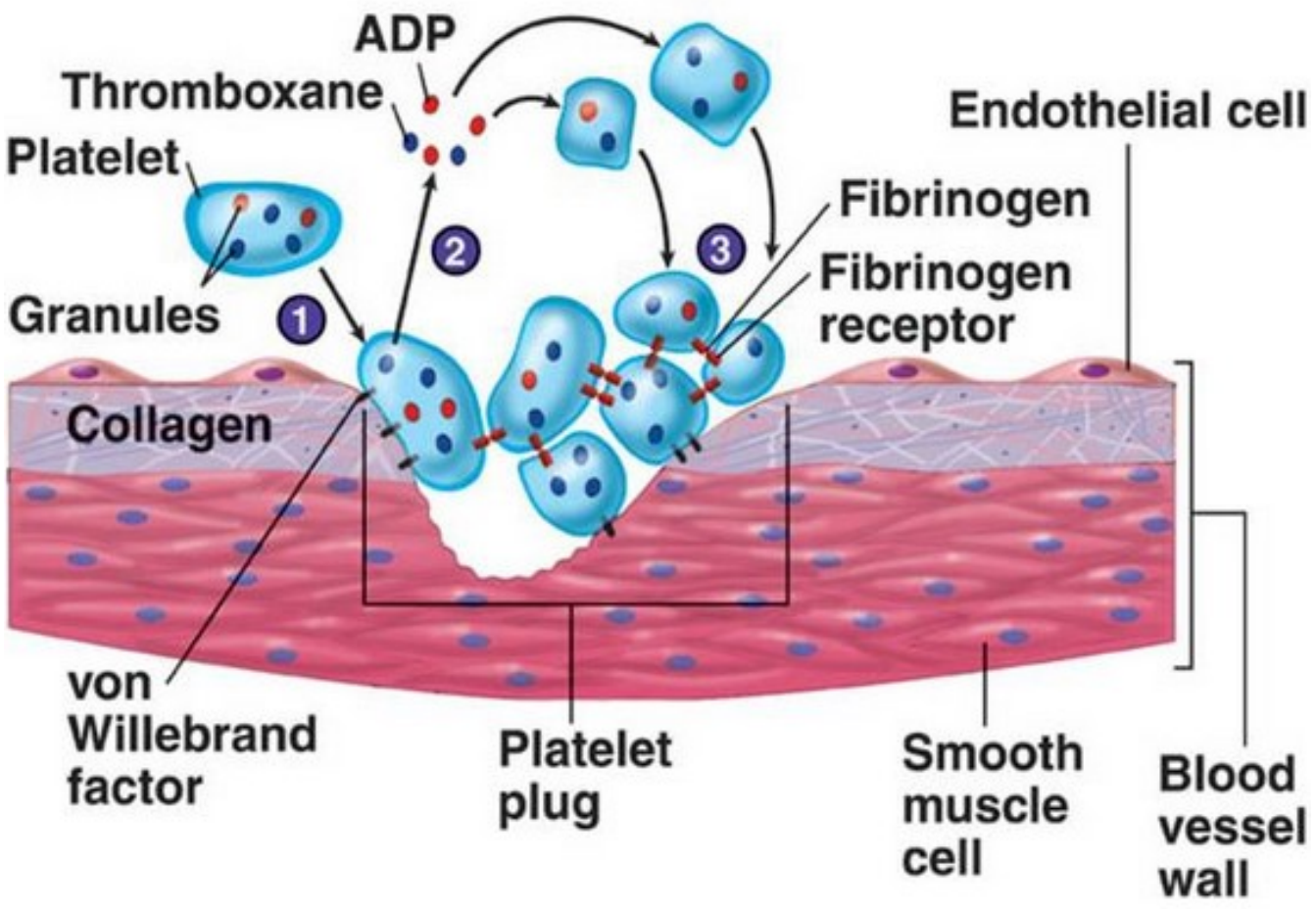
Low antithrombin levels

↓

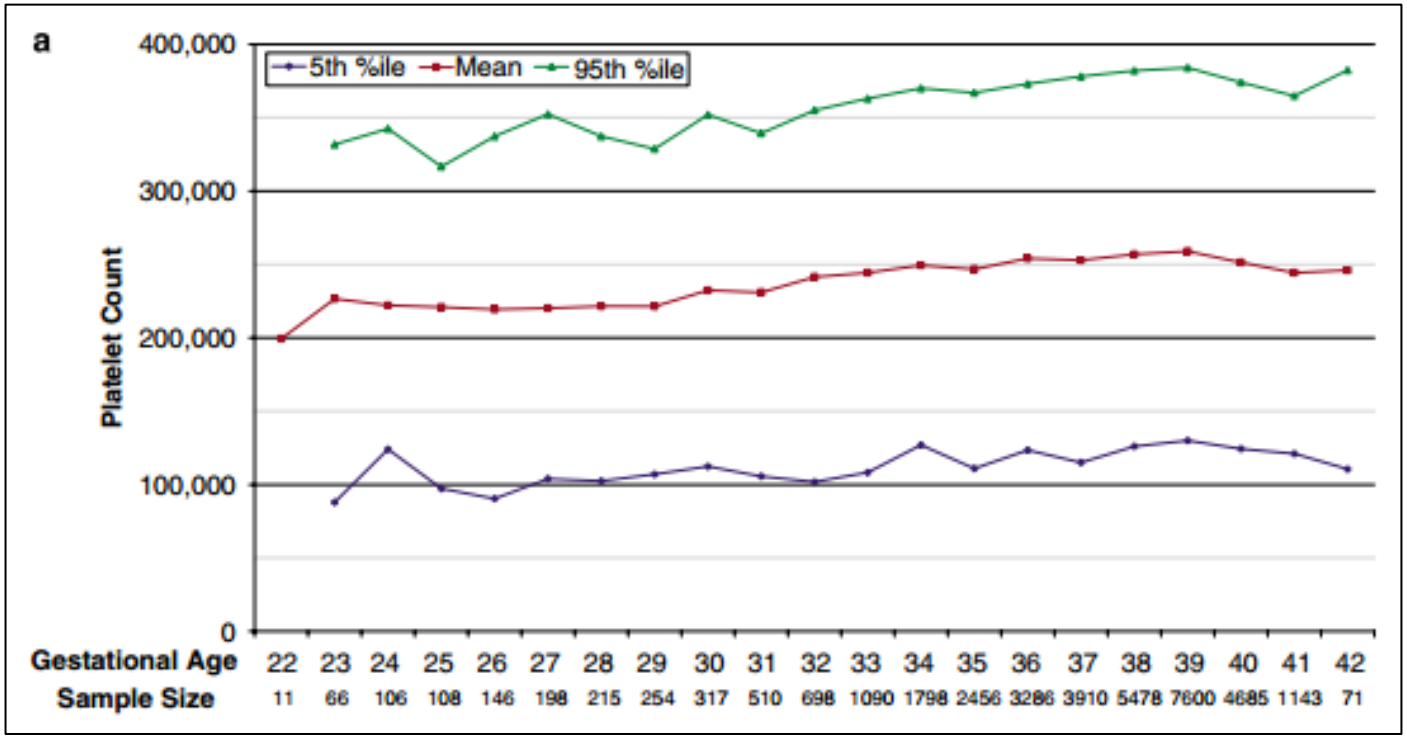
Less inhibition of angiogenesis

Andrew et al Blood 1987; Monagle et al Sem Fetal Neonatal Med 2011

Primary hemostasis



Number of neonatal platelets



Platelet count increases with gestational age

Wiedmeier et al J Perinatol 2009

Platelet aggregation

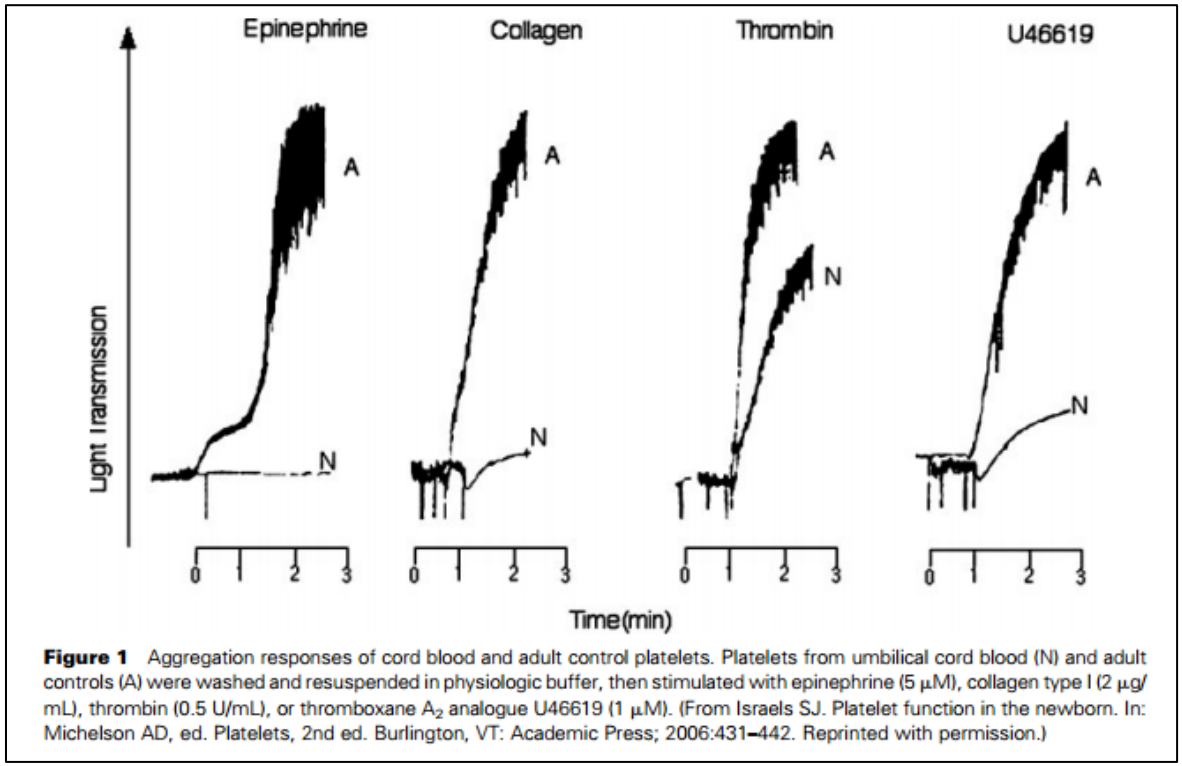


Figure 1 Aggregation responses of cord blood and adult control platelets. Platelets from umbilical cord blood (N) and adult controls (A) were washed and resuspended in physiologic buffer, then stimulated with epinephrine (5 μM), collagen type I (2 μg/mL), thrombin (0.5 U/mL), or thromboxane A_2 analogue U46619 (1 μM). (From Israels SJ. Platelet function in the newborn. In: Michelson AD, ed. Platelets, 2nd ed. Burlington, VT: Academic Press; 2006:431–442. Reprinted with permission.)

- Hypo-responsiveness persists for 2 to 4 weeks
- More pronounced in preterm neonates
- Different mechanisms

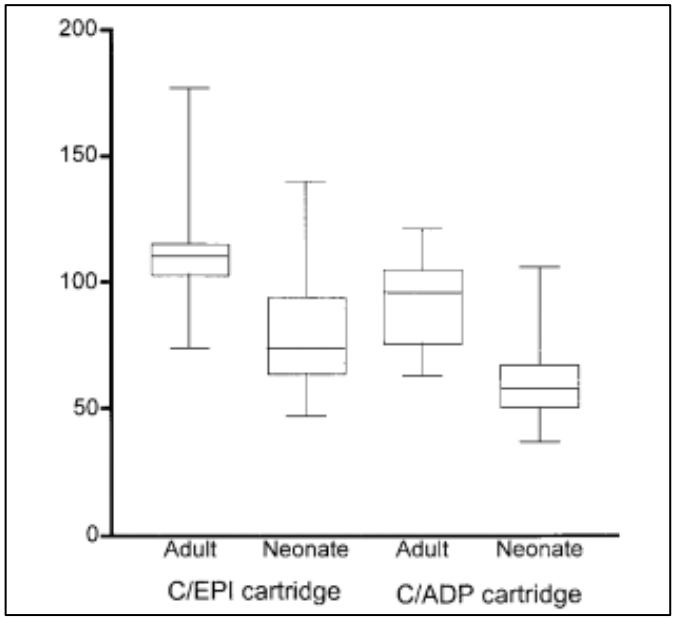
Israels SJ, Sem Thromb Haemost 2009

Global coagulation tests

		Closure time	
Subjects	*n*	Col/Epi (s)	Col/ADP (s)
Adult controls	25	106 (84–150)	83 (64–98)
Cord blood	70	75 (50–112)	58 (43–98)

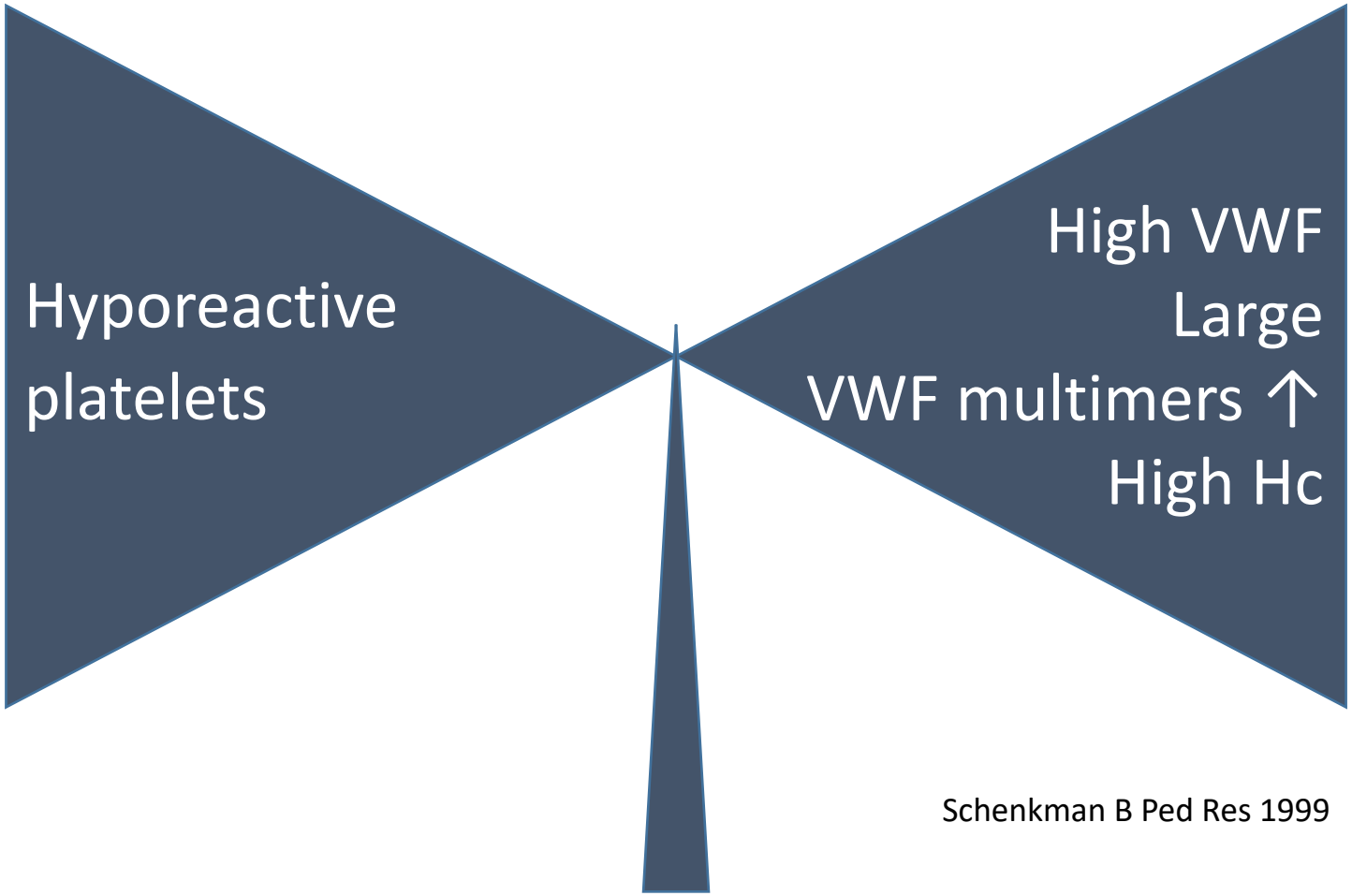
Data are median values (ranges).

Roschitz et al 2001



Israels et al. J Pediatr 2001

Balanced primary hemostasis in healthy neonates



Schenkman B Ped Res 1999

Reaction on thrombocytopenia in BM

Sola-Visner MC et al. Pediatr Res 2007

Adults:

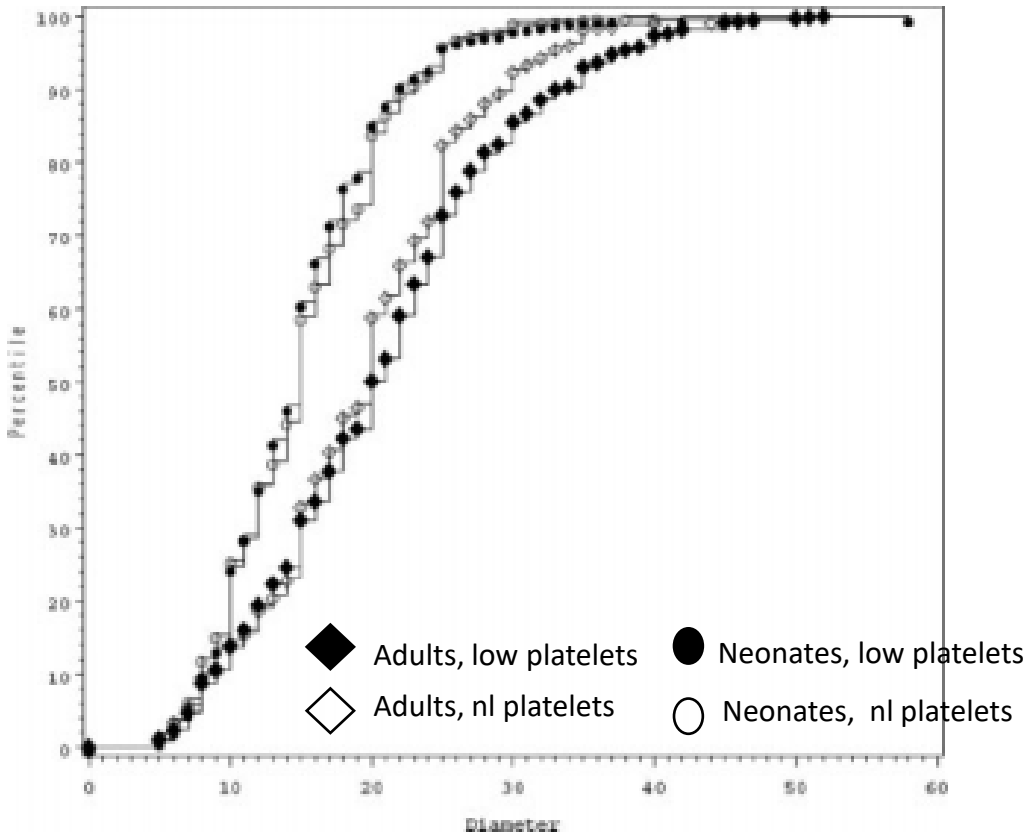
Increase MK mass:

- MK number
- MK size and ploidy

Neonates:

Increase MK mass:

- MK number



Neonatal primary hemostasis: Lack of reserve capacity

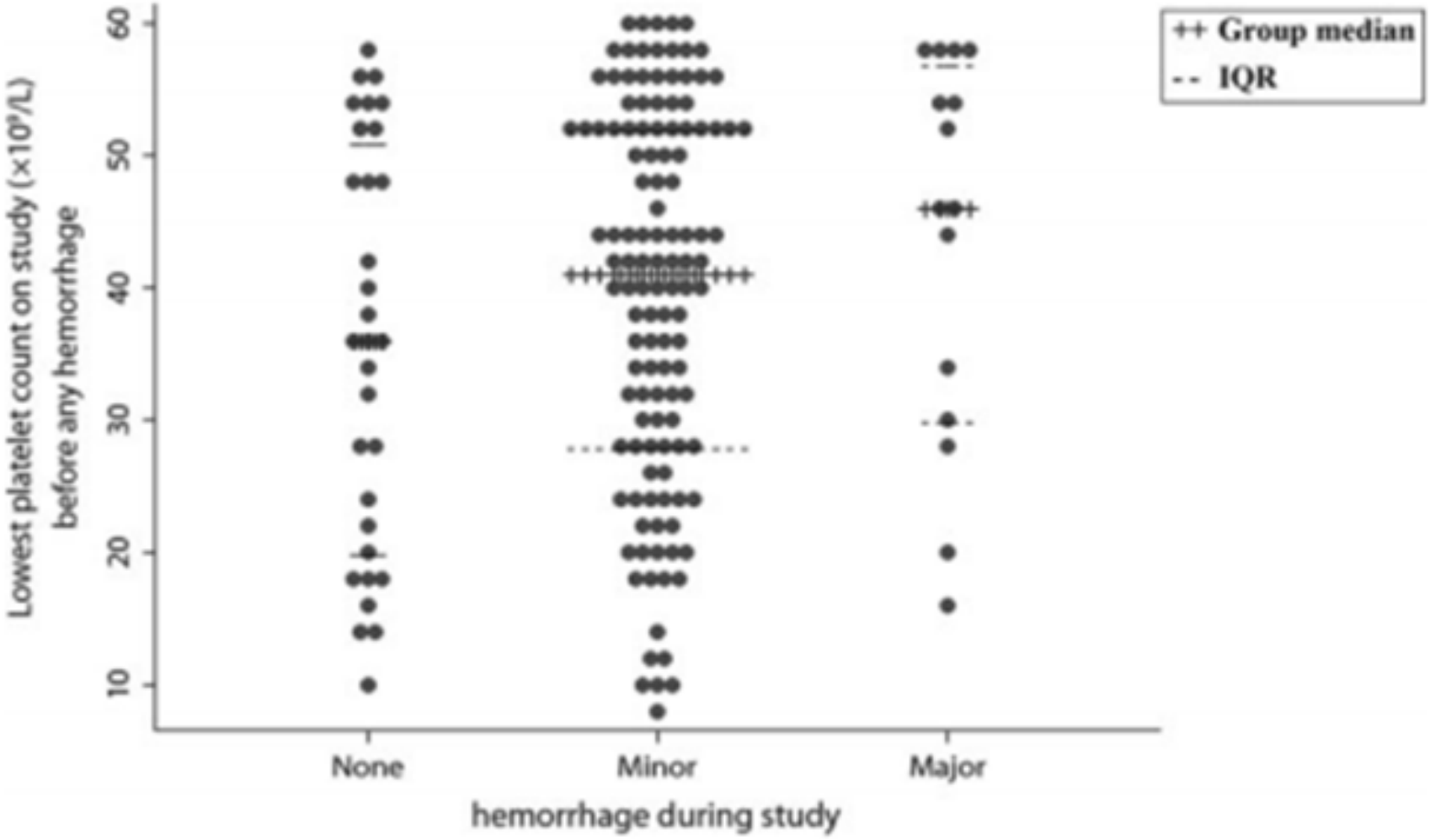
Thrombocytopenia in neonates

- Major concern: major bleeding
- Platelet transfusions only specific treatment
- Stanworth et al 2009:

Platelet Tx: 70% of neonates with platelets < $60x10^9/L$

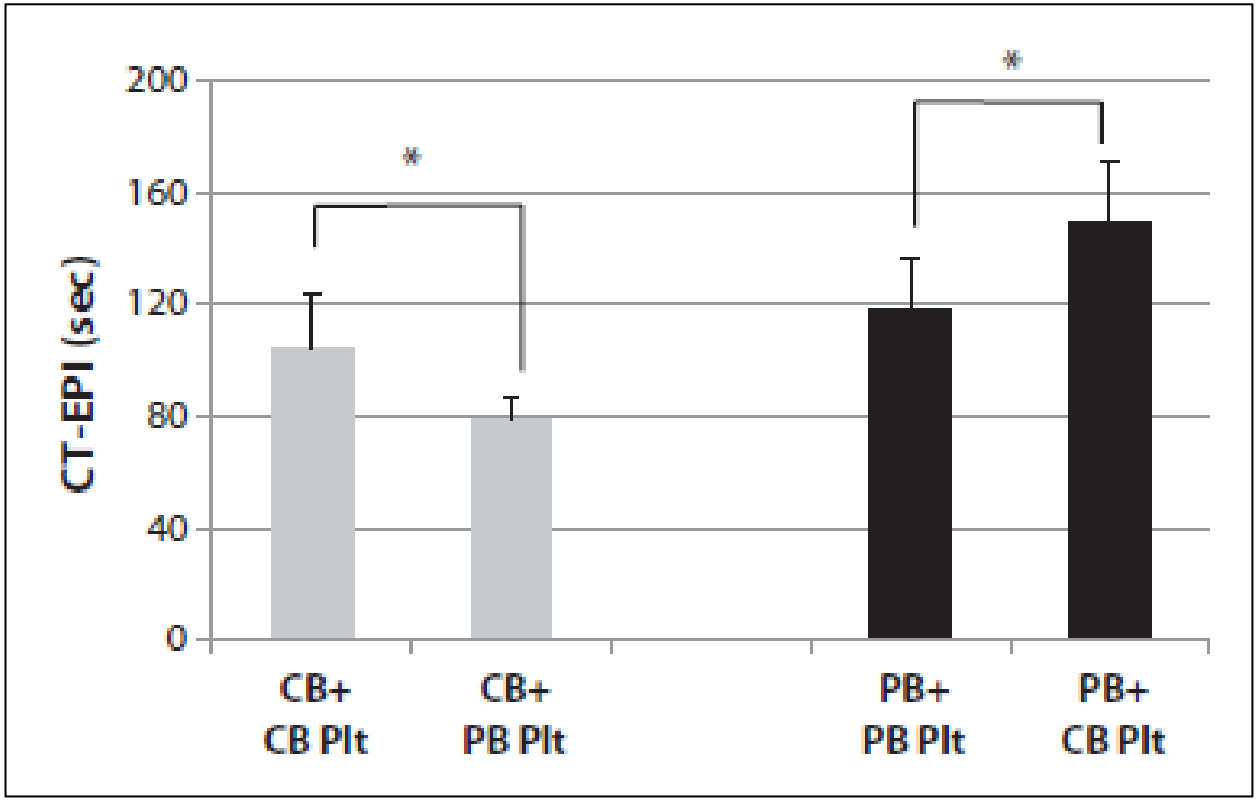
- No evidence of efficacy of prophylactic transfusions
- No relationship between platelet count and bleeding

RISK OF BLEEDING



Stanworth *et al*. Pediatr 2009.

Transfusion risk

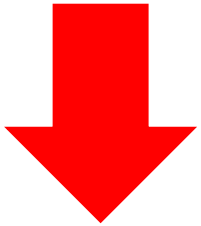


Ferrer-Marin et al. JTH 2011

No evidence of efficacy of prophylactic platelet transfusions

No correlation between platelet count and bleeding

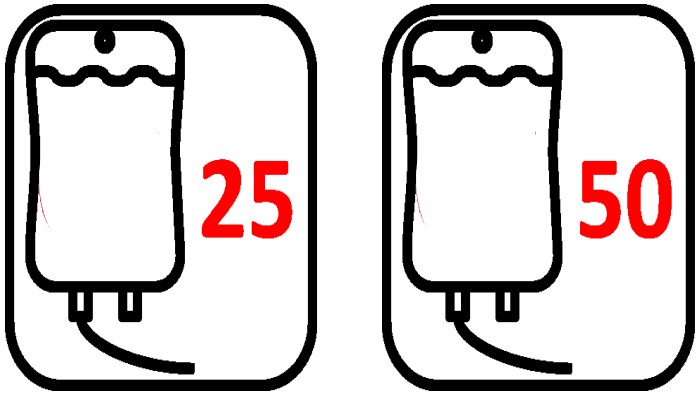
Potential risks of transfusion



Various platelets transfusion thresholds

RCT TRANSFUSION THRESHOLDS

PRETERM BABIES < 34 WEEKS



Curley et al, Neonatol 2014;106:102-106

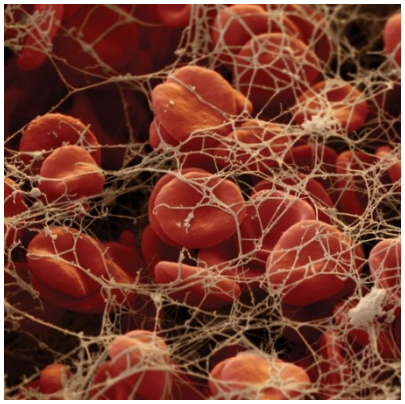
NHS Blood and Transplant

amc

Sanquin Blood Supply

Secundary hemostasis

Qualitative differences



- Plasminogen, VWF, fibrinogen
- Fetal fibrinogen:

  More sialic acid ⟶

  Delayed fibrin polymerization

  Increased thrombin clotting times

Ganakis DK et al Ann NY Aca Sc 1983

Hemostatic system in de fetus

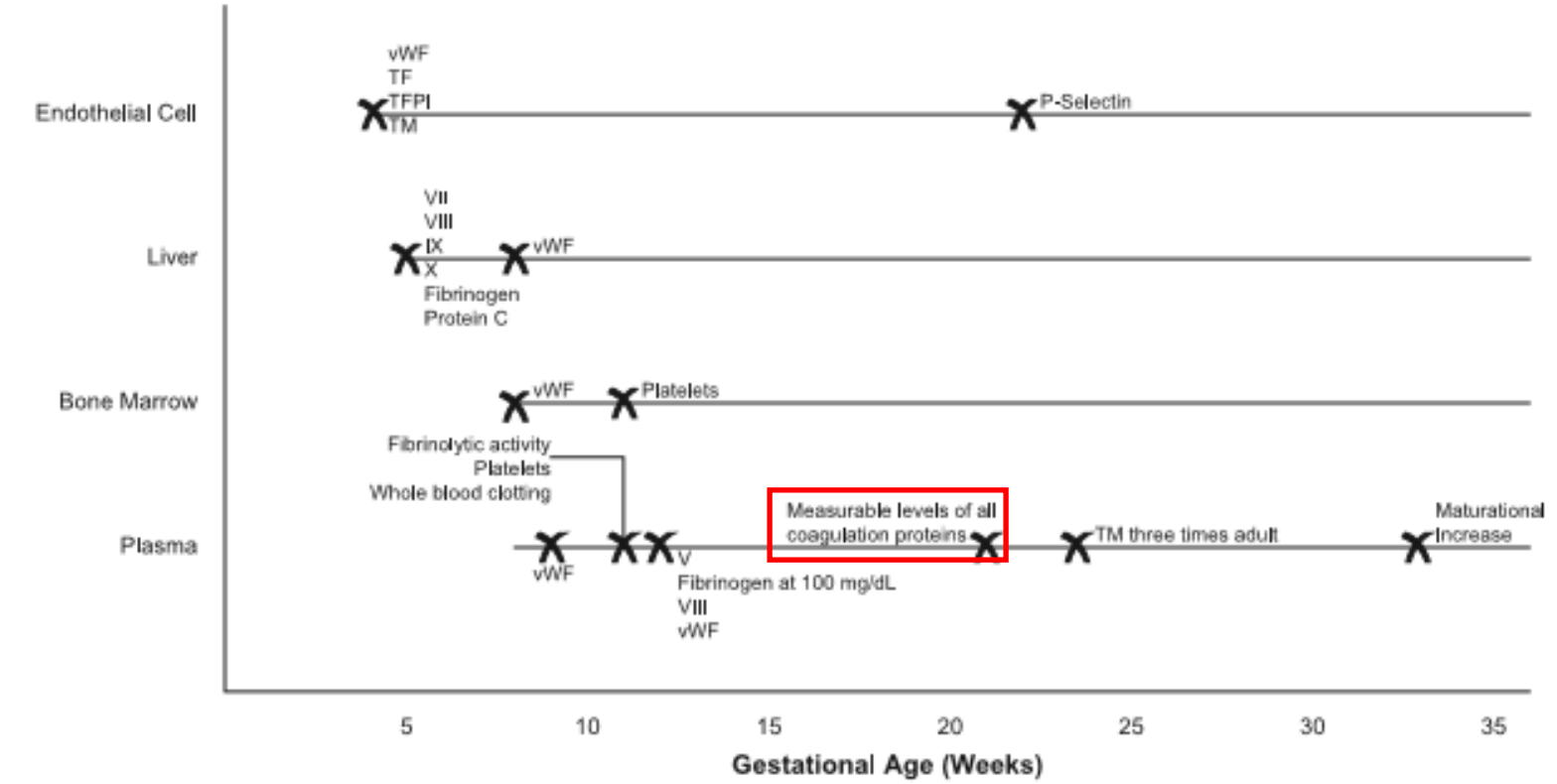


Figure 1 Developmental expression of the fetal hemostatic system. This graph depicts the temporal expression of fetal coagulation proteins and platelets in endothelial cells, hepatic cells, bone marrow and plasma during gestation.

Manco-Johnson MJ. Throm Res 2005

Maturation coagulation proteins

	Neonatal age	Age adult values
	Decreased Increased	
Procoagulants		
FII, FVII, FIX, FX		16 yr
FXI, FXII		1 yr, 16 yr
FV, FVIII, fibrinogen, FXIII		
Anticoagulants		
AT, PS, PC		3 mo, 1 mo, 16 yr
α2macroglobulin		adult
Fibrinolysis		
Plasminogen		6 mo

Andrew M, Blood 1978; Monagle P, TH 2006; Appel I, JTH 2012; Attard C, JTH 2013.

Global coagulation tests

APTT

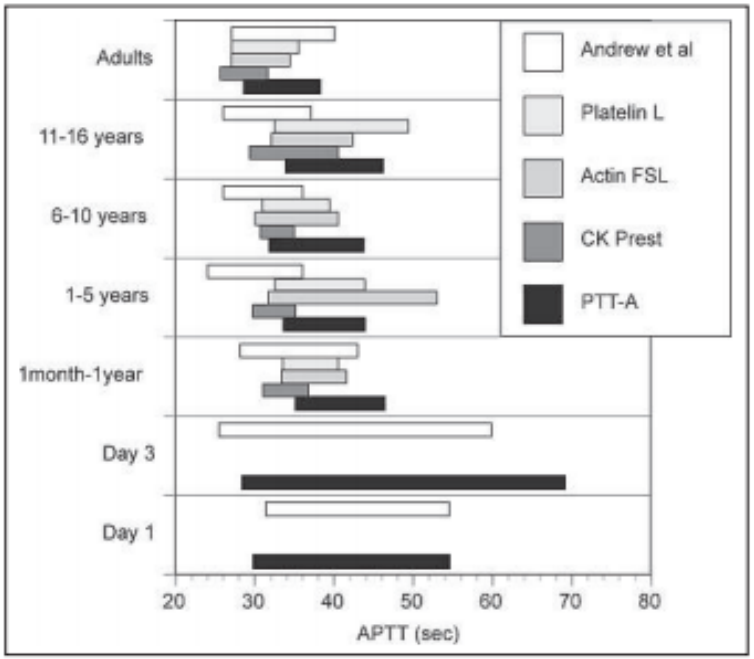
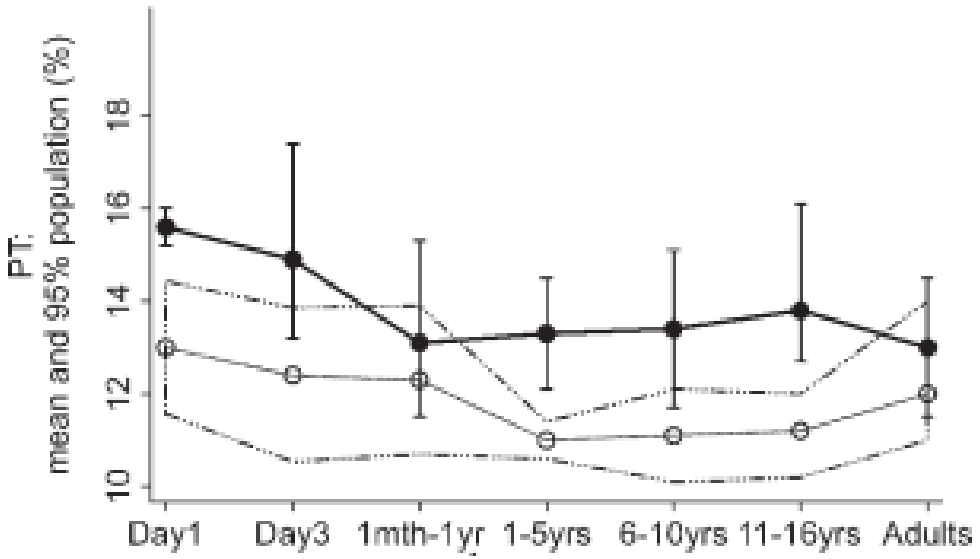


Figure 1: Age-specific APTT reference ranges for healthy neonates and children using the STA analyser and four different commercially available APTT reagents. Reference ranges are compared to those published by Andrew et al. Day 3 results for Andrew et al are actually day 5.

PT



No increased bleeding tendency !

Monagle P et al; TH 2006

Interpretation of laboratory results

- No age-related reference ranges specific to reagents and instruments used

Developmental hemostasis: recommendations for laboratories reporting pediatric samples

V. IGNJATOVIC,*† G. KENET‡§ and P. MONAGLE*†¶ ON BEHALF OF THE PERINATAL AND PAEDIATRIC HAEMOSTASIS SUBCOMMITTEE OF THE SCIENTIFIC AND STANDARDIZATION COMMITTEE OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS

Murdoch Childrens Research Institute, Royal Childrens Hospital, Parkville, Victoria; †Department of Paediatrics, Royal Childrens Hospital, The University of Melbourne, Parkville, Victoria, Australia; ‡Thrombosis Unit, National Hemophilia Center, Sheba Medical Center, Tel-Hashomer; §The Sackler Medical School, Tel Aviv University, Tel Aviv, Israel; and¶Department of Clinical Haematology, Royal Childrens Hospital, Parkville, Victoria, Australia

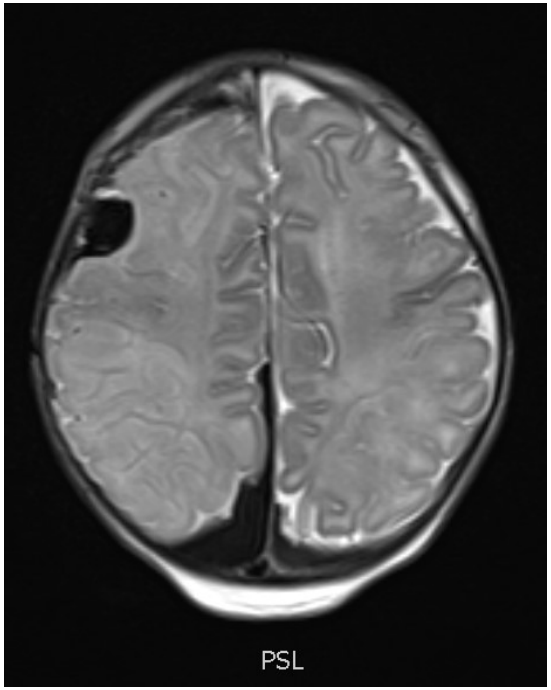
- Diagnosis of congenital factor deficiencies is not possible in the neonatal period

Low vitamin K dependent factors

Poor vitamin K transfer across placenta

Little vitamin K in breast milk

Vitamin K deficient bleeding



Antithrombotic treatment

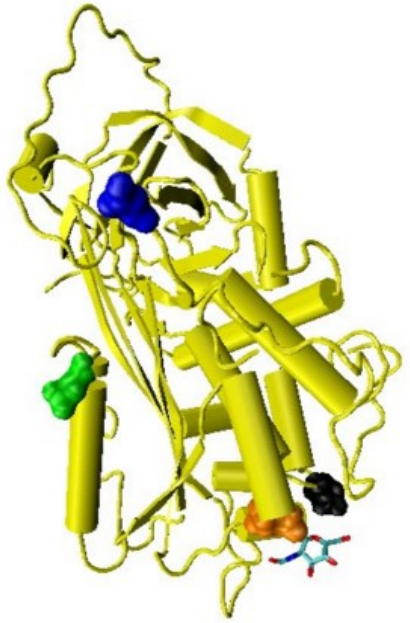
Heparin: potentiates inhibitory effects of AT

Neonates:

- Low levels AT, and FII, FX
- Increased volume of distribution
- Increased clearance rate

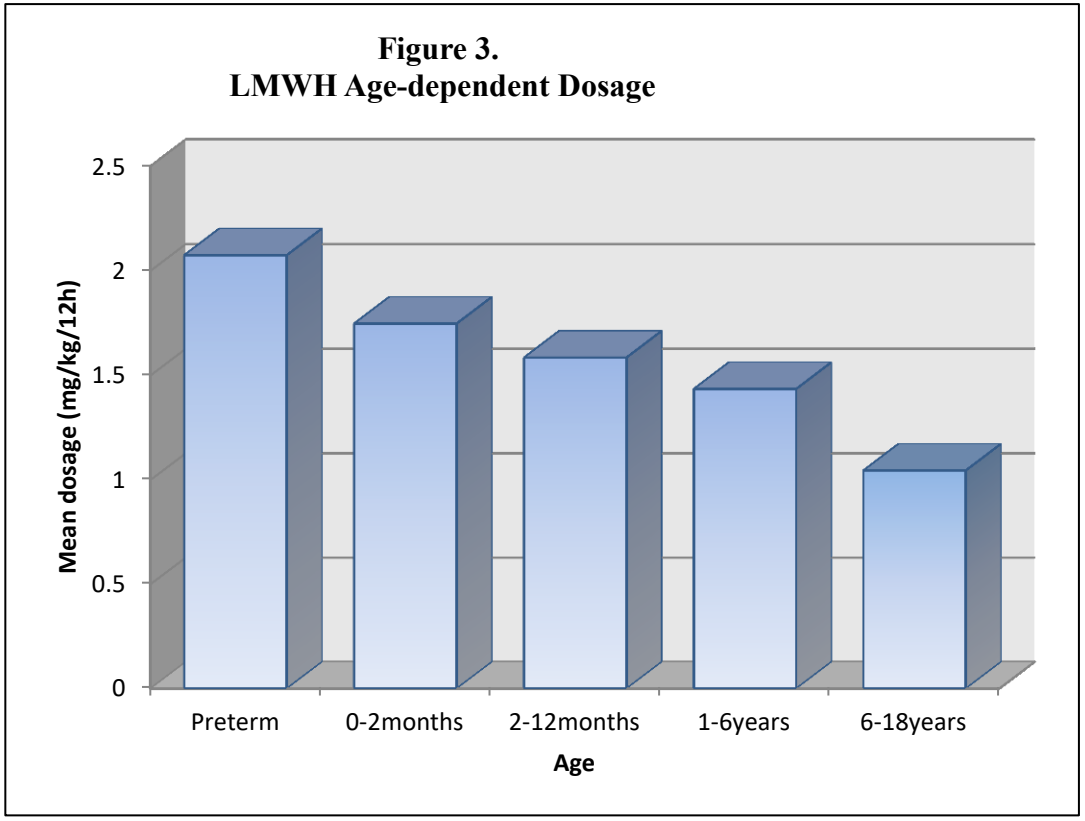
↓

Higher dosages of UFH and LMWH



Literature review dosages LMWH

- Search until Oct 2015
- A total of 18 studies with 1095 children treated with enoxaparin, anti Xa 0.5-1.0 U/mL



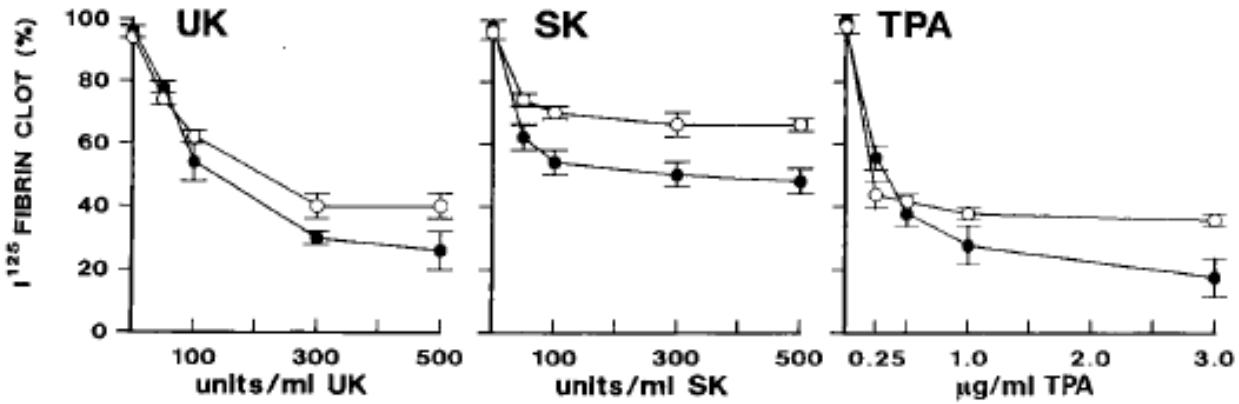
Vitamin K antagonists

Tabel 1: Results

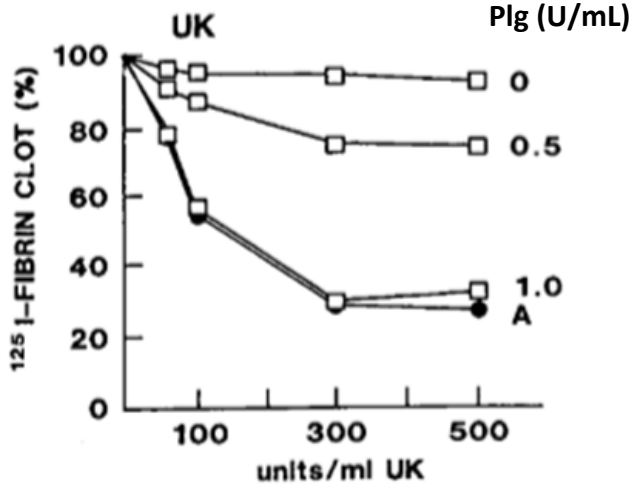
	Initial treatment		Maintenance treatment	
	Phenprocoumon (n=65)	Acenocoumarol (n=49)	Phenprocoumon (n=70)	Acenocoumarol (n=37)
PHARMACODYNAMICS				
Dosages mg/kg, median (range)				
<1 year	0.15 (0.11-0.58)	0.14 (0.06-0.41)	0.13 (0.04-0.31)	0.09 (0.06-0.22)
1-5 years	0.09 (0.07-0.16)	0.10 (0.06-0.13)	0.05 (0.04-0.08)	0.07 (0.02-0.13)
6-12 years	0.13 (0.06-0.28)	0.06 (0.06-0.08)	0.05 (0.02-0.07)	0.07 (0.04-0.22)
13-18 years	0.08 (0.02-0.17)	0.05 (0.01-0.09)	0.03 (0.02-0.07)	0.05 (0.01-0.09)
p-value[1]	*0.002*	*<0.001*	*<0.001*	*0.026*

N Spoor et al, TH 2012

Thrombolytic therapy



In vitro: neonates: low plasma plasminogen levels limit the response to thrombolytic agents



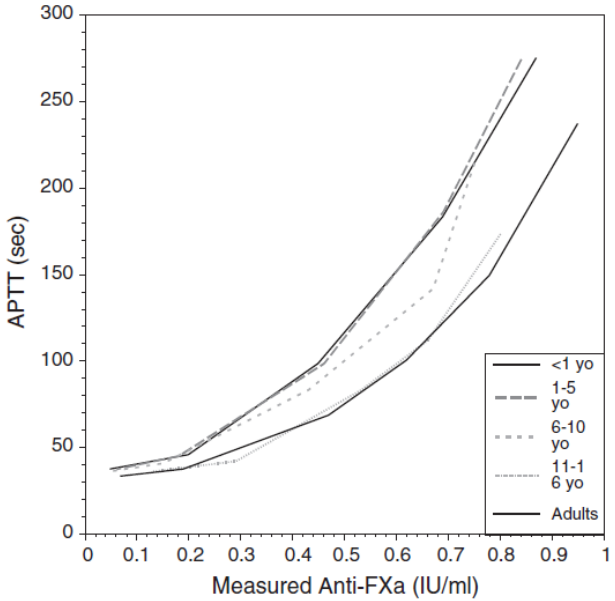
Addition of plasminogen increases clot lysis in neonatal plasma

Andrew et al. TH 1992

Monitoring anticoagulant therapy

Table 1 Therapeutic ranges for APTT and Anti-FIIa that correlate with Anti-FXa of 0.35–0.70 IU/ml

Assay	Therapeutic ranges corresponding to measured Anti-FXa of 0.35–0.70 IU/ml for specific age groups (mean and SD)				
	<1 year	1–5 years	6–10 years	11–16 years	Adults
APTT (s)	82–177[#] (6) (15)	78–200[#] (6) (25)	75–154 (16) (32)	54–142 (16) (38)	55–118[#] (18) (35)

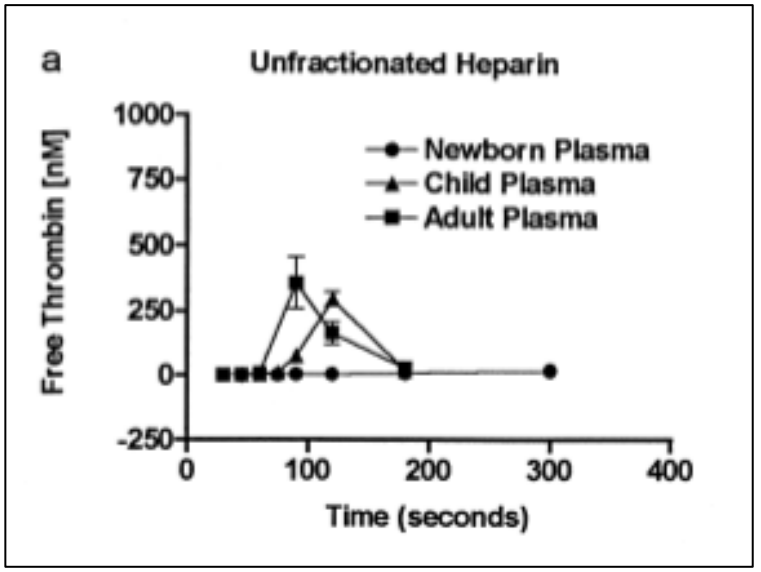


APTT ranges corresponding to therapeutic heparin levels are higher in children

Ignjatovic V et al. Throm Res 2006; 118; 741-5

Therapeutic target ranges (TTR)

- Association between TTRs and clinical outcome not proven
- Studies: Neonates and infants: reduced concentrations heparin and warfarin required to inhibit thrombin generation



Chan et al TH 2002

Schechter JTH 2012:

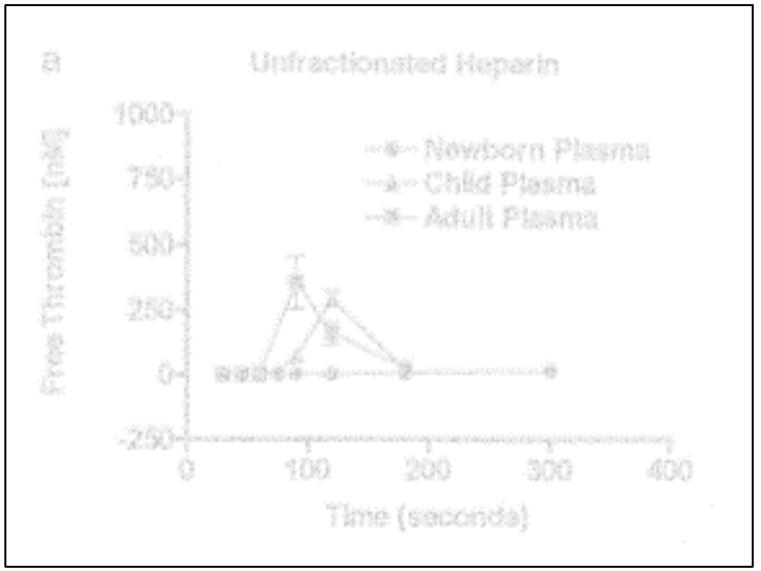
100 neonates and infants with UFH:

15% within TTR in 24 hrs, 17% never in TTR

Outcome: 70% response, 30% no change

Therapeutic target ranges (TTR)

- Association between TTRs and clinical outcome not proven
- Studies: Neonates and infants: reduced concentrations heparin and warfarin required to inhibit thrombin generation



Chan et al TH 2002

Schechter JTH 2012:

100 neonates and infants with UFH:

15% within TTR in 24 hrs, 17% never in TTR

Outcome: 70% response, 30% no change

Summary

- Developmental hemostasis: unique balance
- Lack of reserve capacity: bleeding and clotting in sick neonate
- Consequences for
  - Interpretation of laboratory results
  - Risk of vitamin K deficient bleeding
  - Platelet transfusions
  - Antithrombotic treatment
- New oral anticoagulants:

  pediatric specific PK and PD modeling, incorporating laboratory and clinical outcome measures