Theme Issue Article

Development of selected coagulation factors and anticoagulants in preterm infants by the age of six months

Marjut Salonvaara[1], Pekka Riikonen[1], Elina Vahtera[2], Eija Mahlamäki[3], Kirsti Heinonen[1], Riitta Kekomäki[2]

[1]Department of Paediatrics, Kuopio University Hospital and Kuopio University, Kuopio, Finland
[2]Finnish Red Cross Blood Service, Helsinki, Finland
[3]Department of Clinical Chemistry, Kuopio University Hospital, Kuopio, Finland

Summary

The development of the coagulation and anticoagulation system in preterm infants was assessed, with special emphasis on extremely low birth weight (ELBW) infants and haemorrhagic or other complications after birth. Coagulation factors II (prothrombin), V (FV), VII (FVII) and X (FX) were analysed at birth and at a corrected age of six months. In addition, antithrombin (AT), protein C (PC) and protein S (PS) were measured at six months, and DNA samples were tested for Factor V Leiden (R506Q). Eighty-two infants, with a median gestational age (GA) of 32 weeks (range 24–36) and a median birth weight of 1562 g (range 695–3520), were studied. Fifteen of these were ELBW infants (range 695-1000g). Prothrombin, FV, FVII and FX reached healthy term six-month-old infant activity levels. Prothrombin and FX did not reach adult values; median activity levels remained at 82% and 78%, respectively. During the follow up, the FV and FVII levels of the ELBW infants (GA 24-27 weeks) increased more than those of the preterm infants born with higher GA ($p < 0.001$). At birth, prothrombin correlated significantly with FV, FVII and FX ($p < 0.001$). FVII at birth and at six months correlated significantly with PC ($p= 0.021$ and $p= 0.009$, respectively). These findings indicate that the gain in the coagulation factor concentrations in infancy is greatest in infants with the lowest GA at birth. Interesting new inter-relations of coagulation factor and physiological anticoagulant levels may indicate that there are still unrecognised pathways in the function of newborn haemostasis.

Keywords

Prematurity, infant, coagulation factor, physiological anticoagulant, development

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Introduction

The haemostatic system in the neonate and especially in the preterm infant is in a dynamic state (1-3). Neonatal intensive care has advanced in recent years, and more extremely low birth weight (ELBW) infants survive, but a significant proportion of these infants may still suffer from neonatal complications, such as intraventricular haemorrhage (IVH) after birth (4).

Most data on coagulation factors in the early weeks of gestation (before 30 weeks) have been obtained from foetuses (5, 6). However, specific coagulation factor assays have been developed which, together with micro techniques, make it possible to study haemostatic factors directly from blood samples from ELBW infants as well. Reference values for coagulation components and anticoagulants in foetuses from 19 to 38 weeks gestation are available (6). These values may facilitate the evaluation of the coagulation status of very premature infants. Foetal haemostasis functions at lower levels of haemostatic factors than newborn infant haemostasis (5, 6). Corresponding postnatal values obtained from preterm infants are higher (2, 3) than the plasma levels of the foetus coagulation factors. Few coagulation studies have included ELBW infants (7).

Correspondence to:
Dr. Marjut Salonvaara
Department of Pediatrics
Kuopio University Hospital
P.O.B. 1777, FIN- 70211 Kuopio
Finland
Tel.: +358 17 172 399, Fax: +358 17 172410
E-mail: marjut.salonvaara@fimnet.fi

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Recent advances in the care of the smallest infants have led to lower mortality, with the result that the number of preterm infants with neonatal problems has increased. True reference ranges for extremely premature infants are lacking because these infants do not usually appear to be healthy (2). Moreover, various complications in pregnancy, such as mother's medication or preterm labour, may affect coagulation status (8). Preterm infants with neonatal problems are prone to develop haemorrhagic and thrombotic complications (9).

In this study, we explored the development of selected coagulation factor activities from birth to six months with an emphasis on ELBW infants. Interesting new inter-relations of coagulation factor and physiological anticoagulant levels were observed.

Materials and methods

The infants included in this study were born at Kuopio University Hospital between 1996 and 1998. The study protocol was approved by the research ethics committee of Kuopio University Hospital. Informed written consent was obtained from parents before they entered the study.

Subjects

Premature infants born at less than 37 weeks gestation and admitted to the neonatal intensive care unit in Kuopio University Hospital were eligible for the study. The gestational age was based on a combination of maternal dates and ultrasound examinations. All infants received 1 mg vitamin K intramuscularly at birth (10, 11). Patients were eligible for the study if all data of the blood samples at birth and at the corrected age of six months were available and the blood samples had been taken properly.

Data collection

After birth: data concerning the major neonatal morbidities were collected from patient charts. Special attention was paid to IVH, necrotising enterocolitis, grade II-V retinopathy of prematurity and infections. Insertion of peripheral central venous lines, duration of ventilation support, and corticosteroid, antibiotic and possible fresh frozen plasma treatment were also recorded.

At the age of six months: the corrected age of six months was determined using post-term age calculated from expected date of delivery. Patient charts were reviewed with special emphasis on haemorrhagic or thrombotic complications, infections, and neurological problems. All children were examined by one of the researchers (MS, a paediatrician) in the out-patient clinic.

Laboratory methods

At birth: Blood samples were drawn from the peripheral arterial or venous catheters immediately after placement within 2 hours after birth. Blood sample for the coagulation factor assays was collected directly into citrate vacuum tubes (1.8 ml, Vacuette, Greiner GmbH, Frickenhausen, Germany). The blood for the coagulation studies was immediately centrifuged at room temperature at 1400 *g* for 30 minutes, then plasma was removed, aliquoted and kept at -80ºC until analysed.

The data and sample collection and laboratory methods for the coagulation factor activities were carried out as described in detail by us recently (12). The laboratory participates regularly in four different external quality assessment schemes.

Table 1: Patient characteristics in each gestational age group.

	GA 24 – 27 n = 15	GA 28 – 30 n = 20	GA 30 – 33 n = 20	GA 34 – 36 n = 27
Birth weight, g				
Median	820	1125	1810	2460
Range	695 – 1180	810 – 1850	1140 –2460	1670 – 3520
Male	12	9	9	17
Apgar <6 at 1 min	14	19	12	11
<6 at 5 min	3	3	0	0
SGA	5	5	8	8
IVH, grade I–IV	7	1	0	0
ROP, grade II–V	4	2	0	0
Antibiotic treatment, days				
Median	23	7	3	0
Range	4 – 71	0 – 34	0 – 15	0 – 31
Corticosteroid treatment, days				
Median	10	6		
Range	0 – 32	0 – 14	0	0
Ventilation support, days				
Median	23	5	1	0
Range	10 – 70	0 – 33	0 – 7	0 – 5

GA gestational age; SGA small for gestational age; IVH intraventricular haemorrhage; ROP retinopathy of prematurity

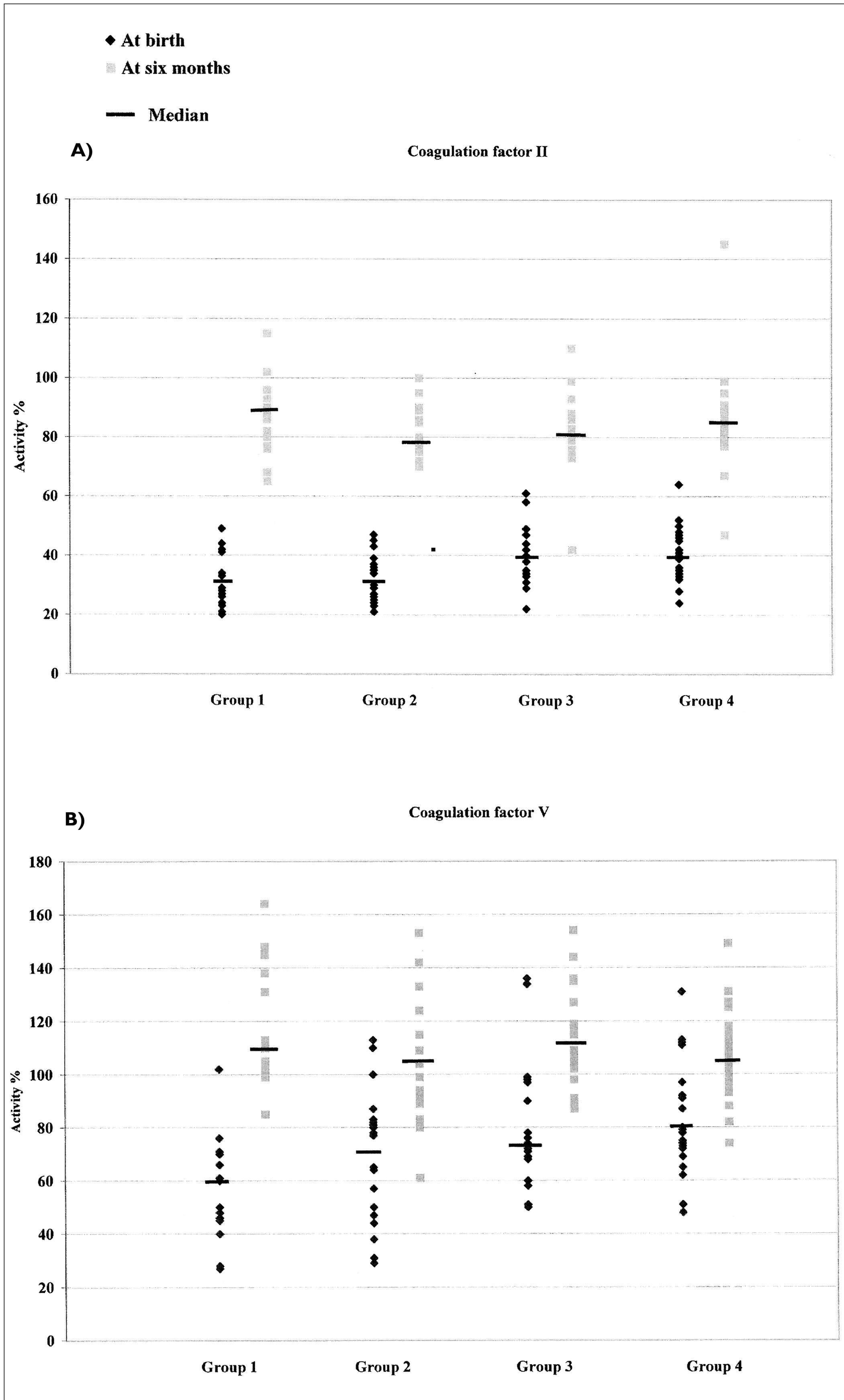

Figure 1: Coagulation factor activities at birth and at the corrected age of six months with median activities for each gestational age (GA) group. Group 1 (n= 15): GA 24-27; Group 2 (n= 20): GA 28-30; Group 3 (n= 20): GA 31-33; Group 4 (n= 27): GA 34-36.

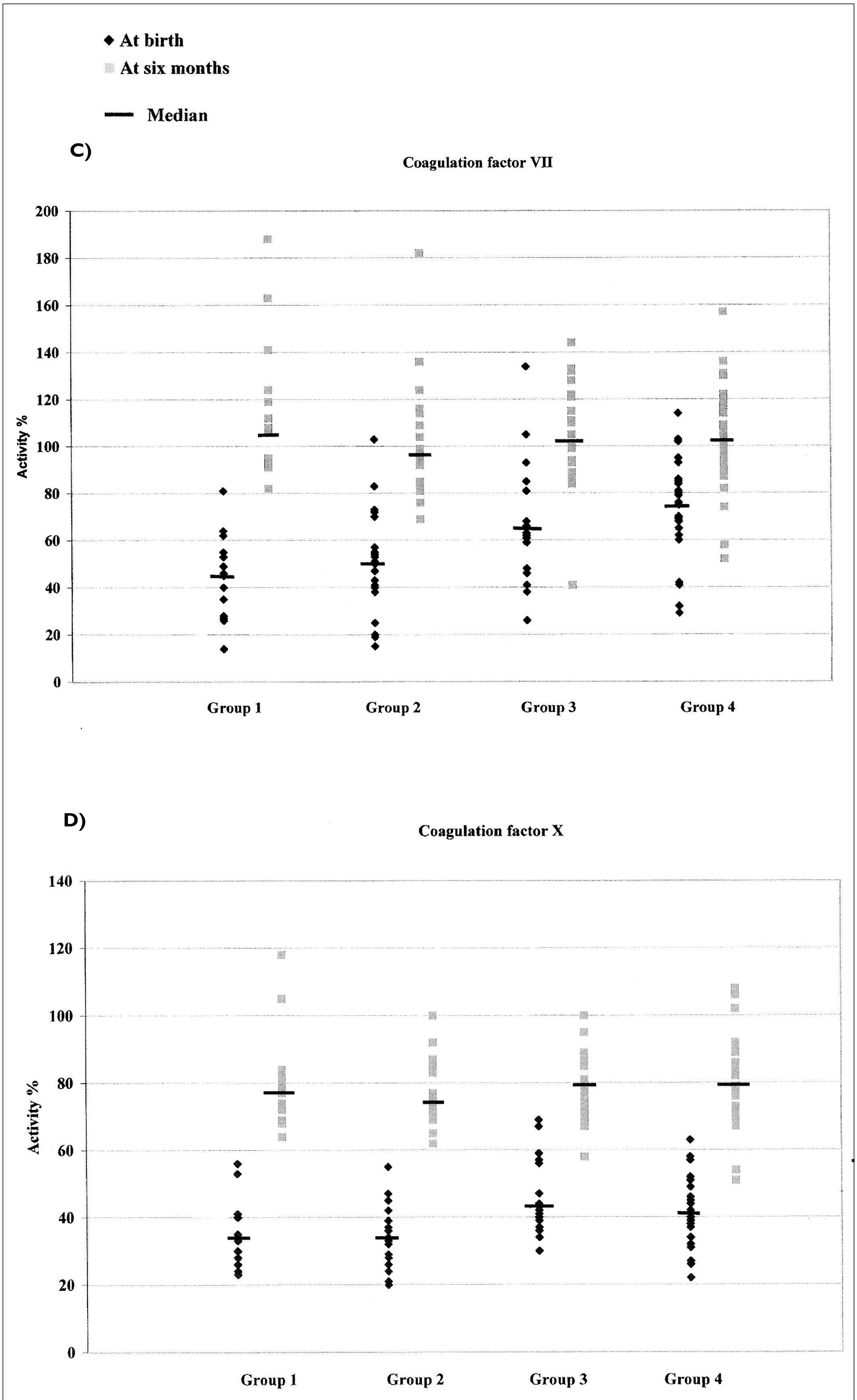

Figure 1: Continued

At the corrected age of six months: blood samples were taken in a citrate tube for children (Vacuette, Greiner GmbH, Frickenhausen, Germany) and sent in ice the same day to the Department of Haemostasis of the Finnish Red Cross Blood Service, Helsinki, for analysis and further assays. The levels of coagulation factors II (FII, prothrombin), V (FV), VII (FVII) and X (FX) were determined with the one-stage method using an STA® Compact® Analyser from Diagnostica Stago (Asnières, France); functional activities of PS, PC and AT were determined according to the manufacturer's instructions with an STA Staclot® Protein S, Staclot® Protein C and Staclot® AT (Diagnostica Stago, Asnières, France), respectively. For PS and AT a frozen plasma pool calibrated with the International Standard of the National Institute for Protein C STA Unicalibrator from Diagnostica Stago were used as standards. The gene defect FV (R506Q) was identified using the restriction fragment length polymorphism (RFLP) technique.

Statistical analysis

Spearman's correlation between each coagulation factor and anticoagulant measured was calculated. Differences in coagulation factor changes from birth to six months of corrected age between gestational age groups, and associations between coagulation factor development, IVH, necrotizing enterocolitis and retinopathy were tested with repeated measures analysis of variance (ANOVA). The Mann-Whitney U-test was used to compare averages of neonatal complications and physiological anticoagulants at six months of corrected age among groups formed on the basis of gestational age. Bonferroni correction was made for multiple comparisons. SPSS 11.0 for Windows was used for all data analyses.

Results

Subjects

Specific demographic information about the study population groups is given in Table 1.

The median gestational age was 32 weeks (range 24 – 36), and the median birth weight was 1562 g (range 695 – 3520). There were 57 singletons, 11 sets of twins and one set of triplets. Fifteen of the infants were ELBW infants (range 695-1000g). Peri- and neonatal complications were diagnosed as follows: small for gestational age (SGA), n=26 (32%); asphyxia at birth, n= 14 (17%); IVH, n=8 (10%); grade II-V retinopathy of prematurity, n= 6 (8%); septic infections, n=11 (13%); and necrotising enterocolitis, n=3 (4%). As expected, the sickest infants were born before 28 gestational weeks (Table 1). They all needed ventilation support after birth.

Peripheral central venous lines were inserted in 13 (16%) infants. No thrombotic events were diagnosed.

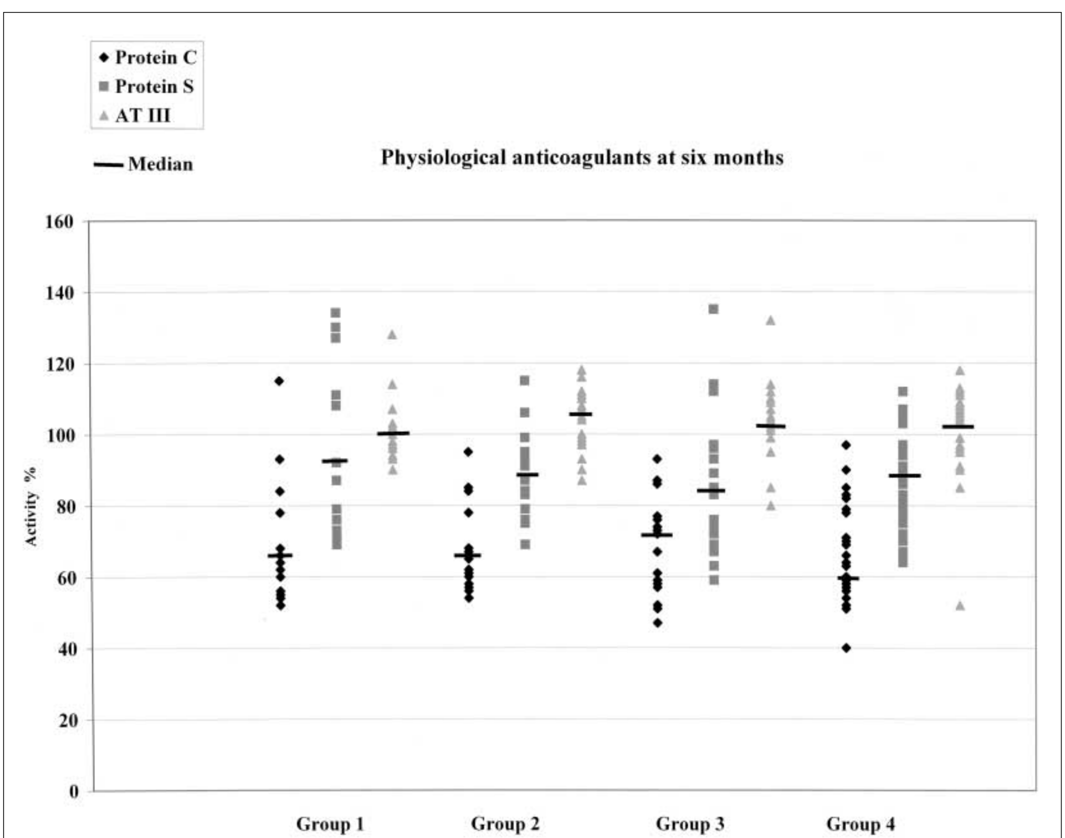

Figure 2: Physiological anticoagulant activities at the corrected age of six months with median activities for each gestational age (GA) group. Group 1 (n= 15): GA 24-27; Group 2 (n= 20): GA 28-30; Group 3 (n= 20): GA 31-33; Group 4 (n= 27): GA 34-36.

Overall haemostasis

The development of the activity of the coagulation factors FII, FV, FVII and FX by six months is presented in Figure 1. The activities of the physiological anticoagulants AT, PC and PS at six months of age are presented in Figure 2. At the corrected age of six months, regardless of the GA at birth, FII, FV, FVII and FX reached the earlier reported levels of coagulation factors in healthy term six-month-old infant (2). Prothrombin and FX did not reach the adult reference values; median activity levels of prothrombin in each GA group remained at 88%, 79%, 80% and 83%, and those of FX at 78%, 75%, 79% and 78%, respectively. FV and FVII reached mean adult plasma concentrations (Fig.1B, 1C) the median activity level of FV in each GA group was 111%, 104%, 112% and 106%, and those of FVII 107%, 98%, 103% and 102%, respectively. The ELBW infants increased significantly ($p<0.005$) more FV and FVII concentration values from birth (median 60% and range 27-102%, median 46% and range 14-81%, respectively) to six months (median 111% and range 85-164%, median 107% and range 82-188%, respectively) than the other three study groups (Fig.1B, 1C).

Mean AT and PS activities reached term infant and adult values at six months (Fig. 2). Mean PC activity reached the term infant value at six months, but was, as expected, lower than the adult value (Fig. 2).

FV Leiden (R506Q) was identified in three (3.6%) infants with GA 30, 31 and 34 weeks. None of them had any thrombotic or haemorrhagic problems after birth. No central venous lines were inserted in these three infants.

At six months: infants with BPD (n=27) had similar coagulation factor and physiological anticoagulant activities as infants without BPD (n=55). Infants with asphyxia at birth (n=14) had lower median coagulation factor FX activity at the age of six months than infants without birth asphyxia (73% vs. 79%, $p=0.048$). Septic infections at birth did not affect coagulation status at six months.

Coagulation status of sick vs. healthy ELBW infants

Although all infants born before 28 weeks of gestation (n=15) had some morbidity, they were divided into two groups: healthy and sick. An ELBW infant was regarded as healthy (n=8) at the time of the first coagulation factor blood samples if the infant had no other difficulties than respiratory distress at birth. Sick ELBW infants had respiratory distress, and IVH later (n=7). Sick ELBW infants had lower median prothrombin activity at birth than healthy infants (27% vs. 38%, $p=0.144$), but the difference was not significant.

At the corrected age of six months, sick infants still had lower median prothrombin activity (80% vs. 92%, $p=0.144$), but the median activities of FV, FVII and FX were similar in both groups. The median AT and PC activities were also similar, but the median PS activity was lower in sick infants than in

Table 2: Spearman's correlation between coagulation factors and physiological anticoagulants in preterm infants without intraventricular haemorrhage (n=74). Coagulation factors were measured at birth and at the corrected age of six months, and physiological anticoagulants at the corrected age of six months. Coagulation factors = FII, (prothrombin), FV, FVII and FX; PC =protein C, PS= protein S, ATIII = antithrombin.

			FII at birth	FV at irth	FVII at birth	FX at birth	FII at six months	FV at six months	FVII at six months	FX at six months	ATIII at six months	PC at six months
Spearman's rho		Correlation Coefficient										
	FV at birth	Correlation Coefficient	,547**									
		Sig. (2-tailed)	,000									
	FVII at birth	Correlation Coefficient	,515**	,686**								
		Sig. (2-tailed)	,000	,000								
	FX at birth	Correlation Coefficient	,585**	,602**	,614**							
		Sig. (2-tailed)	,000	,000	,000							
	FII at six months	Correlation Coefficient	,195	,105	,158	,071						
		Sig. (2-tailed)	,096	,375	,178	,546						
	FV at six months	Correlation Coefficient	,040	,313**	,191	,131	,210					
		Sig. (2-tailed)	,732	,007	,103	,265	,072					
	FVII at six months	Correlation Coefficient	,007	,046	,183	,101	,405**	,429**				
		Sig. (2-tailed)	,951	,698	,119	,393	,000	,000				
	FX at six months	Correlation Coefficient	,092	-,008	,154	,192	,667**	,210	,437**			
		Sig. (2-tailed)	,434	,943	,191	,101	,000	,072	,000			
	ATIII at six months	Correlation Coefficient	-,016	,121	,150	-,042	,466**	,207	,183	,344**		
		Sig. (2-tailed)	,892	,305	,201	,725	,000	,077	,119	,003		
	PC at six months	Correlation Coefficient	,123	,131	,268*	,165	,444**	,065	,303**	,401**	,459**	
		Sig. (2-tailed)	,298	,267	,021	,159	,000	,581	,009	,000	,000	
	PS at six months	Correlation Coefficient	,000	-,130	-,158	-,092	,446**	,277*	,323**	,327**	,162	,060
		Sig. (2-tailed)	,998	,270	,178	,437	,000	,017	,005	,004	,167	,614

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3: Coagulation study differences at birth and at the corrected age of six months comparing ELBW infants to the infants born at 34-36 weeks gestation. Coagulation values are activities (%). Values are expressed as means (SD).

	ELBW infants (n = 15)	GA 34 – 36 (n = 27)	p*
At birth:			
Factor II	32 (9)	41 (8)	0.007
Factor VII	42 (18)	73 (22)	0.000
Factor X	34 (10)	41 (10)	0.031
Factor V	58 (21)	85 (22)	0.001
At the corrected age of six months:			
Factor II	86 (13)	84 (16)	NS
Factor VII	111 (29)	102 (23)	NS
Factor X	79 (15)	80 (14)	NS
Factor V	115 (24)	107 (16)	NS
Antithrombin	100 (7)	100 (13)	NS
Protein C	69 (17)	66 (13)	NS
Protein S	92 (22)	86 (14)	NS

GA gestational age; ELBW extremely low birth weight infants (BW < 1000g);

* Mann-Whitney U-test was used; NS not significant (p>0.05)

healthy ones (73% (range 70-92%) vs. 110% (range 59-135%), p=0.080). There were no thrombophilic infants among these ELBW infants with IVH.

Infants with retinopathy (n=6) had higher median FV activity at six months than those without retinopathy (142% (range 80-153%) vs. 109 (range 61-164%), p=0.135).

Correlations between coagulation factors and anticoagulants

The correlation between the coagulation factors and anticoagulants at birth and at six months in the infants without IVH (n=74) are given in Table 3. At birth, prothrombin correlated significantly with coagulation factors FV, FVII and FX, and at six months with FVII and FX. Notably, FV at birth correlated significantly with FV at six months (p= 0.007). Prothrombin correlated with physiological anticoagulants AT, PC and PS at the corrected age of six months. FVII at birth and at six months correlated with PC measured at six months (p=0.021 and p=0.009, respectively). FVII at six months also correlated significantly with PS (p=0.005).

Discussion

The main findings in the present study are that prothrombin, FV, FVII and FX reach six-month-old term infant values at the corrected age of six months, even in ELBW infants. New technology has made it possible to take blood samples from ELBW infants for the analysis of several haemostatic factors (12). The coagulation defects seen in extreme prematurity were corrected by impressive gains in coagulation factor activities. To our knowledge, this is the first prospective follow-up study of the development of the specific coagulation factors and physiological anticoagulants in ELBW infants.

Earlier studies (1-3, 5, 6) have shown that the coagulation system in foetuses and preterm infants develops rapidly toward term infancy. The problem in studies concerning preterm infants has been the limited number of patients, with a lack of the smallest infants. Andrew et al. (2) studied 67 infants born between 30 and 33 weeks of gestation of which only 26 were available for follow-up study at six months. Reverdiau-Moalic et al. (6) studied the coagulation status of 64 healthy human foetuses at the age of 19-38 weeks of gestation, but they did not have any follow-up of the infants. Reference ranges for preterm infants >30 weeks of gestation and full-term infants have been determined mainly according to these studies (13, 14).

The vitamin K-dependent factors prothrombin, FVII and FX, which have varying half lives in circulation, were selected for the study. Factor II has the longest natural half life, and factor VII the shortest; they therefore reflect well the vitamin K-dependent coagulation factor synthesis in the liver. In addition, FV was analysed despite its lability, because of the expected early maturation of synthesis (2, 5, 6). At the corrected age of six months, prothrombin and FX were at low adult values, but FV and FVII reached high adult values, and the gain in the concentration by the age of six months was greatest in the ELBW infants. This gain may be considered a sign of developmental catch up seen in, for example, the weight gain of ELBW infants.

In the present study FV at birth in infants without IVH correlated with prothrombin, FVII and FX at birth. FV at birth also correlated with FV at six months. This may underscore the importance of FV among other early developing factors of haemostasis, such as FVIII and platelets (2, 6, 15). The synthesis of coagulation factors has been assessed for FV in hepatocytes from 5- to 10-weeks GA embryos (6). FVa binds with FXa on a membrane surface to form the prothrombinase complex. This is the essential activator of prothrombin to thrombin (16). The consumption coagulopathy of preterm infants may not affect all coagulation factors similarly, so the correlations may suggest true physiologic regulation.

No correlation was found between vitamin K-dependent coagulation factors and FV by van Hylckama Vlieg et al. (17), but they did find that FV was more correlated with FVIII and fibrinogen in healthy adults. In our study, neither FVIII nor fibrinogen was included in the analyses.

The capacity to form thrombin is centrally regulated by prothrombin during infancy (14). In this study, prothrombin correlated with physiological anticoagulants at the corrected age of six months. The data may suggest that synthesis of physiological anticoagulants may be carefully regulated to match especially prothrombin formation.

Recently, van Hylckama Vlieg et al. (17) found clustering between FVII and FX and between prothrombin and PC in healthy adults. They presented evidence for inter-relations between the levels of coagulation factors in the clotting cascade. Our results from preterm infants are in line with the findings of van Hylckama Vlieg et al. (17) from adult subjects. In our study, vitamin K- dependent coagulation factors correlated with each other among infants without IVH. FVII also correlated with PC. This correlation may be explained by the fact that vitamin K is an essential cofactor for both FVII and PC (19). FVII and PC have short plasma half lives in relation to the other vitamin K-dependent factors. Van Hylckama Vlieg et al. (17) suggest that the genetic basis for the regulation of the coagulation system may lie outside the genes coding for these factors.

In our patients, mean PC activity reached the six-month-old term infant values by the corrected age of six months, but was, as expected, at the mean level of 60 – 70 % of adult values (2, 20). Accordingly, only 25 (30%) of 82 infants at six months had PC levels within adult reference range values (73-126%). These PC levels did not depend on the GA of the infant at birth, and only one infant had IVH after birth. Hence, low PC activity at six months refers to immature synthesis in the liver (19, 20).

As in earlier studies (2, 3, 19), AT and PS were at adult levels at the corrected age of six months. Median PS activity at six months did not depend on the GA of the infant at birth (Figure 2). Interestingly, infants with IVH had lower mean and median PS activity concentrations at six months than infants without IVH. This was even more obvious when only infants with GA less than 28 weeks were included in the comparisons, even though the difference was not significant (p=0.08). Poor utilization of vitamin K cannot be the explanation, since other vitamin K-dependent factors were normal. PS has been reported to circulate in two forms: a free active form, and an inactive form that circulates bound to C4b binding protein (C4b – BP) (21, 22). It has been shown that during acute phase response the plasma concentrations of C4b – BP isoforms lacking the β chain increase more than those containing the β chain. The stability in the levels of free PS concentrations may therefore depend on the concerted increases of PS and the C4b – BP β chain (23). In our study, only functionally active PS was measured, leaving open the possible role of higher values of the C4b – BP levels in infants with IVH. Since there is no indication that IVH at birth per se would be an especially proinflammatory condition over an extended period of time, the likelihood of C4b – BP bias is small.

In summary, our prospective follow-up study determined the postnatal development of prothrombin, FV, FVII and FX at birth and at six months, and the six month levels of the physiological anticoagulants AT, PS and PC in 82 preterm infants born between 24 and 36 weeks of gestation. A notable gain in coagulation factor levels among ELBW infants during the follow-up period was found. This finding suggests the preservation of a developmental capacity in spite of immaturity at birth in ELBW infants. Interesting associations with clinical conditions and correlations between haemostatic factors such as FV and the development of FVII and PC may indicate that there are still unrecognized pathways in the function of newborn haemostasis.

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References

1. Hathaway WE. The bleeding newborn. Semin Hematol 1975; 12: 175–88
2. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the healthy premature infant. Blood 1988; 72: 1651–57
3. Andrew M, Paes B, Milner R, et al. Development of the human coagulation system in the healthy full-term infant. Blood 1987; 70: 165–72
4. Piecuch RE, Leonard CH, Cooper BA, et al. Outcome of extremely low birth weight infants (500 to 999 grams) over a 12-year period. Pediatrics, 1997; 100; 633–9
5. Forestier F, Daffos F, Galactéros F, et al. Hematological values of 163 normal fetuses between 18 and 30 weeks of gestation. Pediatr Res 1986; 20: 342 -6
6. Reverdiau-Moalic P, Delahousse B, Body G, et al. Evolution of blood coagulation activators and inhibitors in the healthy human fetus. Blood 1996; 88: 900–6.
7. Barnard D, Simmons M, Hathaway W. Coagulation studies in extremely premature infants. Pediatr Res 1979; 13: 1330–5
8. Shankaran S, Bauer CR, Bain R, et al. Prenatal and perinatal risk and protective factors for neonatal intracranial hemorrhage. Arch Pediatr Adolesc Med 1996; 150: 491-7
9. Mautone A, Girdano P, Montagna O, et al. Coagulation and fibrinolytic systems in the ill preterm newborn. Acta Paediatr 1997; 86: 1100–4.
10. Puckett RM, Offringa M. Prophylactic vitamin K for vitamin K deficiency bleeding in neonates

(Cochrane Review). In: The Cochrane Library, Issue 1 2003; Oxford: Update Software.

11. Crowther CA, Henderson-Smart DJ. Vitamin K prior to preterm birth for preventing neonatal periventricular haemorrhage (Cochrane Review). In: The Cochrane Library, Issue 1 2001; Oxford : Update Software.
12. Salonvaara M, Riikonen P, Kekomäki R, et al. Effects of gestational age and pre-and perinatal events on the coagulation status in premature infants. Arch Dis Child Fetal Ed 2003; 88: F324-F328.
13. Williams MD, Chalmers EA, Gibson BE. Guideline. The investigation and management of neonatal haemostasis and thrombosis. Br J Haematol 2002; 119: 295–309.
14. Kuhle S, Male C, Mitchell L. Developmental hemostasis: pro-and anticoagulant systems during childhood. Semin Thromb Hemost 2003; 29: 329-37.
15. Kühne T, Imbach P. Neonatal platelet physiology and patophysiology. Eur J Pediatr 1998; 157: 87-94.
16. Mann KG, Kalafatis M. Factor V: a combination of Dr Jekyll and Mr Hyde. Blood 2003; 101: 20–30.
17. Van Hylckama Vlieg A, Callas PW, Cushman M, et al. Inter-relation of coagulation factors and D-dimer levels in healthy individuals. J Thromb Haemost 2003; 1: 516-22.
18. Nicolaes GAF, Dahlbäck B. Factor V and thrombotic disease. Description of a Janus-Faced Protein. Arterioscl Thromb Vasc Biol 2002; 22: 530–8.
19. Fair DS, Marlar RA. Biosynthesis and secretion of factor VII, protein C, protein S, and the protein C inhibitor from a human hepatoma cell line. Blood 1986; 67: 64–70.
20. Andrew M, Vegh P, Johnston M, et al. Maturation of the hemostatic system during childhood. Blood 1992; 80: 1998–2005.
21. Malm J, Bennhagen R, Holmberg L, et al. Plasma concentrations of C4b-binding protein and vitamin K-dependent protein S in term and preterm infants: low levels of protein S-C4b-binding protein complexes. Br J Haematol 1988; 68: 445–9.
22. Sthoeger D, Nardi M, Karpatkin M. Protein S in the first year of life. Br J Haematol 1989; 72: 424–8.
23. Garcia de Frutos P, Mohammed Alim RI, Härdig Y, et al. Differential regulation of α and β chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. Blood 1994; 84: 815-22.