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Coagulation Physiology and Abnormalities in Newborn Infants

Association with Intraventricular Haemorrhage and Central Venous Catheter-related Thrombosis

Doctoral dissertation

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ABSTRACT

INTRODUCTION: Perinatal and neonatal intensive care has advanced in recent years. A significant proportion of the smallest and the sickest infants survive, but they still suffer from neonatal complications such as intraventricular haemorrhage or thrombotic complications. The development of haemostasis, especially in extremely low birth weight infants (ELBW), is still poorly characterized. Moreover, the possible associations of haemostatic factors with the haemorrhagic or thrombotic complications in neonates have not yet been studied in detail.

AIMS OF THE STUDY: The main objective of this study was to investigate the development of the coagulation and anticoagulation system in preterm infants, with special emphasis on haemorrhagic complications after birth. A further objective was to investigate the anticoagulation status of infants with central venous catheter (CVC) -related thrombotic complications during infancy.

PATIENTS AND METHODS: The patients were infants born less than 37 weeks of gestation and who needed neonatal intensive care after birth. Coagulation factors II (FII), V (FV), VII (FVII) and X (FX) were measured at birth and at the corrected age of six months. The physiological anticoagulants antithrombin (AT), protein C (PC) and protein S (PS) were determined at the corrected age of six months. Factor V Leiden mutation was also investigated. The associations between coagulation status and perinatal and neonatal variables were examined. Infants with CVC inserted after birth and having had symptomatic thrombotic complications within three months of age were studied for prothrombotic risk factors.

RESULTS: The coagulation status of 125 preterm infants was studied at birth, and 82 of these infants could be followed-up until the corrected age of six months. The lowest mean levels of FII, FV, FVII and FX (31%, 57%, 43%, and 35%, respectively) and mean platelet count (181x 10^9/l) at birth were detected in preterm infants born at 24-27 weeks gestation. The values increased with higher gestational age (GA). Asphyxiated infants had significantly lower mean FII, FV, FVII and FX concentration and platelet count, and higher mean INR values, than infants without asphyxia at birth. Small for gestational age infants had significantly lower mean FV and FVII activities and platelet count than appropriate for gestational age infants. At the corrected age of six months, regardless of the GA at birth, FII, FV, FVII and FX reached the healthy term six-month-old infant coagulation factor levels. IVH in very low birth weight infants (n=38) was associated with birth weight <1000g (p=0.012) and low FII activity (p=0.024) at birth.

At birth FII correlated significantly with FV, FVII and FX, and at six months FII correlated with FVII and FX. FII also correlated with AT, PC and PS at the corrected age of six months. FVII at birth and at six months correlated significantly with PC at six months (p=0.021 and p=0.009, respectively). FVII at six months also correlated significantly with PS (p=0.005). These correlations may indicate new as yet unknown inter-relations between coagulation and anticoagulation systems.

Symptomatic catheter-related thrombosis was detected in 10/44 (23%) infants. A positive family history with thromboembolic episodes at young age was found in 3/10 families. All the infants with thrombosis had undergone abdominal surgery. Only one prothrombotic factor (FV Leiden mutation) was demonstrable within the patient population.

CONCLUSIONS: A notable gain in coagulation factor levels among ELBW infants during the follow-up period could be verified. IVH in VLBW infants was associated with low FII activity at birth. New inter-relations between coagulation factors and physiological anticoagulants could be demonstrated. Newborns with CVC in association with abdominal surgery seemed to be at great risk of CVC-related venous thrombosis.

National Library of Medicine Classification: WS 420, WH 310, WL 355, WB 365
Medical Subject Headings: infant, newborn; infant, very low birth weight; infant, small for gestational age; fetal growth retardation; cerebral hemorrhage; blood coagulation; blood coagulation disorders; thrombosis; blood coagulation factor inhibitors; catheterization, central venous

To my family

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Hämeenlinna, December 2004

Marjut Salonvaara

LIST OF ABBREVIATIONS

AGA	Appropriate size for gestational age
APC	Activated protein C
APTT	Activated partial thromboplastin time
AT	Antithrombin
BW	Birth weight
BPD	Bronchopulmonary dysplasia
C4bBP	C4b binding protein
CP	Cerebral palsy
CVC	Central venous catheter
DIC	Disseminated intravascular coagulation
DVT	Deep venous thrombosis
ELBW	Extremely low birth weight
FII	Coagulation factor II (prothrombin)
FV	Coagulation factor V
FVII	Coagulation factor VII
FVIII	Coagulation factor VIII
FX	Coagulation factor X
GA	Gestational age
HDN	Hemolytic disease of newborn
ICD -10	International Classification of Diseases
INR	International normalised ratio
IUGR	Intrauterine growth restriction
IVH	Intraventricular haemorrhage
LMWH	Low molecular weight heparin
MABP	Mean arterial blood pressure
NEC	Necrotizing enterocolitis
NICU	Neonatal Intensive Care Unit
PC	Protein C
PS	Protein S
PT	Prothrombin time
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SD	Standard deviation
SGA	Small for gestational age
TAT	trombin-antithrombin-complex
TE	tromboembolism
UFH	Unfractioned heparin
US	Ultrasound
VLBW	Very low birth weight
VTE	Venous thromboembolism

LIST OF ORIGINAL PUBLICATIONS

I. Salonvaara M. Riikonen P, Kekomäki R, Vahtera E, Mahlamäki E, Halonen P, Heinonen K. Effects of gestational age and prenatal and perinatal events on the coagulation status in premature infants. Arch Dis Child Fetal Neonatal Ed 2003; 88: F319-23.

II. Salonvaara M, Riikonen P, Kekomäki R, Vahtera E, Mahlamäki E, Kiekara O, Heinonen K. Intraventricular haemorrhage in very low birth weight preterm infants: association with low prothrombin activity at birth. Accepted (Acta Paediatr 2005; in press).

III. Salonvaara M, Riikonen P, Vahtera E, Heinonen K, Kekomäki R. Development of selected coagulation factors and anticoagulants in preterm infants by the age of six months. Thromb Haemost 2004; 92: 688-96.

IV. Salonvaara M, Riikonen P, Kekomäki R, Heinonen K. Clinically symptomatic central venous catheter – related deep venous thrombosis in newborns. Acta Paediatr 1999; 88: 642-6

CONTENTS

1. INTRODUCTION

Over the past century, significant advances have been made in the understanding of the human coagulation system. The coagulation cascade was first described in the 1960s (Davie et al. 1964, Macfarlane 1964). Since then, a vast amount of information concerning coagulation factors and their role in coagulation has been reported.

Although acquired disorders most often present in sick term or preterm infants, many inherited disorders manifest in otherwise healthy infants (Williams et al. 2002). Recognition of the clinical status in which bleeding occurs is therefore an important clue to the underlying diagnosis.

The haemostasis of the newborn is still poorly understood, especially that of extremely low birth weight (ELBW) infants. The plasma concentrations of most haemostatic proteins are significantly different in foetuses, preterm and term neonates, infants and adults (Andrew et al. 1987 and 1988, Reverdiau-Moalic et al.1996). The haemostatic process is dynamic and evolves throughout infancy and childhood (Andrew et al. 1990a).

Because of the recent advances in perinatal and neonatal intensive care, most of the smallest and sickest infants survive (Lemons et al. 2001, Tommiska et al. 2003), so the number of infants with neonatal problems is increasing. Understanding of the evolution of the coagulation system in neonates is needed to ensure accurate diagnosis and appropriate interventions for preterm infants and sick neonates (Andrew 1997).

The purpose of this work was to evaluate the selected coagulation factors at birth and at six months of age in preterm infants. Development of coagulation status, and relations between coagulation factors and morbidity especially in ELBW infants were emphasised.

In addition, neonatal thromboembolic events associated with central venous catheters (CVC) and other prothrombotic risk factors were also studied.

2. REVIEW OF THE LITERATURE

2.1. Selection of the literature

The review of the literature was based on a computer-assisted search of publications up to the end of October 2004.The citations and abstracts were searched from PubMed, a web-based retrieval, which was developed by the National Center for Biotechnology Information (NCBI) at the National Library of Medicine (NLM), located at the National Institutes of Health (NIH), Bethesda, MD, USA. PubMed provides access to bibliographic information that includes MEDLINE. This is the NLM's premier bibliographic database containing bibliographic citations and author abstracts from biomedical journals published in the United States and 70 other countries. The database contains over 12 million citations dating back to the mid-1960s. Coverage is worldwide, but records in this review are only from English-language sources.

The following terms were used in the search from the database: infant, neonate, newborn, prematurity, preterm infants, very low birth weight, extremely low birth weight, small for gestational age, intrauterine growth retardation, intracerebral h(a)emorrhage, intraventricular h(a)emorrhage, blood coagulation, coagulation factors, coagulation disorders, thrombosis, blood coagulation inhibitors, central venous catheter, low molecular weight heparin, thrombolytic therapy.

2.2. Haemostasis

2.2.1. Normal haemostasis

The blood coagulation system was called a "warterfall" by Davie and Ratnoff (1964) and a "cascade" by Macfarlane (1964). Today's modern concept of the scheme of blood coagulation is a complex network of interactions regulated by positive and negative feedbacks (Davidson et al. 2003).

Haemostasis in a healthy individual is a balanced system where the haemorrhagic tendency is counteracted by thrombotic factors including a process where haemorrhage caused by vascular injury is arrested. The general purpose of the coagulation proteins is to generate thrombin and make a fibrin plug to stop bleeding (Davidson et al. 2003).

The vessel wall, platelets and coagulation factors have a linked interaction. This primary haemostasis is activated by injury, which activates platelet adhesion and

aggregation. Tissue factor (TF) activated by vessel wall injury activates coagulation proteins. That is called secondary haemostasis (Roberts et al. 1998).
The activation of the coagulation system and the natural anticoagulation system and fibrinolysis is shown in **Figure 1**.

Vitamin K-dependent coagulation factors II (FII), VII (FVII), IX (FIX) and X (FX), protein C (PC) and protein S (PS) are synthesized in the liver. Coagulation factors have different elimination half-lives: FII (prothrombin) has the longest half-life, 60-70 hours, while FVII has the shortest half-life, 3-6 hours. FIX has a half-life of 18-24 hours and factor X (FX) 30-40 hours (Roberts et al. 2001).

FV is stored in platelet α-granules and secreted upon platelet stimulation (Nicolaes and Dahlbäck 2002). Twenty percent of the circulating FV is found in platelet alpha granules (Roberts et al.2001). The half-life of FV is 12 hours (Roberts et al. 2001). FV is the inactive precursor of factor Va, which contributes to the blood clotting fraction by binding with FXa (Mann and Kalafatis 2003). The activation of FV to FVa is essential to its biologic function. FV Leiden and other FV gene mutations result in abnormal FV function. They result in either low circulation levels of the protein or the complete deficiency of FV. FV is needed for normal blood clotting physiology and the impaired evolution of FV activation can cause severe pathology (Mann and Kalafatis 2003).

FV and FVIII are related proteins and share common biosynthetic pathways. FV presents itself in two forms. In its activated form it has essential functions in the procoagulant pathway; on the other hand, the nonactivated form has anticoagulant properties as an activated protein C (APC) cofactor in the regulation of FVIIIa activity (Nicolaes and Dahlbäck 2002). APC is as an inhibitor of the activities of FVa and FVIIIa (Roberts et al. 2001).

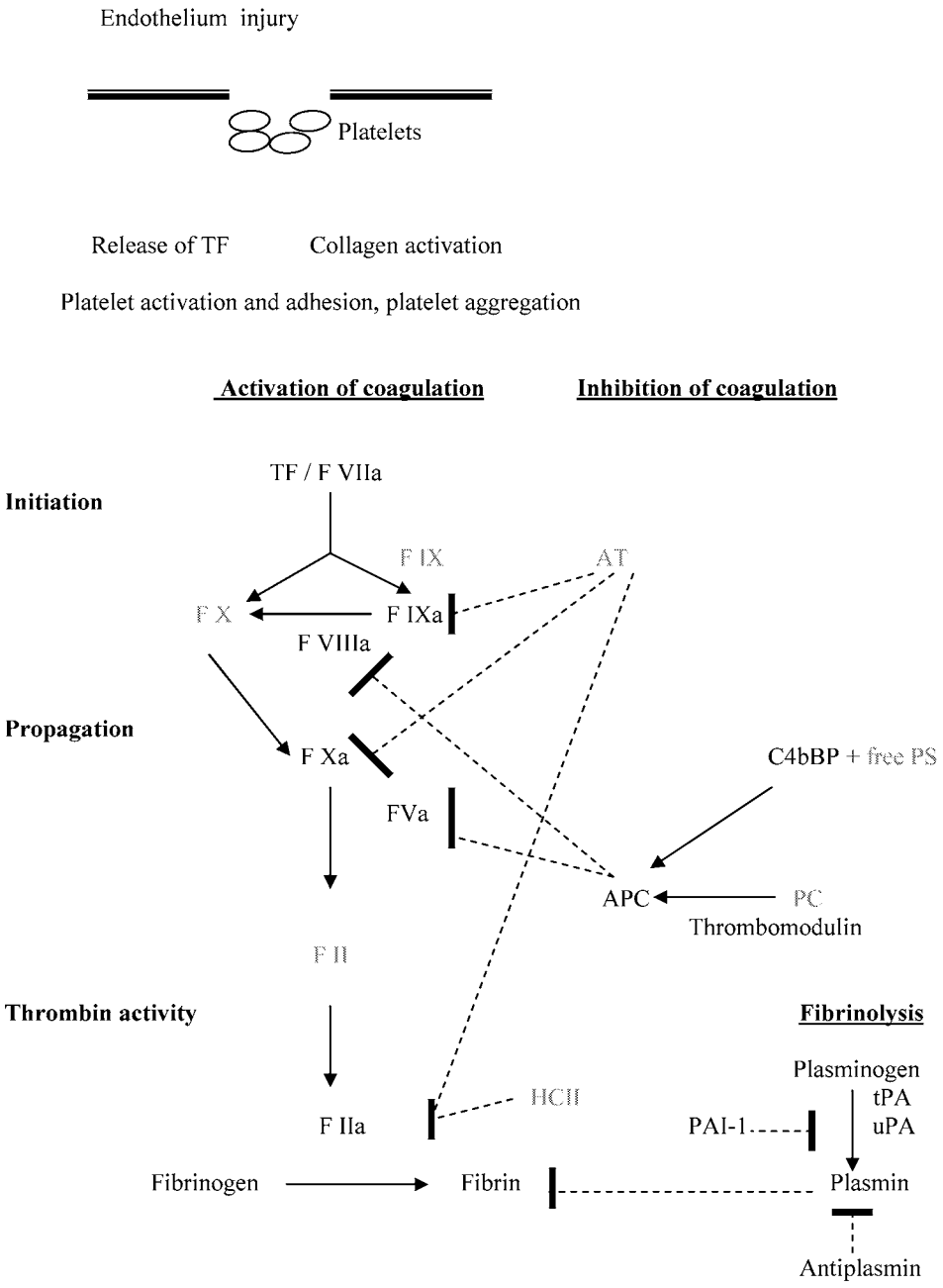

Figure 1. Normal haemostasis. Tissue factor (TF) is released from the subendothelium initiating the reaction involving factor VIIa. Activated protein C (APC) degrades the thrombin-activated forms of factors Va and VIIIa. Inhibition of thrombin is marked with dotted lines (----). The decreased plasma levels of procoagulants and inhibitors in foetuses and preterm infants are marked in orange.

PC is produced in the liver and has a half-life of 6 hours (Roberts et al. 2001). It is a vitamin K-dependent zymogen which circulates as a two-chain, biologically inactive species. The active enzyme, APC, is generated by thrombin-mediated cleavage. It also has multiple anti-inflammatory functions (Esmon 2003). This physiologic event requires thrombomodulin as a thrombin cofactor.

APC-resistant individuals lack an essential APC cofactor, which is suggested to be intact FV. This holds the potential to function in synergy with PS as an APC cofactor in the activated FVIII (FVIIIa). Thus, FV, PS, and APC regulate the activity of the complex of FIX and FVIIIa, which activate FX (Nicolaes and Dahlbäck 2002).

Certain plasma proteins interacting with endothelial cell surfaces and fibrin regulate the generation and inhibition of thrombin. Antithrombin (AT) is produced in the liver and has a half-life of 72 hours (Roberts et al. 2001). It inactivates thrombin as well as activated coagulation factors FXa, FIXa, FXIa and FXIIa (Lane and Caso 1989).

PS is a vitamin K-dependent glycoprotein, which is produced mainly in the liver, but it has also been identified in megacaryocytes and endothelial cells. PS plasma half-life is about 42 hours (Roberts et al. 2001). In human plasma, about 40% of PS circulates as free PS and about 60% of PS circulates in complex with C4b-binding protein (C4bBP) (Rezende et al. 2003). The formation of this complex results in loss of PS cofactor function. Only free PS has APC cofactor activity (Rezende et al. 2003).

Fibrinogen is synthesized by hepatocytes. It is found in plasma and in platelet α-granules. Fibrinogen is an acute-phase reactant and its synthesis can be increased with strong inflammatory stimulus. Its half-life is 3 to 5 days. It has a plasma concentration of 2.0-4.0 g/l (Roberts et al. 2001).

To achieve normal haemostasis, thrombin generation is regulated by a number of inhibitors (Mann et al. 2003). The activation of the fibrinolytic system occurs when plasminogen is converted into plasmin by plasminogen activators, such as tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) (Rijken 2003). These plasminogen activators are inhibited by plasminogen activator inhibitor-1

(PAI-1). This is synthesized by endothelial cells and hepatocytes and is present in plasma and on the surface of platelets. Plasmin is primarily inhibited by plasmin inhibitor (α2-antiplasmin). The activation of plasminogen by tPA is enhanced by fibrin. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a recently discovered fibrinolysis inhibitor that presents a link between coagulation and fibrinolysis. TAFI is synthesized in the liver (Bouma and Meijers 2003).

2.2.2. Haemostasis in the foetus

Placenta is probably the source of the increased thrombin generation noted during pregnancy (Andrew et al. 1990b, Delorme et al. 1992). Components of haemostasis are synthesised by the foetus and they do not cross the placenta from the mother into the foetus (Cade et al. 1969). The components of primary haemostasis are well developed in foetuses. Thrombopoietin, the major regulator of platelet homeostasis, is detectable in the foetal liver at six weeks of gestation (Sola et al. 1999). The platelet count of foetuses between 18 and 30 weeks of gestation is within the normal range for adults of 150 to 450 x 10^9/l (Israels et al. 2003).

Plasma concentrations of many coagulation factors change during foetal life (Delorme et al. 1992). Most coagulation proteins appear in the blood circulation by approximately 10 weeks of gestation age (GA) and increase gradually with GA (Bleyer et al. 1971, Reverdiau-Moalic et al. 1996). Some of these result from decreased gene expression in the immature liver (Hassan et al. 1990). Synthesis of fibrinogen and FVIII has been found in 5- to 10-week GA embryos (Forestier et al. 1986, Hassan et al. 1990, Reverdiau-Moalic et al. 1996).

Foetal haemostatic system functions are at lower levels than in the newborn infant. It is likely that the haemostatic system is balanced, since bleeding and thrombotic complications are not a feature of normal foetal life (Kuhle et al 2003b).

2.2.3. Haemostasis in pretem and newborn infants

The significance of and variation in the levels of the different components of the coagulation system for ELBW infants have not been fully described (Forestier et al. 1986, Andrew et al. 1990a). Because of the difficulties in obtaining blood from the smallest preterm infants, most of the reference data for preterm infants born before 28

weeks of gestation are obtained from foetuses (Forestier et al. 1986, Reverdiau-Moalic et al. 1996). There is a lack of strong evidence for many of the recommendations suggested, as the appropriate clinical and laboratory trials have not been undertaken in neonates. Therefore, reference values for haemostasis of the foetus and newborn have been collected from incomplete and heterogenous data (Hathaway and Corrigan 1991, Williams et al. 2002, Kuhle et al. 2003b).

The haemostatic system in neonates markedly differs from that of adults (Andrew et al. 1987 and 1988). By six months of age, most components of the coagulation system have attained normal adult values. The accurate diagnosis of haemostatic disorders in newborn infants must be made in the context of age-dependent physiological values of the haemostatic components (Andrew et al. 1990a, Kuhle et al. 2003b).

Some of the selected studies presenting foetal and neonatal haemostatic data after birth are presented in **Table 1**.

2.2.4. Vitamin K-dependent factors: FII, FVII, FIX and FX, PC and PS, and vitamin K prophylaxis

Coagulation factors. The low concentrations of vitamin K-dependent coagulation factors at birth are due to defective gamma-carboxylation of the precursor proteins (Suttie et al.1980, Suttie 1993). Plasma concentrations of FII, FVII, FIX and FX are less than 70% of normal adult values (Andrew at al. 1990a). The rate of thrombin generation is delayed and decreased in newborns compared with adults. The amount of thrombin generated is affected by the plasma concentrations of prothrombin, i.e. factor II (Andrew et al. 1990b). Thrombin generation remains reduced throughout childhood (Andrew et al. 1994c). Plasma concentrations of all four vitamin K-dependent coagulant factors are decreased for many weeks postnatally (Andrew 1997).

Protein C and Protein S pathways. PC and PS systems develop through infancy, achieving normal adult levels only by adolescence (van Teunenbroek et al. 1990, Kuhle et al. 2003b). The PC pathway plays a significant role in the physiological regulation of coagulation (Petäjä and Manco-Johnson 2003). Petäjä et al. (1998) studied the PC pathway of newborn infants, and they showed low levels of PC. APC showed no

Table 1. Selected haemostatic data of foetuses and newborn infants

Authors	Patient type	N	Blood sample type	Age	Haemostatic data studied	Comments
Heikinheimo 1964	8 foetuses 12 fullterm	23	cord blood	foetuses in utero; newborns at birth	screening tests, coagulation factors II, V and VII, fibrinogen	foetuses GA 12-16 wk; foetuses were generally found to have the same coagulation factor content as full-term newborns
Chessells and Wigglesworth 1971	GA 34-40 wk	9	cord blood at birth; follow up umbilical artery/ peripheral vein	0-3 and 24 hours	screening tests, platelets, fibrinogen, FDP	asphyxiated infants at birth showed evidence of disordered haemostasis (DIC)
Condie 1976	23 from normal pregnancy 16 from pre-eclamptic mothers	39	cord blood	at birth	coagulation factors X, XI, and XII, fibrinogen, fibrinolysis	GA of the infants was not reported; no significant difference between normal pregnancy and pre-eclampsia
Barnard et al. 1979	30 premature (GA 24-31 wk); 24 healthy fullterm	54	Preterm: umbilical artery Fullterm: cord blood	preterm: 0-3 hours; fullterm at birth	screening tests, platelet count, coagulation factors, AT, FDP	FII and FVII were significantly lower in preterm infants than in term infants
Lox et al. 1985	55 healthy fullterm 52 pre-eclamptic fullterm	107	cord blood	at birth	screening tests, fibrinogen, coagulation factors	Significant correlations in pre-eclamptic mothers and their neonates in APTT, factors II, V, and VII

GA = gestational age; FDP = fibrinogen degradation products; APTT = activated partial thromboplastin time; DIC = disseminated intravascular coagulation; SGA= small for gestational age; RDS = respiratory distress; AT= antithrombin; PTT = partial thromboplastin time; PT = prothrombin time

Table 1. Selected haemostatic data of foetuses and newborn infants (continued)

Authors	Patient type	N	Blood sample type	Age	Haemostatic data studied	Comments
Dube et al. 1986	159 preterm (GA ≤ 36 wk) 208 fullterm 18 post-term (GA ≥ 42 wk)	385	cord blood	at birth	screening tests, fibrinogen, FDP	term SGA neonates had significantly prolonged PT and low plasma fibrinogen; FDPs were higher in preterm infants
Forestier et al. 1986	fetuses: 18-20 wk (n=25) 21-22 wk (n=55) 23-25 wk (n= 61) 26-30 wk (n=22)	163	cord blood	fetuses in utero; 63 at birth	blood cell counts, coagulation factors II, V, VIII, IX	no significant increase in coagulation factor concentrations with increasing GA
Andrew et al. 1987	healthy full-term	40 - 79	Venous blood	days 1, 5, 30, 90, 180	screening tests, fibrinolysis, coagulation and inhibition systems	number of patients varied from 29 to 70 in each test; values used as reference data
Andrew et al. 1988	healthy preterm (GA 30-36 wk)	40 - 96	Venous blood	days 1, 5, 30, 90, 180	screening tests, fibrinolysis, coagulation and inhibition systems	number of patients varied from 40 to 96 in each test; values used as reference data
Kazzi et al 1989	preterm 52 GA 23-34 (controls) 51 GA 22-34 (antenatal vitamin K-treatment)	103	cord blood	at birth	screening tests, vitamin K-dependent coagulation factors	no difference between the groups in coagulation profile at birth
Pietersma-de Bruyn et al. 1990	17 preterm (GA 29-34 wk) 7 SGA (GA 35-41)	24	cord blood; venous blood later	at birth, days 3, 7 and 28	coagulation factors II and X, AT, platelet count,	no significant difference between coagulation factors; vitamin K did not affect coagulation studies

GA = gestational age; FDP = fibrinogen degradation products; SGA= small for gestational age; RDS = respiratory distress; AT = antithrombin; PT = prothrombin time

Table 1. Selected haemostatic data of foetus and newborn infants (continued)

Authors	Patient type	N	Blood sample type	Age	Haemostatic data studied	Comments
Shah et al. 1992	40 healthy (GA 30-38 wk) 30 sick (GA 30-38 wk) 20 sick preterm (GA 25-29 wk)	90	Healthy: venous blood; sick: umbilical arterial catheter	0-12 hours after birth	screening tests, fibrinogen, AT, α_2 – macroglobulin, heparin cofactor II	plasma from sick neonates had significantly lower levels of both AT and α_2 - macroglobulin compared with healthy neonates
Seguin and Topper 1994	preterm (GA 24-29 wk)	52	Arterial or venous blood	0-12 hours after birth	APTT, PT, fibrinogen	PT and APTT prolonged
Thorp et al. 1995	preterm (GA 24-33 wk) 90 controls; 98 antenatal vitamin K and phenobarbital therapy	188	cord blood	at birth	coagulation factors II, VII, IX, X and PT, PTT	antenatal vitamin K and phenobarbital therapy did not improve cord blood levels of coagulation factors and PT and PTT
Reverdiau-Moalic et al. 1996	fetuses: 19-23 wk (n=20) 24-29 wk (n=22) 30-38 wk (n=22) newborns (n=60)	164	cord blood	fetuses in utero; newborns at birth	screening tests, coagulation system, coagulation inhibitors	GA of the newborns was not reported; values used for reference data
Mautone et al. 1997	preterm (GA 30- 34 wk)	63	Venous blood	1 day and one week	screening tests, platelets, fibrinogen, AT, TAT, D-dimers, PAI, tPA	Newborns divided into 4 groups: RDS, asphyxia, infection, healthy (control group); both coagulation and fibrinolysis were activated in RDS and sepsis
Hannam et al. 2003	preterm (GA 26-32) 16 SGA + 16 controls	32	Arterial blood	few hours after birth	INR, liver function, fibrinogen	SGA infants had significantly higher INR

APTT = activated partial thromboplastin time; AT = antithrombin; PTT = partial thromboplastin time; PT = prothrombin time; PAI = plasminogen activator inhibitor; tPA = tissue-type plasminogen activator; INR = internatonal normalized ratio; TAT = trombin-antithrombin-complex

significant difference between the cord and adult plasmas. Van Teunenbroek et al. (1990) found a rapid rise in PC activity and antigen levels by the age of 7-9 months. Thereafter, the progression was slower toward adolescence.

The incidence of heterozygous PC deficiency in healthy individuals has been estimated to be 1:300-500 (Miletich et al. 1987, Tait et al. 1995). Homozygous PC or PS deficiency is clinically seen as purpura fulminans in newborn infants (Marlar and Neumann 1990, Salonvaara et. al. 2004). The incidence of this defect is estimated to be at least 1: 500 000 live births (Marlar and Neumann 1990).

PS has an important role as a cofactor to APC in the degradation of coagulation factors Va and VIIIa. Foetal development of PS has been found to occur in the absence of C4bBP (Melissari et al. 1988). C4bBP was observed at the end of intra-uterine life Malm et al. (1988) found that the concentration of C4bBP in cord blood of preterm infants is very low, and it increased toward full term. Term newborns have relatively low levels of C4bBP and high levels of free PS and PS anticoagulant activity (Schwarz et al. 1988). Total PS level in the healthy term newborn on the first day of life is lower than in adults but higher than in the preterm infant. At one year of age, the mean level is still significantly lower than in adults (Sthoeger et al. 1989).

Vitamin K. Vitamin K deficiency can cause bleeding in an infant in the first weeks of life (Shapiro et al. 1986). It is characterized by a prolonged prothrombin time in association with decreased activity of vitamin K-dependent coagulation factors (Sutor 1995). Vitamin K is usually given prophylactically after birth for the prevention of haemorrhagic disease of the newborn (HDN). A single 1.0 mg dose of intramuscular vitamin K after birth is effective in the prevention of classic HDN (Puckett and Offringa 2003). Antenatal administration of vitamin K_1 to pregnant women prior to preterm birth does not improve the haemostatic defects nor does it reduce the incidence or severity of intraventricular haemorrhage (IVH) in their infants (Kazzi et al. 1989, Crowther and Henderson-Smart 2003).

2.2.5. Coagulation factors V and VIII, platelets and fibrinogen

FV and FVIII. Plasma concentrations of FV and FVIII at birth are similar or increased compared with adults (Andrew et al.1987 and 1988). Inherited FV deficiency is extremely uncommon (Nicolaes and Dahlbäck 2002). Newborns with a severe deficiency of FV or FVIII can be diagnosed at birth because concentrations of these factors are then clearly less than normal physiological values (Andrew et al.1987 and 1988b).

The FV Arg506Gln mutation (FV Leiden) affects the anticoagulant APC-cofactor activity of FV in the APC-catalysed inactivation of FVIIIa. As a result of the mutation, the regulation of thrombin formation via the PC pathway is impaired (Bertina et al.1994). The frequency of APC resistance is 2-10 % in the normal population (Hillarp et al. 1995).

Platelets. Thrombopoietin is the main haematopoietic growth factor for platelet production (Chang et al. 1996, Porcelijn et al. 2002) Preterm infants have impaired thrombopoietin response in thrombocytopenia (Watts et al. 1999). There is a relative deficiency in neonatal platelet function (Kühne and Imbach 1998). Acquired and inherited neonatal diseases may influence foetal and neonatal platelet physiology, causing thrombocytopenia and / or platelet dysfunction (Kühne and Imbach 1998, Beiner et al. 2003).

Fibrinogen. Plasma concentrations of fibrinogen are similar in neonates and adults (Roberts et al. 2001). However, Hamulyak et al. (1983) found that fibrinogen in neonates is in the "foetal form", i.e. the phosphorus content of cord blood fibrinogen is 3-4 times higher than that of adult fibrinogen, and thrombin clotting time of cord blood fibrinogen was prolonged.

2.2.6. Physiological anticoagulants: antithrombin and α-2-macroglobulin

Antithrombin. The prevalence of inherited heterozygous AT deficiency in the general population is reported to be 1:250 (Bauer 1998). Thrombosis during childhood in heterozygous AT deficiency is rare, but can occur when acquired risk factors are involved (Andrew et al. 2001). Homozygous deficiency usually presents in newborn

with severe clinical manifestations (Kuhle et al. 2001). Acquired AT deficiency has been linked to nephrotic syndrome (Andrew and Brooker 1996, Citak et al. 2000). Plasma concentrations of AT are decreased in newborns at the time of birth and achieve average adult values by 3 months of age (Andrew et al. 1990a). In preterm infants, AT activity is reduced, depending on GA (Andrew et al. 1987 and 1988) and complications such as RDS, NEC, sepsis and DIC (Peters et al. 1984a, Manco-Johnson 1989, van den Berg et al. 1989). Infants with low levels of AT have been shown to have a higher incidence of IVH (McDonald et al. 1984a).
Cvirn et al. (1999) noted that a reduction of AT and PC concentration in newborn and adult plasma resulted in increased thrombin generation. Their results show that the anticoagulant effect of AT and PC in newborn and adult plasma is the same.

α-2-macroglobulin. Plasma concentrations of α-2-macroglobulin at birth are higher than adult values and are up to twice adult values at six months of age (Andrew et al. 1987 and 1988). Ling et al. (1994) suggested that α-2-macroglobulin is as important inhibitor, as AT in cord plasma. α-2-macroglobulin also acts as a procoagulant by inhibiting APC in both cord and adult plasma (Cvirn et al. 2001b). Mitchell et al. (1991) studied AT deficient children aged 2 to 13 years and report that there was a significant correlation between the α-2-macroglobulin level and ability to inhibit thrombin in AT-deficient patients.

2.2.7. Coagulation screening tests

Coagulation screening tests are commonly used to measure overall capacity for thrombin generation (Lusher 1996, Jennings and Cooper 2003). The most commonly used tests are prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen concentration. PT provides a measure of the FVIIa /Tissue Factor pathway; APTT provides a measure of contact factor pathway. International Normalised Ratio (INR) corrects differences in reagent sensitivities in PT tests (Pinto et al. 1993, Andrew et al. 1995a). The slow rate of thrombin generation in newborns is reflected in prolonged PTs and APTTs (Andrew et al. 1987 and 1988, Hathaway and Corrigan 1991). Schmidt et al. (1992a) showed that early thrombocytopenia was the strongest predictor of coagulopathy in newborns. Due to differences in the haemostasis of newborn infants and adults, the sensitivity of

screening tests at birth is poor. Abnormal coagulation screening tests support the diagnosis of coagulopathy in newborn infants, but normal screens do not exclude the activation of the coagulation and fibrinolytic systems (Schmidt et al. 1993).

2.3. Definitions

2.3.1. Obstetric and maternal risk factors

Pre- and perinatal risk factors. Prenatal risk factors are those influencing the foetus *in utero*, i.e. before birth. Perinatal risk factors have effects from the 20th week of gestation to the age of seven days after birth (Macfarlane et al. 1999).

Pre-eclampsia. Pregnant women who repeatedly have a systolic blood pressure $\geq$ 140 mmHg and a diastolic pressure $\geq$ 90 mmHg are defined as having hypertension. Hypertension accompanied with proteinuria of at least 0.3 g per 24 hours is classified as pre-eclampsia (Report of the National High Blood Pressure Education Program, 2000).

Birth asphyxia. Apgar scores at one and at five minutes are valuable criteria when attempting to define perinatal asphyxia, and newborn acid-base status is an important additional criterion (Levene et al. 1986). Asphyxia diagnosis in ICD-10 is not defined by the cord blood pH values. ICD-10 defines severe birth asphyxia by Apgar scores 0-3 at 1 minute, and mild birth asphyxia is defined by Apgar scores 4-7. An earlier version, ICD-9, also had criteria of cord blood pH below 7.00 in severe birth asphyxia and pH 7.20-7.00 in mild birth asphyxia.

Traditionally, a cord blood pH cut-off of less than 7.20 has been used (Weber 1980, Winkler et al. 1991, Samueloff et al. 1994). Some studies recommend umbilical artery pH below 7.00 as a definition of asphyxia (Goldaber et al. 1991). In preterm pregnancy, Apgar scores are directly related to gestational age, so Apgar scores are not sufficient criteria for the diagnosis of asphyxia in preterm infants (Catlin et al. 1986, Stark et al. 1990).

2.3.2. Neonatal risk factors

Preterm birth. Preterm birth is defined as birth at less than 37 weeks gestation. Infants born at less than 28 weeks gestation are defined as extremely immature preterm infants (ICD-10).

Low birth weight. Definitions depending on the birth weight (BW) of the infants are also used (ICD-10). Low birth weight infants weigh less than 2500 gram at birth. Very low birth weight (VLBW) infants weigh less than 1500 gram at birth. ELBW infants weigh less than 1000 gram at birth.

Small for gestational age (SGA) infants are those who have a birthweight either below the 10^{th} centile or more than two standard deviations (SD) below the gestational age-adjusted mean birth weight (ICD-10). Ethnic growth curves are used in defining SGA (Pihkala et al. 1989).

2.3.3. Prematurity-related diseases

Respiratory distress syndrome (RDS) is immaturity of the lungs, which is charactarised by poorly developed lung structure and immature surfactant synthesis (Hallman et al. 2002). RDS is common in infants born before 30 weeks of gestation, but it might be a significant problem up to 34 weeks of gestation (Lewis et al. 1996).

Retinopathy of prematurity. Retinal vascularization starts at 16 weeks of gestation. Vascularization is completed at term. Retinopathy of prematurity (ROP) is an ischemia-induced proliferative retinopathy, which can be recognized in the preterm infant when morphological changes are seen at the junction of between vascularized and non-vascularized retina (Mechoulam and Pierce 2003). There is an international classification of ROP into five stages (An international classification of retinopathy of prematurity, 1984).

Necrotizing enterocolitis (NEC). NEC primarily affects premature newborns. The clinical staging for infants with NEC is divided into three stages according to clinical and x-ray findings (Bell et al. 1978).

Intraventricular haemorrhage (IVH). IVH and periventricular haemorrhages are graded according to the classification of Papile et al. (1978) as follows: Grade I: subependymal haemorrhage; Grade II: IVH without ventricular dilatation; Grade III: IVH with ventricular dilatation; Grade IV: IVH with parenchymal haemorrhage.

Periventricular leukomalacia (PVL). PVL results in necrosis of the periventricular white matter and damage to the corticospinal fibers in the internal capsule. The pathogenesis of PVL is related to an incomplete state of development of the vascular supply to the brain, impaired cerebrovascular autoregulation and vulnerability of oligodendroglial precursor cells in white matter (Volpe 2001).

Disseminated intravascular coagulation (DIC). DIC is a process charactarized by widespread activation of the coagulation system with the formation of soluble or insoluble fibrin, and in which coagulation factors and platelets are consumed with secondary activation of fibrinolysis. This results in the generation of excess thrombin and plasmin, and may contribute to multiple organ failure (Levi and Ten Cate 1999).

2.4. Mortality and morbidity in prematurity

Because of the improvements in antenatal and neonatal intensive care during the last decades, ELBW mortality has declined significantly, the survival rate now being up 70 to 80% (Järvenpää et al. 1991, Tommiska et al. 2001, Harper et al. 2002). This improvement does not seem to associate with major morbidity, i.e. chronic lung disease, NEC and IVH in the smallest infants (Hack and Fanaroff 2000, Lemons et al. 2001, Tommiska et al. 2003). RDS is a leading cause of mortality and morbidity in premature infants. Maternal administration of corticosteroids reduces the occurence of RDS in preterm births (Spinillo et al. 1995a).

The incidence of IVH decreases with increasing GA, being about 30 to 50 % in ELBW infants (Allen et al 1993, Lemons et al 2001, Tommiska et al. 2001). Depending on the patient population, IVH contributes significant morbidity and mortality in newborn intensive care units; up to 20-40% of preterm infants suffer from some type of IVH (McDonald et al. 1984b). Papile et al. (1978) studied infants with birth weight less than 1500 grams and found an overall incidence of IVH of 43%. Later, a Canadian report (Synnes et al. 2001) concerning infants treated in neonatal intensive care units (NICU) revealed an overall incidence of IVH of 29%. They noted the association between IVH and use of vasopressors and treatment of acidosis.

2.5. Arterial and venous catheters in neonatal intensive care

Better survival of extremely premature and VLBW infants with immature gastrointestinal tract and high requirements for blood products, parenteral nutrition and antibiotic treatment means that prolonged intravenous access is often a major problem in NICUs (Andrew et al. 2001).

Central venous cannulation in infants can be difficult, and repeated attempts might damage the vessel wall causing relatively high risk of thrombosis. Hind et al. (2003) report success with the use of two-dimensional ultrasound (US) for internal jugular vein cannulation.

Catheters may be thrombogenic because of the damage to the vessel wall and disruption of the blood flow. Some intravenously-given fluids may also promote vessel damage (Andrew et al. 2001). To avoid occlusion and/or thromboembolism, heparinisation of the catheters is used. Barrington (2000) report that heparinisation of the infusate decreases the incidence of catheter occlusion, but does not affect the frequency of thrombosis. The Cochrane database review (Shah et al. 2002) shows that there is not enough knowledge to recommend heparin for routine prophylaxis to prevent occlusion or thrombosis in peripherally inserted central venous catheters (CVC).

2.5.1. Central catheters

Catheterisation of the umbilical artery has been used since the 1960s (Gupta et al. 1968). The umbilical arteries are easily cannulated for a few days after birth and the catheters can be left in place for at least two weeks. The most common problem with vascular catheters is infection. Other complications are bleeding and thrombosis (Möller et al. 1995, Hentschel et al. 1999).

The umbilical vein is usually needed for resuscitation of the newborn (Möller et al. 1995). CVCs via subcutaneous chemoports or non-tunneled or tunneled CVCs are used later in infancy when prolonged parenteral nutrition is needed (Andrew et al. 1995b).

2.5.2. Peripheral catheters

Because of the complications of umbilical artery catheterisation, peripheral arterial lines are preferred. The radial, posterior tibial and dorsalis pedis arteries are the most commonly used sites (Randel et al. 1987). Peripheral arterial catheters should be inserted when multiple and repeated samples are required. Arterial blood sampling is indicated for monitoring blood gases in infants with respiratory distress. Peripheral ischemia is a major complication at peripheral sites (Möller et al.1995).

Venous access in a less than 1000-gram premature infant is difficult. Peripheral veins are quickly exhausted because the thin, fragile veins are easily perforated, and develop thrombophlebitis. Peripherally inserted central catheters (PICC) are now used in these neonates to provide long-term vascular access (Crowley et al. 1997, Dubois et al. 1997).

2.6 Risk factors for coagulation abnormalities

Haemorrhagic tendencies are seen in the neonatal period. There are several reasons why the newborn infant may have bleeding complications. These include physiological deficiencies, prenatal influences, immaturity of vessels and tissues, birth trauma, asphyxia and sepsis (Hathaway 1975). Abnormal coagulation screens in sick newborn infants strongly support a diagnosis of DIC (Shirahata et al. 1998). However, normal screen tests do not exclude activation of the coagulation and fibrinolytic systems (Schmidt et al. 1993).

2.6.1. Obstetric and maternal factors

Prenatal and perinatal risk factors, such as maternal medication and morbidity, may disturb haemostasis and increase haemorrhagic incidents in the newborn (Shapiro et al. 1986, Shankaran et al. 1996). Multiple pregnancy is a known risk factor for preterm birth. Complications at birth are more frequent in multiple than in singleton pregnancy (Shankaran et al. 2002, Smith et al. 2002).

Pre-eclampsia. Hypertension and pre-eclampsia are one of the reasons for SGA, because they affect maternal organs. Generalized endothelial damage and dysfunction worsen placental circulation (Roberts et al. 1989, Roberts and Cooper 2001). However,

recent studies have shown that pre-ecclampsia is an etiologically heterogenous disorder that occurs in at least two different subsets: one with normal placental function, and another with placental dysfunction (Brouhgton Pipkin and Roberts 2000, Rasmussen and Irgens 2003).

Some infants whose mothers have pregnancy-induced hypertension or pre-eclampsia have an associated thrombocytopenia (Thiagarajah et al. 1984, Beardsley 1991). Preterm infants with intrauerine growth restriction (IUGR) especially are at greater risk of adverse outcome (Beiner et al. 2003). Sainio et al. (2000) found no correlation between levels of thrombopoietin in amniotic fluid and cord plasma or platelet counts. Megakaryocyte mass is decreased in infants with thrombocytopenia associated with IUGR or pregnancy-induced hypertension (Paul et al. 2002).

Higgins et al. (2000) found no significant differences between antithrombin-thrombin complex and fibrin degradation in cord blood of the pre-eclamptic mother and of normal pregnancy, although the trend was toward higher levels in pre-eclampsia. They suggest that foetal circulation is protected from at least some of the haemostatic disturbance from the mother's side. Kupferminc et al. (2000) found thrombophilia in 67% of women with severe pre-eclampsia. On the other hand, Grandone et al. (2002) report that mothers carrying the FV G1691A or FII G20210A mutation have a significantly higher risk of delivering neonates with lower birth weight.

Asphyxia. Low et al. (2003) used an artery base deficit of >12 mmol/l as evidence of a significant antepartum metabolic acidosis and asphyxia in preterm pregnancy. Andres et al. (1999) suggest that umbilical artery pH <7.00 with a metabolic component is the most important variable in neonatal morbidity.

Megakaryocytes, i.e. precursors for platelets, are susceptiple to asphyxia leading to thrombocytopenia in asphyxiated neonates (Kühne and Impach 1998, Murray 2002).

Chessells and Wigglesworth (1971) and Chadd et al. (1971) studied severely asphyxiated infants, and report that the more severe the hypoxia, the more abnormal the DIC-associated clotting tests. El Beshlawy et al. (2004) studied asphyxiated infants and found a marked decrease in the level of AT, PC and PS hypoxic-ischemic neonates before TE complications.

Mode of delivery. The labor stress of vaginal delivery may influence the levels of physiological anticoagulants. PC, PS, and AT activities were higher in infants born vaginally than those delivered by caesarean section. Fibrinogen concentrations and plasminogen activity were higher in infants born vaginally than in those born by caesarean section (Franzoi et al. 2002).

Intra-amniotic infection. Amnionitis or chorioamnionitis may induce premature birth (Mercer 2003). Infection of the mother can transfer to the foetus before birth and cause sepsis in the preterm baby after birth (Laugel et al. 2003). Some recent studies suggest that coagulation disorders and intrauterine exposure to infection or inflammation are associated with the risk of cerebral palsy (CP) in normal birth weight (Grether and Nelson 1997) and term infants (Nelson 2002), but not in very preterm infants (Grether et al. 2003). Infectious agents such as *Toxoplasma gondii* and *rubella*, *cytomegalo* (Feusner et. al 1983), and *herpes simplex* viruses can cause significant haematologic alterations in the foetus (Haggerty 1985). The usual haematologic manifestations are thrombocytopenia and anemia or DIC (Feusner et. al 1983).

Diabetes mellitus. Infants of diabetic mothers can have haematologic abnormalities such as increased red cell mass hyperviscosity syndrome, increased platelet aggregation, and increased incidence of thombosis (Shannon et al. 1986, Katzman 1989, Cordero et al. 1998, Giannakopoulou et al. 2002). Sibai et al. (2000) found that 20% of the mothers with gestational diabetes had pre-eclampsia. The incidence of pre-eclampsia rose significantly with increasing severity of diabetes.

Antenatal corticosteroids. Antenatal corticosteroids are used to prevent respiratory distress syndrome (Crowley 2000). Leviton et al. (1993) found a reduced risk of postnatal IVH in low birth weight newborns with antenatal corticosteroid treatment. However, recent studies have also reported adverse effects of corticosteroids when used in repeated doses (Shinwell et al. 2000, Whitelaw and Thoresen 2000). The brain development of these preterm infants was delayed, with probable effects on neurodevelopment. Baud et al. (1999) found an association between antenatal exposure to betamethasone and decreased risk of PVL among very premature infants, whereas dexamethasone was associated with a higher risk of PVL.

2.6.2. Neonatal factors

SGA. Peters et al. (1984b) report that SGA infants had significantly lower AT values than appropriate size for GA (AGA) infants. Von Kries et al. (2001) identified foetal thrombophilia as an additional cause of low birth weight in term infants. On the other hand, Verspyck et al. (2002) report that there was no difference in the prevalence of inherited thrombophilia between SGA and AGA infants.

RDS. Elevated levels of coagulation activation markers have been observed in RDS (Schmidt et al. 1992b, Aronis et al. 1998). Low plasma levels of AT have also been observed in RDS (Schmidt et al. 1992b).

Sepsis. Sometimes in sepsis, the coagulation system is activated to the extent of DIC, and a consumption of coagulation factors and inhibitors occur causing DIC (Levi and Ten Cate 1999). Aronis et al. (1998) documented DIC in 16.7% of the septic VLBW infants.

NEC. Sepsis and/or DIC are usually associated with NEC. Feusner et al. (1983) found thrombocytopenia and hypofibrinogenaemia with normal screening tests and coagulation factors V and VIII in preterm infants with NEC. A single case of FV Leiden mutation in an infant with NEC and mesenteric vein thrombosis has been reported (Göpel et al. 1999). Hypoxic-ischemic infants with decreased levels of AT, PC and PS may develop NEC (El Beshlawy et al. 2004).

2.6.3. Bleeding problems

2.6.3.1. Intraventricular haemorrhage

The pathogenesis of IVH is multifactorial (Wallin et al. 1990). Prematurity is a major risk factor (Duda Dykes et al. 1980, Wallin et al. 1990, Piecuch et al. 1997), and premature infants have impaired cerebrovascular autoregulation, and this might also be associated with IVH or PVL (Volpe 2001). Marked changes in arterial blood pressure affect cerebral blood flow (Grönlund et al. 1994, Meek et al. 1999), cerebral oxygenation (van de Bor and Walther 1991, Bohin et al. 1995, von Siebenthal et al. 1999), and changes in cerebral blood pressure and perfusion are considered to be major risk factors for IVH (Tsuji et al. 2000, Cordero et al. 2002).

McDonald et al. (1984a) followed 50 preterm infants from birth with serial coagulation and real-time cranial US studies. They found significant associations of lower values of fibrinogen, platelet count, AT and FVIII in the first four hours of life.

Beverley et al. (1984) found that preterm infants with IVH had prolonged APTT and reduced FII, FVII and FX activity at the age of 48 hours.
Prothrombotic risk factors have been associated with hydrocephalus and IVH in newborns (Riikonen and Kekomäki 1998, Petäjä et al. 2001, Aronis et al. 2002).

In the preterm infant, hypoxic-ischaemic brain injury resulting in IVH and focal white matter necrosis is well documented (Vannucci 2000, du Plessis and Volpe 2002). The incidence of major handicap (CP or mental retadation) in preterm infants is mostly associated with IVH and/or PVL (Papile et al. 1983, Piecuch et al. 1997). An association has been found between premature rupture of the membranes and IVH, PVL and cerebral palsy (Spinillo et al. 1995b, de Vries et al. 1998).

2.6.3.2. Pulmonary and gastrointestinal bleeding

Pulmonary haemorrhage is a rare complication of RDS, and is most often associated with high mortality. De Carolis et al. (1998) studied 79 preterm infants with GA < 30 weeks, and report 10 pulmonary haemorrhages (12.6 %). They found that hypocoagulability was one of the risk factors associated with haemorrhage. Bhandari et al. (1999) found that SGA preterm infants with pulmonary haemorrhage had a 100% mortality rate.

Kuusela et al. (2000) report that newborn infants treated in the NICU have a high frequency of stress-induced gastric haemorrhage. Mechanical ventilation and hypotension increased the risk. They did not study the coagulation system.

2.6.4 Thrombotic events

The most common locations of venous thrombi in neonates are the renal veins, vena cava and cerebral veins. Neonates also have high rates of catheter-related thrombosis. Arterial vascular occlusions are mainly ischemic strokes, as are catheter-related

thromboses in the aorta or femoral arteries (Nowak-Göttl et al. 2003, Kuhle et al. 2004).

2.6.4.1 Incidence

Thrombotic disorders are relatively rare in infants. On the other hand, compared with older children, neonates and young infants are more predisposed to thrombosis (Andrew 1995). The incidence of deep venous thrombosis (DVT) has been reported to be 5.3/10,000 per year in hospitalised children and 0.07/10,000 in the general population of children in Canada (Andrew et al. 1994a). An international registry of symptomatic venous thromboembolism (VTE) in newborns reports an incidence of 2.4 per 1,000 admissions to NICU (Schmidt and Andrew 1995). A German prospective two-year registry reports the incidence of symptomatic neonatal thromboembolic events (TE) to be 5.1 per 100,000 births (Nowak-Göttl et al. 1997b). In the Netherlands, van Ommen et al. (2001) report the incidence of VTE to be 14.5 per 10,000 infants aged 0 to 28 days.

The Canadian Pediatric Ischemic Stroke Registry (De Veber and Andrew 2001) reports sinovenous thrombosis in children, and found an incidence of 0.67 cases per 100 000 children/year. Neonates were most commonly affected. Sometimes sinovenous thrombosis is neurologically asymptomatic in the newborn (Golomb et al. 2001, Lynch et al. 2002). Sinovenous thrombosis is often accompanied by infarction or IVH (Wu et al. 2002).

Renal vein thrombosis is the most common non-CVC-related VTE in infancy (Schmidt and Andrew 1995, Nowak-Göttl et al. 1997b). **Table 2** presents a selection of thromboembolic studies in newborns.

2.6.4.2. Risk factors

In neonatal venous thrombosis, a "trigger factor" is usually present. Such factors include difficult delivery, hypotension, hypovolemia, asphyxia, diabetes of mother, maternal antiphospholipid syndrome, sepsis, SGA, congenital heart disease, operations, and CVCs (Nowak-Göttl et al. 2003). Neonates with severe genetic or acquired deficiences of PC, PS and AT, which are often combined with factor V Leiden mutation, have an increased risk of thrombosis (Petäjä et al. 1996, Manco-Johnson 1997, Edstrom and Christensen 2000).

2.6.4.3. Catheter-related thrombosis

The main complications in CVCs are pericatheter thrombosis and infection (Harms et al.1995, Andrew et al. 2001, Hentschel et al. 1999). Over 80% of VTE in newborns are secondary to CVCs (Schmidt and Andrew 1995, van Ommen et al. 2001). Thrombotic complications have been observed in 1-22% of neonates requiring CVCs (Mehta et al. 1992, Tanke et al. 1994). Asymptomatic CVC-related venous TEs are also of clinical importance. It has been reported that up to 24% of 'silent', i.e. asymptomatic, aortic thrombi were not detected until autopsy (Schmidt and Andrew 1988). The signs of thrombosis depend on the location and size of the thrombus, and whether its location is arterial or venous (Edstrom and Christensen 2000). Acute catheter-related thrombosis includes blockage of the CVC along with swelling, pain and discoloration of the limb involved (Massicotte et. al 1998).

Increased incidence of venous thrombosis has been found in premature and other high-risk infants (Sadiq et al 1987), especially if an indwelling catheter is used in combination with surgery (van Ommen et al. 2003). Petäjä et al. (1996) examined neonates in association with cardiac surgery, and found low concentrations of PC and AT, indicating an acquired deficiency state which was associated with thrombotic complications.

PICCs seem to have lower frequency of TEs than CVCs (Neubauer 1995, Dubois et al. 1997). Recently, there have been reports that catheter migration may pose a significant risk for cardiac tamponade or pleural effusion (Darling et al. 2001, Leipälä et al. 2001, Nowlen et al. 2002).

2.6.4.4. Thrombophilia

A person having a positive family history, early age at onset and a frequent recurrence of thrombosis is said to have congenital thrombophilia (Lane et al. 1996). The frequency of congenital prothrombotic disorders in children with thromboembolic events varies from 10 % to 62 % depending on the study design, patient population, and laboratory tests used in the study (Revel-Vilk and Massicotte 2003). Genetic defects in coagulation inhibitors can cause thrombosis in infancy, although the situation becomes

Table 2. Studies of neonatal thromboembolism

Authors	N	Study design	Age	Risk factors	Diagnosis	Main findings
Grisoni et al. 1986	107	single-centre: retrospective	neonates	CVC		23/107 (21%) CVC-related thrombosis, birth weight <1000g and requiring repeated catheterizations were associated with risk of complications
Sadiq et al. 1987	48	single-centre	GA 31.8 ± 6.9 wk	CVC, sepsis	Two dimensional echocardiography, venography	10 catheter-related venous thrombotic events in 7 infants; 2/7 asymptomatic
Payne et al. 1989	12	single-centre	GA 29 – 43 wk	UAC-related thrombosis	Echocardiography	all symptomatic infants; n= 4 (33%) arterial thrombosis without UAC, n=1 (8%) AT deficiency
Mehta et al. 1992	42	single-centre: prospective	GA 24 – 39 wk	CVC-related thrombosis	Two dimensional echocardiography	n=9 (14%) central venous thrombosis; AT values were lower in thrombotic children, but related to GA
Tanke et al. 1994	193	single-centre: prospective	GA 27 – 42 wk	CVC-related thrombosis	Echocardiography	n= 25 (13%) central venous thrombosis; higher incidence in infants with umbilical catheters
Schmidt and Andrew 1995	97	multi-centre: retrospective	GA 27 – 42 wk	CVC, dehydration, systemic infection	Doppler US, angiography	Results from a Canadian and International registry; VTE prevalence 2.4/1000 NICU admissions; 89% CVC-related thrombosis
Nowak-Göttl et al. 1997	79	multi-centre: consecutive	GA 25 – 42 wk	CVC, dehydration, asphyxia, maternal diabetes, CHD	Doppler US, angiography, magnetic resonance angiography	Results from a German two-year registry; incidence of symptomatic thromboembolism 5.1/ 100 000 births

GA = gestational age; UAC = umbilical artery catheter; CVC = central venous catheter; CHD = congenital heart disease; US = ultrasonography; CT = computer tomography; AT= antithrombin VTE; = venous thromboembolism

Table 2. Studies of neonatal thromboembolism (continued)

Authors	N	Study design	Age	Risk factors	Diagnosis	Main findings
Günther et al. 2000	91	multi-centre: case-control, prospective	term infants	perinatal asphyxia, dehydration, septicaemia, maternal diabetes	US, CT, MRI	infants with ischaemic CNS stroke; acquired and genetic prothrombotic risk factors were studied; increased lipoprotein(a), FV mutation, PC deficiency were significant risk factors
Heller et al. 2000	65	multi-centre: case-control	neonates			German Childhood Thrombophilia Study; 31 RVT; 24 PVT; 10 HVT; FV G1691A mutation 21% vs 8% in controls; additional risk factors in 27/65 patients
van Ommen et al. 2001	47	multi-centre: consecutive	GA 25 – 42 wk	CVC, infection, asphyxia, CHD, surgery, hypovolemia, nephrotic syndrome, maternal diabetes	Echocardiography, US, venography, CT	Results from a Dutch two year registry; incidence of VTE 14.5/ 10 000 infants; 94% CVC-related; 1/18 tested infants had FV Leiden; 1 acquired AT deficiency
deVeber and Andrew 2001	160	multi-centre: consecutive	newborns to 18 years of age	head and neck disorders, acute or chronic systemic illness	CT, MRI	Results from six first years of the Canadian Pediatric Ischemic Stroke Registry; incidence of cerebral sinovenous thrombosis 0.67/ 100 000; neonates most commonly affected; prothrombotic states in 41%
Revel-Vilk et al. 2003	171	single-centre: consecutive	newborns (21 preterm) to 16 years of age	CHD, infection, cancer, CVC	Confirmatory diagnostic imaging	prevalence of FV Leiden, prothrombin G20210A polymorphism, PS, PC and deficiency and increased Lp(a) not different from general Caucasian population
Kosch et al. 2004	59	multi-centre: case-control, consecutive	newborns (14 preterm)	sepsis, CVC, birth asphyxia	color duplex US, CT, MRI	RVT and prothrombotic risk factors were studied; 68% had at least 1 prothrombotic risk factor

GA = gestational age; UAC = umbilical artery catheter; CVC = central venous catheter; CHD = congenital heart disease; US = ultrasonography; CT = computer tomography; RVT = renal venous thrombosis; PVT = portal vein thrombosis; HVT = hepatic vein thrombosis; AT = antithrombin

more common in later childhood (Nowak-Göttl et al. 1997a, van Ommen and Peters 2003).

The congenital prothrombotic disorders that are clinically significant prior to thrombotic events in newborns include deficiencies of AT (Seguin et al. 1994, Sánchez et al. 1996), PC and PS (Marlar and Neumann 1990, Pegelow et al. 1992), increased lipoprotein (a) level (Sträter et al. 1999, Nowak-Göttl et al. 1997a) and the presence of prothrombin gene G20210A and FV gene mutations (Schobess et al. 1999, Nowak-Göttl et al.1996, Hundsdoerfer et al. 2003).

Elevated plasma levels of FVIII predict recurrent VTE in adults. Goldenberg et al. (2004) found that elevated levels of FVIII at diagnosis and a persistent elevation after standard anticoagulant therapy predicted a poor outcome in children with thrombosis. On the other hand, Kenet et al. (2003) studied preterm infants with thrombophilia, and did not find any increased risk for neonatal complications.

PC and PS deficiencies and the FV Leiden mutation are the three genetic abnormalities of the PC pathway associated with thrombophilia. Both PC and PS defects are frequently associated with the FV Leiden mutation (Aiach et al. 1997). The APC resistance phenotype, i.e. FV gene mutation, is the most important cause of venous thrombosis, and is present in 20 % to 60 % of adult patients with venous thromboembolism (Dahlbäck 1995). Hagstrom et al. (1998) retrospectively analyzed the records of 33 neonates with thromboembolic disease. None of the infants with DVT were FV Leiden positive. On the other hand, 6/22 (27%) infants with arterial central nervous system thrombosis had the FV Leiden mutation. In cerebral venous thrombosis, around 20% of infants and children have the FV Leiden mutation, or increased Lp(a), or PC deficiency (Vielhaber et al. 1998).

PC and PS deficiencies are autosomally transmitted with variable penetrance in two main groups: families with symptomatic heterozygous individuals, and families with asymptomatic heterozygotes and with homozygotes developing fatal thrombotic complications (Miletich et al. 1987, Levo et al. 2000). Homozygous individuals suffer from severe purpura fulminans in the neonatal period, and cerebral and/or ophthalmic damage even *in utero* (Marlar et al. 1989, Marlar and Neumann 1990, Kirkinen et al. 2000). Mintz-Hittner et al. (1999) report the same vitreoretinal findings as in ROP in infants with compound heterozygous PS deficiency. Greiner et al. (1999) investigated adult patients with retinal vein occlusion and report a similar prevalence of the FV Leiden mutation as in patients with DVT.

Corrigan and Jeter (1992) studied newborns with severe congenital heart disease and found significantly increased levels of PAI-1 antigen, plasminogen activator inhibitor (PAI) and tissue-type plasminogen activator (tPA) in stressed newborns, showing their ability to activate the fibrinolytic mechanism. Andrew et al. (1992a) found decreased clot lysis in cord plasma of full-term infants. In the cord system, in contrast to the adult system, less plasminogen was consumed in response to thrombolytic agents, reflecting the lower concentration of plasminogen.

Koren et al. (2003) noted that cerebrovascular accident is the major thrombotic event in infants, and a combination of prothrombotic factors may cause such events. Frequently the etiology of the symptomatic ischemic stroke in neonates is a combination of acquired and genetic risk factors (Günther et al. 2000, Andrew et al. 2001).

There is little information concerning the recurrence of VTE in newborns. In older infants, recurrent VTE occurs in 5-13%, depending on the treatment and prothrombotic risk factors (Andrew et al. 2001). Nowak-Göttl et al. (2001) followed children with first venous thromboembolism: recurrent VTE occurred in 21% of children with genetic thrombophilia within a median time of 3.5 years. Kosch et al. (2004) followed children with neonatal RVT for a median time of 4 years, and 4% of the children suffered a second TE. All children with recurrent TEs had at least one prothrombotic defect.

Post-thrombotic syndrome symptoms may occur as long as 5-10 years after the initial event. Recently, post-thrombotic syndrome has been described in children aged 1 month to 18 years, with an estimated incidence of 12-63% (Monagle et al. 2000, Kuhle et al. 2003a); some of them developed VTE as newborns.

2.6.4.5. Diagnosis of DVT

Symptoms that usually cause suspicion of DVT are swelling, pain or discoloration of the related limb, dysfunction of the CVC, or superior vena cava syndrome. In arterial thrombosis the limb is pale, cold and pulseless (Andrew et al. 1997). Clinical presentation of renal vein thrombosis includes a palpable abdominal mass, macrohematuria, proteinuria, hypertension and renal failure (Andrew and Brooker 1996). Typical clinical symptoms of neonatal stroke are diffuse or focal neurologic signs and seizures (de Veber and Andrew 2001).

Imaging

The diagnosis of DVT in neonates is complicated by the small size of the patients, the widely diverse locations, underlying disorders and the lack of validated noninvasive diagnostic radiographic tests (David and Andrew 1993).

Ultrasound. Male et al. (2002) report US sensitivity of 37 % and venography sensitivity of 79 % in DVT. US was more sensitive than venography in jugular veins, but insensitive for DVT in intrathoracic thrombosis in children. US is most commonly used for the diagnosis of renal vein thrombosis (Hibbert et al. 1997, Male et al. 2003). Revel-Vilk et al. (2004) used US for the diagnosis and follow-up of DVT in 190 children. The location of the thrombus was as follows: extremities in 69% of the children, abdomen in 14%, right atrium in 3%, and multiple in 14%.

Cranial US is of value in defining the centrally located vascular lesions of PVL in preterm infants (Andrew et al. 2001, Debillon 2003). Inder et al. (2003) report low sensitivity (26 %) and a low positive predictive value (36 %) for the presence of noncystic white matter injury in preterm infants, as detected on magnetic resonance imaging at term.

Radiographic studies. Depending on the location of thrombosis, US with venography is the most appropriate method for DVT diagnosis in neonates (Revel-Vilk and Massicotte 2003). Roy et al. (2002) report asymptomatic thrombosis in 30 % of newborns with umbilical venous catheters: echocardiographic diagnosis was insensitive, and contrast venography was needed for accurate diagnoses.

Magnetic resonance imaging is useful for the diagnosis of IVH and PVL (Counsell 2003). Radiographic studies of neonatal stroke include computer tomography, magnetic resonance imaging, and magnetic resonance angiography (de Vries et al. 1989, Valkama et al. 2000, Counsell 2003). Cerebral sinovenous thrombosis is increasingly diagnosed due to the knowledge of the associated clinical symptoms and improved cerebrovascular imaging (Hüppi 2002, Chan et al. 2003).

Laboratory tests

Symptomatic patients should be investigated in comparison with age- and gender-matched healthy controls with attention to ethnicity (Rees 1996, Hakala et al. 1995, van Ommen et al. 2003). However, sometimes the acquired thrombo-embolic complications mask the inherited deficiency (Sutor and Uhl 1997, Bonduel et al. 2000). The PC pathway especially should be adequately evaluated because of the physiologically low PC values in childhood (Nowak-Göttl et al. 1996). Otherwise healthy thrombophilic children who are carriers of a one identified thrombophilic abnormality appear to be at very low risk of thromboembolic complications (Tormene et al. 2002).

2.7. Treatment

2.7.1. Fresh frozen plasma and specific component therapy

Fresh frozen plasma (FFP) has been used in the hope of preventing bleeding and thrombotic complications, especially IVH in neonates (Beverley et al. 1985). It has also been used as a volume expander to avoid or to treat hypotension and hypovolemia in preterm infants (Northern Neonatal Nursing Initiative Group 1996a and 1996b). FFP failed to fully prevent IVH in neonates.

Johnson et al. (1982) treated preterm infants who had RDS and abnormally prolonged PTT with fresh frozen plasma (FFP). FFP failed to fully improve coagulation screening test values. Hyytiäinen et al. (2003) studied sick premature infants who had FFP

infusions after birth and found that routine screening tests recognised poorly the extent of thrombin formation. They report the effect of thrombin downregulation in only some of the infants after FFP infusions. Turner et al. (1981) studied the coagulation status of low birth weight preterm infants with RDS and term infants with asphyxia soon after birth. The correction of haemostatic defects with prothrombin preparation or FFP did not change the mortality or morbidity of those infants.
Transfusion-related acute lung injury (TRALI) is a serious complication of blood transfusion which has been rarely diagnosed in infants. Pathogenesis of TRALI may be connected to the presence of anti-HLA and/or antigranulocyte antibodies in the plasma of donors (Popovsky 2001).

Greisen and Andreasen (2003) compared the effect of recombinant factor VIIa (rFVIIa) and FFP on prothrombin time. They found that rFVIIa partially normalised prothrombin time in preterm babies. Olomu et al. (2002) successfully treated two VLBW infants with severe pulmonary haemorrhage with rFVIIa.

Treating purpura fulminans due to homozygous PC deficiency, PS deficiency or activated PC resistance with FFP or PC concentrate is useful in the acute phase (Marlar et al. 1989, Dreyfus et al. 1991, Pipe et al. 1996, Salonvaara et al. 2004).

Brangenberg et al. (1997) report that preterm infants given a single dose of 50-200 IU/kg AT on the first day of life had lower incidence of IVH (13% vs. 20-26%) than reported in epidemiological studies. Mean AT activity before replacent therapy was 40% and rose to 90%. In contrast, Fulia et al. (2003) found no significant difference in the incidence of IVH between AT treated preterm infants and a placebo treated group. Kreuz et al. (1999) treated children with DIC with AT concentrate 80 U/kg/day along with heparin therapy. An increase in plasma AT concentration (median 45% to 84%) was recorded 24 h after the initial substitution, followed by normalisation of PT, APTT and platelet count. Gross et al. (1982) studied 33 neonates with DIC, and no difference was seen in APTT, platelet count or fibrinogen levels between the exchange transfusion group, FFP group and control group. They suggest that the outcome of DIC was more dependent on the treatment of the primary cause of the illness.

2.7.2. Heparin prophylaxis

Heparin-bonded CVCs can be used as prophylaxis to prevent CVC-related thrombosis (Krafte-Jacobs et al. 1995, Pierce et al. 2000). In a meta-analysis of randomized controlled studies (Randolph et al. 1998) unfractioned heparin at a concentration of 1U/ml infused continuously through peripheral venous and arterial catheters was effective in reducing thrombus formation. In many NICUs heparin is used routinely to maintain the patency of indwelling arterial catheters (Lesko et al. 1986, Randel et al. 1987, van Lingen et al. 1992). In contrast to Lesko et al. (1986), who report a four-fold increase in the incidence of IVH in infants receiving heparin, Chang et al. (1997) report that 1U/ml of heparin does not increase the incidence or severity of IVH or significantly alter the coagulation profile in premature infants.

There is no evidence of the efficacy or safety of routine primary heparin prophylaxis with CVCs in children, but children with long-term TPN at home and immunocopromised children may benefit from antithrombotic prophylaxis (Andrew et al. 1995b, Henrickson et al. 2000).

2.7.3. Treatment of thromboembolism

Unfractioned heparin. Unfractioned heparin (UFH) has been the most commonly used anticoagulant for acute thromboembolic disorders for decades (Moll and Roberts 2002), even though there are only a few studies of UFH in newborns (Andrew et al. 2001, Monagle et al 2001). The dose of UFH required to achieve the therapeutic APTT (=1.5 to 2 times normal values) is higher for newborns than for older children (Andrew et al. 1994b). Whether the target therapeutic APTT used for older children is optimal for newborns remains unknown (Sutor et al. 1997). Similarly, the appropriate duration of therapy with UFH is unknown (Chan et al. 2003).

The clinically important side effects of UFH include major bleeding (Moritz et al. 2003) and heparin-induced thrombocytopenia (HIT) (Ranze et al.1999, Severin and Sutor 2001, Schmugge et al. 2002).

Low-molecular-weight-heparin. In recent years low-molecular-weight-heparins (LMWH) have been widely used for prophylaxis and treatment of thromboembolic events (Moll and Roberts 2002). They are considered more convenient in clinical use than UFH, since less monitoring is needed and the dose response can be predicted.

They also cause less osteoporosis and HIT than UFH (Moll and Roberts 2002). Revel-Vilk et al. (2004) studied 107 neonates with DVT, 39 of them preterm infants. The mean dose of enoxaparin was 1.5 $\pm$ 0.5 mg/kg/dose. Complete resolution of the thrombus was reported in 58%. Complete resolution of arterial thrombi was reported in 85% at the end of the follow-up.

Streif et al. (2003) found that preterm infants required higher doses of enoxaparin than full term infants to maintain anti-factor Xa levels in the target range. Dix et al. (2000) used a mean dose of 0.83-1.76 mg/kg/12 h s.c. to achieve target anti-factor Xa level between 0.5 and 1.0 U/ml. Major bleeding occurred in 4% of the newborns receiving therapeutic LMWH, two of whom were extremely preterm infants. Monagle et al. (1998) successfully treated a homozygous PC deficiency with LMWH.

Vitamin K antagonists. At present, vitamin K antagonists are the only oral anticoagulants used in children (Moll and Roberts 2002). Warfarin is widely used in the treatment of congenital heart disease (Marzinotto et al. 2000), but it has several disadvantages: a narrow therapeutic window, large inter-individual dosing differences, interactions with dietary changes and other medication, the potential for serious bleeding, the need for monitoring and for patient compliance (Moll and Roberts 2002, Newall et al. 2003), and osteoporosis in long-term treatment (Cheung et al. 2001). Monitoring of a whole blood prothrombin time/international normalized ratio (PT/INR) was assessed for the therapeutic response in children treated at home (Massicotte et al. 1995, Marzinotto et al. 2000).

Thrombolytic agents. Systemic thrombolytic therapy is indicated for arterial occlusions, massive pulmonary embolism; in cases of danger to life (Nowak-Göttl et al. 1999). The largest age group, which is treated, is infants, and the main indication is cardiac catheterization. In neonates thrombolytic therapy should be used with caution due to risk of bleeding (Gupta et al. 2001). There is most experience of streptokinase (SK), urokinase (UK) and tissue-type plasminogen activator (t-PA) in the thrombolytic treatment of neonates (Kirk and Qureshi 1989, Nowak-Göttl et al. 1999, Manco-Johnson et al. 2000, Gupta et al.2001, Hartmann et al. 2001). Although SK is cheap, it has the potential for allergic reactions. UK and tPA are nowadays widely used (Monagle et al. 2001).

A low-dose infusions of tPA (0.01-0.6mg/kg/per hour) is enough for neonates (Rimensberger et al. 2001, Wang et al. 2003). The usual complication is bleeding. Severe bleeding is rare. The incidence of bleeding requiring treatment is about 20% of pediatric patients (Monagle et al. 2001, Hartmann et al. 2001).

3. AIMS OF THE STUDY

The main purpose of this study was to evaluate the physiological development of haemostatic factors in preterm infants in infancy.

The specific aims of this study were:

1. to prospectively determine the levels of the specific coagulation factors in premature infants at birth and to investigate the same coagulation factors and physiological anticoagulants at the corrected age of six months (Studies I and III)

2. to assess prospectively the effects of gestational age and pre- and perinatal events on the coagulation status in this study (Study I)

3. to study the interactions between the specific coagulation factors at birth and mortality and morbidity (Studies II and III)

4. to determine the incidence of clinically symptomatic CVC-related DVT in newborns and small infants, and to identify retrospectively the most common clinical and some of the genetic risk factors for CVC-related DVT among children with thrombotic complications (Study IV).

4. MATERIALS AND METHODS

4.1. Subjects

4.1.1. Patients in the coagulation studies (Studies I-III)

All infants born before 37 weeks of gestation at Kuopio University Hospital and admitted to the NICU during February 1996 to May 1998 were eligible for the study. The exclusion criterias for this study were:

1. Written informed consent was not obtained from the parents.
2. Blood samples for the study were not taken within two hours of age.
3. The infant had received intravenous infusions of heparin, blood or plasma products or intravenous medications before or concurrently with the drawing of blood samples.

Birth asphyxia was defined as venous cord blood < 7.26 or Apgar scores <7 at 5 minutes.

Figure 2 shows the numbers of the study subjects: 161 patients were eligible for the study. Thirty-five children were excluded from the study because of protocol violation: written informed consent was not obtained in three cases, and blood samples were not taken within two hours of age in 32 cases. One baby was omitted from the analysis of the coagulation studies because of a distinct inconsistency in the preanalytical factors.

Study I. Patients were divided into four GA groups, according to the hypothetically different stage of the coagulation development. The four groups were: Group 1: Infants with GA < 28 wks; Group 2: Infants with GA 28-30 wks; Group 3: Infants with GA 31-33 wks; Group 4: Infants with GA 34-36 wks. These infants represented 34 % of all preterm infants born at Kuopio University Hospital at the time of the study. Group 1 represents 98 % of all the ELBW infants born at Kuopio University Hospital at the time of the study. The final study group consisted of 125 children. The characteristics of these patients are shown in Table 1/**I**.

Study II. These infants were a subgroup of those in Study I. The study group was formed from the infants (n=38) with GA below 30 weeks. The characteristics of these patients are shown in Table 1/**II**.

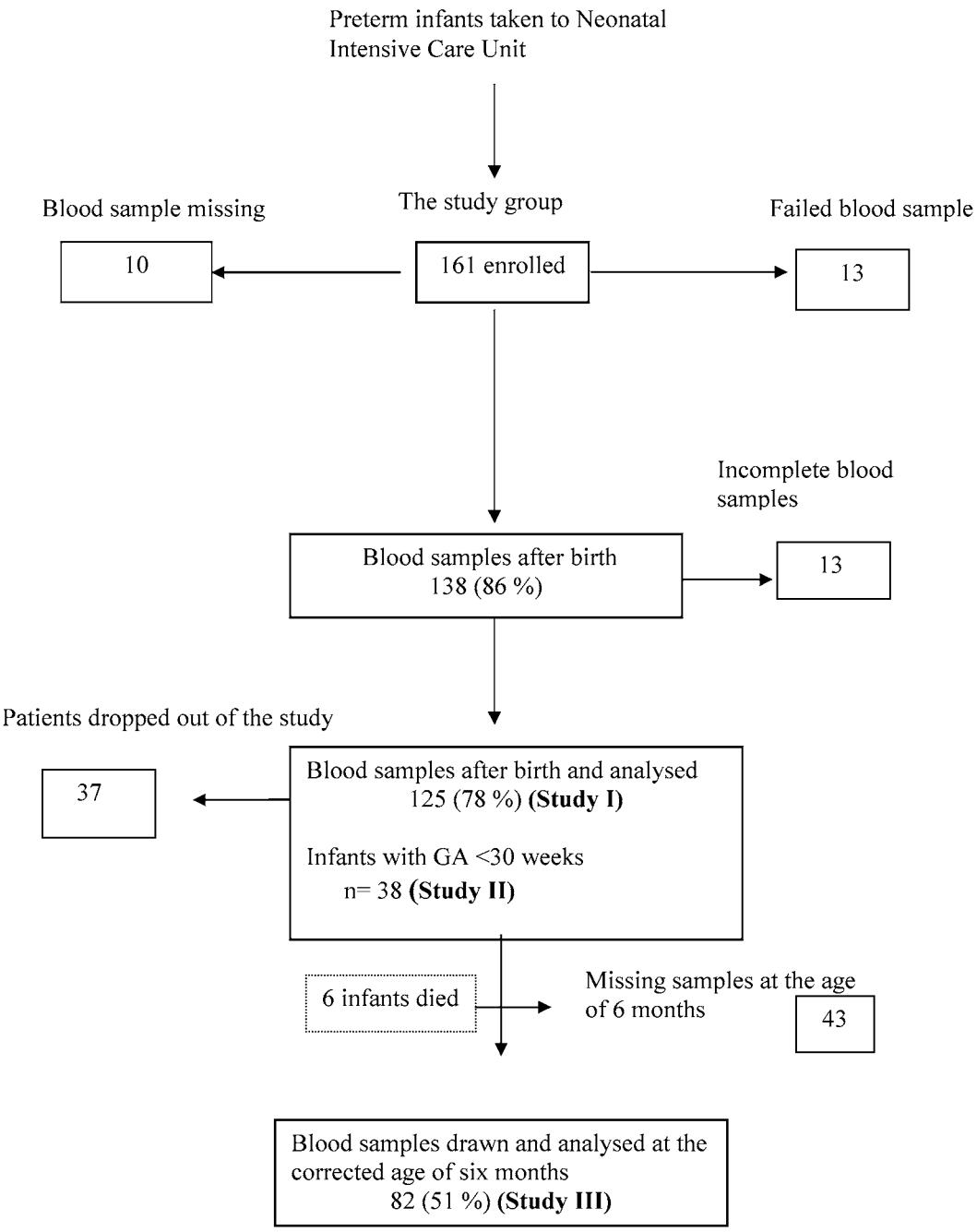

Figure 2. Study population in the coagulation studies (Studies I-III).

Study III. The study population consisted of the infants who participated in Study I and whose blood samples were analysed again at the corrected age of six months. This final group consisted of 82 children, which is 66 % of the patients in Study I. The loss of patients was due to missing
blood samples either after birth or at the age of six months, and to the fact that the parents of the healthiest infants, i.e. preterm infants with GA > 34 wks, did not want to participate in the study at the age of six months. Six infants died during the study period: two infants with GA 26 and 27 wks, one infant with GA 28 wk, two infants with GA 31 and 33 wks, and one infant with GA 35 wk.

Eighty-two percent of the infants with GA 24-30 weeks who were alive at the corrected age of six months participated in the final stage of the study. As expected, most of the drop-outs (n=37) were from the oldest GA groups. The characteristics of these patients are shown in Table 1/**III**.

4.1.2. Patients in the thrombosis study (Study IV)

The infants represent 1.2% of all neonates treated in the NICU during the study period. The subjects were 44 consecutive patients treated in Kuopio University Hospital who required insertion of an indwelling CVC on clinical grounds at the age of 0-90 days during January 1990 to December 1995. The characteristics of these patients are shown in Table 1/**IV**.

4.2. Methods

4.2.1. Data collection

Studies I-III. Data were collected prospectively from the patient charts according to the protocol GA, BW, sex, mode of delivery, Apgar scores, venous cord blood pH.
The mothers' patient charts were reviewed (Studies I-II) for maternal medication, the presence or absence of pre-eclampsia or hypertension in pregnancy and any possible medication for infection or amnionitis.
The infants' patient charts were reviewed (Study III) for neonatal morbidity, chronic lung disease, infections, haematological problems or neurological problems.

Study IV. The patient charts of the children having had a CVC inserted during the study period were reviewed. The medical records and the following data were collected: GA, BW, sex, Apgar scores, postnatal diagnoses and surgical procedures done. Also noted were the age at CVC insertion, days with CVC, the number and agents of CVC related infections, reasons for CVC removal, thrombotic complications, laboratory data at the time of thrombosis, treatment, and final outcome. Parents were interviewed to obtain a full family history of thrombotic events, and information concerning the children's health and medication.

4.2.2. Clinical examination

The clinical status of the newborn infant was recorded at the time of blood sampling after birth (Studies I-II). Clinical examination was performed by a paediatrician to all infants (Study III, n=82; Study IV, n=10) at the corrected age of six months (MS) (Study III) and at the time of the blood sampling (MS) (Study IV).

Arterial blood pressure measurements. Continuous arterial blood pressure was recorded (HP Model 64S, Hewlett-Packard GmbH, Boeblingen, Germany) via peripheral arterial catheter. Mean arterial blood pressure (MABP) values were analysed.

Ultrasound examinations. Cardiac US was carried out with a VingMed (Horten, Norway) within the first three days after birth. The examination was performed by either a pediatric cardiologist or a neonatologist. For cranial US, infants were scanned with an ALOKA SSD 1200 (Tokyo, Japan) by a pediatric radiologist in the NICU routinely within three days after birth, twice a week during the first two weeks of life, weekly for a month, and thereafter monthly until discharge.

4.2.3. Laboratory methods

Blood samples for coagulation factor assays were taken according to instructions (Finnish Red Cross Blood Service: Guidance on clinical laboratory tests). By using controlled procedures in blood puncture activation of the coagulation system was diminished.

Blood samples at birth. Blood samples for coagulation studies were taken within two hours after birth (Studies I-III). Blood samples were obtained by standard technique for Vacuette® Greiner Coagulation tubes (3.2% citrate) for neonates from either peripheral arterial (i.e. radial or tibial artery) or venous catheters immediately after replacement before any heparin had been infused. These samples were immediately centrifuged at 1400 *g* for 30 minutes; then plasma was removed, divided into aliquots, and kept at -80°C for future assays.

Once every two to three months the blood samples were sent in dry ice to the Department of Haemostasis of the Finnish Red Cross Blood Service, Helsinki, Finland. They were analysed batchwise together with routine assays.

Blood samples at the corrected age of six months. Blood samples for coagulation studies were taken at the follow-up visit and were sent to the Department of Haemostasis +4°C and delivered within 4 to 8 hours. Thereafter, these blood samples were treated similarly to the samples at birth.

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4.2.3.1. Coagulation screening tests

Studies I-III. Samples for determining prothrombin time (INR) and activated partial thromboplastin time (APTT) were drawn into neonatal citrate tubes (CTAD 0.1 ml; Becton Dickinson; 0.9 ml). Samples for the platelet count and hemoglobin were taken into EDTA Microtainer tubes (Becton Dickinson; 0.25 ml). The blood samples were taken to the Deparment of Clinical Chemistry in Kuopio University Hospital and were analyzed routinely within a few hours after sampling. APTT was measured using a Thrombolyzer® (Behnk Elektronik, Norderstedt, Germany). Platelet counts were measured using a Coulter STKS analyzer (Coulter Corporation, Miami, FL, USA).

Study IV. Prothrombin time was measured using the Stago Prothrombincomplex Assay (SPA 20) from Diagnostica Stago (Asnières, France) according to the manufacturerer's instructions with an STA Compact Analyzer from Diagnostica Stago.

4.2.3.2. Specific coagulation factor and anticoagulant assays

The activity of vitamin K-dependent coagulation factors II, V, VII, and X was analysed using a one stage method with an STA Compact Analyzer. Factor deficient plasma and Neoplastine CI Plus were purchased from Diagnostica Stago. A frozen normal adult

plasma pool was used as standard, and calibrated with the International Standard (National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK) for factors II, VII and X.

The functional activities of PS, PC and AT were determined according to the manufacturer's instructions with STA Staclot® Protein S, Staclot® Protein C and Staclot® AT (from Diagnostica Stago), respectively. For PS and AT, a frozen adult plasma pool calibrated with the International Standard of the National Institute for Protein C STA Unicalibrator from Diagnostica Stago were used as standards.

4.2.3.3. DNA test for FV Leiden

For the identification of FV Leiden either after birth or at six months of age, the polymerase chain reaction amplification and restriction fragment length polymorphism technique was used (Bertina et al. 1994).

If the gene defect was found, parental guidance was given and the same test was offered to all family members.

4.2.4. Statistical analyses

SPSS for Windows (SPSS Inc., Chicago, Illinois, USA) was used for all data analyses. A p-value less than 0.05 was considered significant.

Studies I-III

The differences in the components between the gestational age groups were first tested with the Kruskall-Wallis test (Study I). Pairwise comparisons between the gestational age groups were performed using the Mann-Whitney U-test (Studies I-III). This was also 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 (Study III). The Wilcoxon signed rank test was used to analyse the coagulation factors between twin A and B (Study I), and between infants with and those without IVH (Study II). The Bonferroni correction was made for multiple comparisons.

Linear regression analysis was used to identify the associations between coagulation factors and the prenatal and perinatal variables (Study I).

The Spearman correlation between each coagulation factor and anticoagulant measured was calculated (Study III). Differences in coagulation factor changes from birth to six

months of corrected age between gestational age groups, and associations between coagulation factor development, NEC, IVH and ROP were tested with repeated measures analysis of variance (ANOVA) (Study III).

Study IV

The Mann-Whitney U-test was used for comparisons.

4.3. Ethics

The study protocol was approved by the Ethical Committee of Kuopio University Hospital.

Written informed consent was obtained from the parents before they entered the studies.

5. RESULTS

5.1. Effects of gestational age and prenatal and perinatal events on coagulation status (I)

The median GA of the infants was 33 weeks, and the median birth weight was 1740 g (range 515-3740). There were 88 singletons, 14 sets of twins, and three sets of triplets. There were 31% SGA newborns and 27% asphyxiated newborns. Fifteen of 34 (44%) asphyxiated infants were also SGA at birth (Table 1/**I**).

The incidence of IVH among all infants (n=125) was 10% (n=13). When only the infants with GA 24-32 were included (n=58), the incidence of IVH was 22%. If only ELBW infants were included (n=23), the incidence of IVH (n=11) rose to 48%.

The median time interval between birth and blood sampling was 40 minutes (range 12-100).

The lowest mean and median levels of coagulation factors II, V, VII and X and platelet count at birth were detected in preterm infants born at 24-27 weeks gestation (n=21). The values increased with higher GA. Compared with the infants born at 34-36 weeks gestation, preterm infants with GA 24-27 weeks and GA 28-30 weeks had significantly lower mean values of FII (31% and 30% vs. 38%) and FVII (43% and 50% vs. 63%) and platelet count ($181x10^9$/l and 214 x 10^9/l vs 255 $x10^9$/l). Preterm infants with GA 24-27 weeks also had significantly lower mean coagulation factor FV activity (57% vs. 76%) when compared with infants with GA 34-36 weeks. No difference was found in values of coagulation screening tests, i.e. APTT and INR.

Asphyxiated infants (n=34) had significantly ($p < 0.05$) lower mean activities of coagulation factors FII (30% vs. 38%), FV (61% vs 75%), FVII (44% vs 62%) and FX (32% vs 42%), and platelet count (202 $x10^9$/l vs 239 $x10^9$/l), and higher mean PT-INR values (1.6 vs 1.3) than infants without asphyxia (n=91) at birth.

SGA infants (n=39) had significantly ($p<0.05$) lower mean activities of coagulation factor FV (63% vs 76%) and FVII (47% vs. 62%) and platelet count (182 $x10^9$/l vs. 250 $x10^9$/l) than AGA infants.

In twin pregnancies (n=14), twin B had lower median activities of coagulation factors II (28% vs. 37%), FV (56% vs. 70%), FVII (54% vs. 59%) and FX (35% vs. 38%) than twin A. Median platelet count did not differ between the twins (235 $x10^9$/l vs. 240 $x10^9$/l).

Besides GA, birth asphyxia was the only prenatal variable that affected coagulation status at birth in the linear regression analysis. Sex, maternal hypertension and/or pre-eclampsia, antenatal corticosteroid therapy, mode of delivery and Apgar scores did not show any significant association with coagulation status.

5.2. Intraventricular haemorrhage in VLBW infants (II)

Thirty-eight infants (median GA 27 weeks, median BW 933 g) were studied. There were 25 singletons, five sets of twins and one set of triplets. There were 12 (32%) SGA infants and six of these were also asphyxiated at birth (Table 1/**II**).

Cranial US was performed routinely within the first three days after birth. Cranial US could not be performed before blood sampling for the coagulation studies. Continuous arterial blood pressure was recorded and MABP results from the 72h after birth were analysed. If hypotension occurred, dopamine alone or dobutamine along with dopamine was initiated.

Thirteen (34%) IVH were diagnosed within three days after birth. In 8/13 (62%) of the infants, IVH occurred within the first day of life. The grades of the IVH were as follows: grade I, one infant; grade II, eight infants; grade III, four infants. The IVH of one infant deteriorated from grade II to grade IV at the age of four days.

Infants with IVH had mean BW 813 gs, whilst infants without IVH had mean BW 1073 gs (p=0.004). BW below 1000 gs was significantly (p=0.012) associated with IVH. Among these infants, IVH also associated with SGA and low Apgar scores (<7) at 1 min. Infants with IVH (n=13) had significantly lower mean MABP (25 mmHg vs. 29 mmHg, p=0.026) in the first three days than infants without IVH (n=25). All infants with IVH needed blood pressure support with dopamine and / or dobutamine after birth. IVH infants had significantly lower prothrombin (FII) activity at birth than infants without IVH (median 25, range 17-42; median 33, range 23-49, respectively, p=0.024).

Infants with IVH also had a trend of lower FV and FVII concentrations than infants without IVH at birth (Fig. 1/**II**).

Neither thrombotic events nor any FV Leiden mutation were observed. No differences were detected in sex, multiple gestation, caesarean section, pre-eclampsia, maternal infection or antibiotics and antenatal corticosteroid treatment between infants with and those without IVH.

Two of these infants died because of IVH (Grades III and IV). One infant with IVH (Grade II) died because of RDS.

5.3. Development of haemostasis (III)

There were 57 singletons, 11 sets of twins and one set of triplets. The median GA was 32 weeks, and the median BW was 1562 g (range 695-3520). Fifteen (18%) infants were ELBW (695-1000g). There were 26 (32%) SGA infants, and 14 (17%) had asphyxia at birth. RDS was diagnosed in 35 (43%), IVH in 8 (10%), ROP (grade II-III) in 6 (8%) and bronchopulmonary dysplasia (BPD) in 27 (33%) infants.

Three (4%) infants had NEC and 11 (13%) had septic infections during hospitalization. Peripheral central venous catheters were inserted in 13 (16%) infants. No thrombotic events were diagnosed.

FV Leiden (R506Q) was identified in three (3.6%) infants with GA 30, 31 and 34 weeks. None of them had IVH or any thrombotic problems after birth. On the other hand, no peripheral central venous catheters were inserted into these infants.

5.3.1. Development of coagulation factor concentrations during infancy

Overall development of the activities of the coagulation factors V, II, VII and X by the corrected age of six months is presented in **Figure 3**.

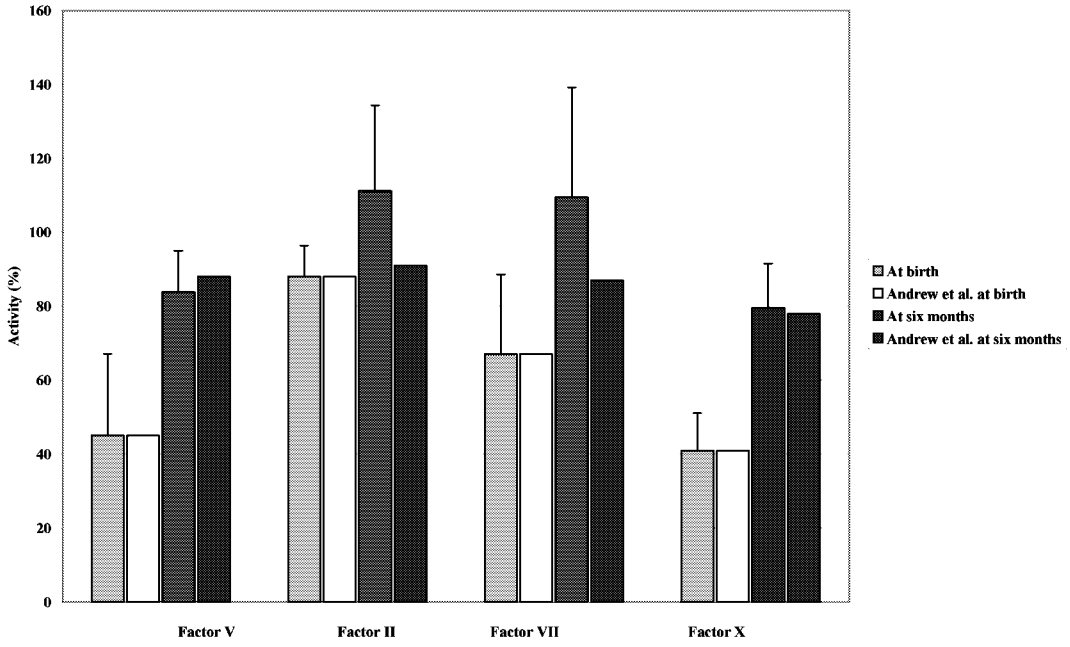

Figure 3. Development of coagulation factors V, II, VII and X in preterm infants. Values are activities (%) and expressed as mean + 1 SD at birth and at corrected age of six months. Mean levels of coagulation factor activities from the studies of Andrew et al. 1987 (preterm infants GA 30-36 at birth) and 1988 (full term infants at six months) are compared with the results of this study.

At the corrected age of six months FII, FV, FVII and FX reached healthy term six-month-old infant activity levels. FII and FX median activity levels remained at 82% and 78%, respectively. FV and FVII reached as high as mean adult plasma concentrations (109% and 101%, respectively).

ELBW infants had the lowest coagulation factor activites at birth, but regardless of the GA at birth, these infants reached the same levels as the other infants by the corrected age of six months. They increased significantly ($p<0.005$) more FV and FVII concentration values from birth to six months than the other preterm infants (Fig 1B and 1C/**III**).

The sickest infants were in the ELBW group (n=15), all of whom needed ventilation support after birth. There were five (33%) SGA infants and six (40%) infants had birth asphyxia. BPD was diagnosed in 14 (93%) infants. At birth, ELBW infants were

regarded as healthy (n=8) at the time of the first coagulation factor blood sampling if they had only RDS and no other difficulties.
Seven ELBW infants had RDS at birth and IVH later. Infants with IVH had a trend of lower median FII activity than healthy infants at birth (27% vs. 38%) and at the corrected age of six months (80% vs. 92%). Other coagulation factor concentrations showed no differences.

If all the infants (n=82) were divided into two groups, BPD (n=27) and no BPD (n=55), there was no significant difference in coagulation factor activities at birth or at six months between these groups, not even in ELBW infants.
Infants with ROP (n=6) had a trend of high median FV activity concentration (142%) at six months.

5.3.2. AT, PC and PS concentrations at corrected age of six months

Mean AT and PS activities reached term infant and adult values (Andrew et al. 1990a) at six months. Mean PC activity reached term infant value at six months, but was, as expected, lower than the mean adult reference value. AT and PC activities were not different between ELBW infants with and those without IVH. Median and mean PS activities were 73 % and 77%, respectively, in infants with IVH.They showed a trend of low activity concentrations compared with the median and mean PS activities (109% and 105%, respectively) of ELBW infants without IVH.
There was no difference in AT, PC or PS activities at six months between infants with and those without BPD.

5.3.3. Correlations between coagulation factors and physiological anticoagulants

New inter-relations between coagulation factors and physiological anticoagulants were found at birth and at the corrected age of six months. At birth, FII correlated significantly with other coagulation factors FV, FVII and FX in infants without IVH (n=74). FV at birth correlated significantly with FV at the corrected age of six months (p=0.007). FII at birth also correlated with AT, PC and PS at six months. FVII at birth and at six months correlated significantly with PC (p=0.021 and p=0.009, respectively). FVII at six months also correlated with PS (p=0.005). Spearman's correlations are shown in Table 2/**III** and significant correlations are shown in **Table 3.**

Table 3. Significant ($p<0.05$) correlations between coagulation factors and physiological anticoagulants at birth and at the corrected age of six months.

Variable	FII at birth	FV at birth	FVII at birth	FII at six month	FV at six months	FVII at six months	FX at six months	AT at six months
FV at birth	.547	.686						
FVII at birth	.515		.686					
FX at birth	.585	.614	.602					
FV at six months		.313	.313					
FVII at six months				0.405	.429			
FX at six months				0.667		0.437		
AT at six months				.466			.344	
PC at six months			.268	.444		.303	.401	.459
PS at six months				.446	.277	.323	.327	

As shown in Table 3, most coagulation factors at birth correlated with each other. At the corrected age of six months, coagulation factors correlated with each other and with physiological anticoagulants.

5.4. Central venous catheter-related thrombosis in infancy (IV)

Fourty-four infants had central venous catheters inserted within 90 days after birth (at the median age of 6 days). Thirty-four (77%) patients had non-tunneled silicone

elastomere catheters initially inserted in the subclavian vein. All catheters were heparinized for DVT prophylaxis with 2 IU of UFH heparin to 1 ml of solution.
The median GA of the infants was 36 weeks. Most of the infants (36/44) had undergone surgery, and 26/36 (72%) had had abdominal surgery. Eight (18%) infants died. No thrombi were detected in autopsy of these children, and DVT was not the cause of death in any children.

Ten infants (23%) had symptomatic catheter-related DVT. There was no difference in median GA between the infants with and those without DVT. The symptoms at the time of thrombosis were as follows: superior vena cava syndrome in 2, swelling and discoloration of the limb in 6, and repeatedly obstructed CVC in 2.

All the infants with DVT had undergone abdominal surgery due to gastroschisis, omphalocele, diaphragmatic hernia, NEC, congenital hyperinsulinism or duodenal atresia. However, two of these infants had DVT even before operation, and two infants had DVT diagnosed long after operation (64 and 272 days, respectively).
The median number of days from catheter insertion to diagnosis of DVT was 19. Infants with DVT (n=10) had a median of 26 catheter days compared with a median of 9 days ($p = <0.005$) in infants without DVT (n=34). CVC had to be removed from all the infants with symptomatic DVT, and also from 8 other infants because of bacteremia and localized infection in 4, and obstruction of CVC in 4.

The DVT diagnosis was verified by venography in all subjects. Thrombotic complications were treated predominantly with streptokinase (50-100 IU/kg/h) and warfarin (0.2-0.3mg/kg/d). Heparin infusion was used in combination with warfarin until INR was in the therapeutical range.

A positive family history with TE episodes at young age (stroke in two and DVT during pregnancy in one) was found in 3/10 families.

Laboratory parameters at the time of DVT were unrevealing. The levels of coagulation inhibitors were evaluated at the median age of 35 months (range 9-69 months). No

deficiencies of PS, PC or AT activities were observed. One child, who had DVT in each of the two CVCs inserted, was carrying the FV Leiden mutation.

6. DISCUSSION

6.1. Selection of patients

Studies I-III. The prospective patient series comprised all the preterm infants born before 37 weeks of gestation between January 1996 and May 1998 treated in the NICU of Kuopio University Hospital (n=161). At birth the final study comprised 125 infants (Study I). At the corrected age of six months (Study III), the number of infants was 82. Our study group represented only 66% of all preterm infants treated in the NICU at the time period, but 98% of all ELBW infants participated in the study. There were two reasons for the patient loss. Firstly, parental consent was not obtained at birth and some of the blood samples failed or were missing. Secondly, at the age of six months, six infants had died, and some of the parents of the healthiest infants did not want to have any blood samples taken from the infant.

This selection bias affects the results of the coagulation studies at birth, because the infants cannot be regarded as totally healthy. These results in our study may not be directly comparable with the reference values of healthy newborn infants in the literature. Andrew et al. (1988) studied healthy preterm infants at the age of one day up to day 180, and their results are used as reference data. The number of infants in their studies varied from 23 to 70. Thus, the number (n=82) of the infants and the coagulation factor levels determined in our study are well comparable with previous studies (Andrew et al. 1988, Shah et al. 1992, Reverdiau-Moalic et al. 1996, Mautone et al. 1997).

Because blood samples for the study were drawn within 2 hours after birth, most of the samples were probably taken before any bleeding problems and other complications occurred. The status of the infants after birth had to be stable enough to allow the blood sample collection before any other treatment than ventilation was performed. Therefore, the coagulation status of this patient series may be taken to represent the healthiest ELBW infants, and the results of our study may represent novel reference values for ELBW patients in general.

Almost every ELBW infant born in our hospital (n=21) at the time of the study were enrolled into the study. The number of ELBW infants born in our hospital varies from

10 to 20 per year. For future studies, national and international multicentre collaboration is needed to obtain a larger population of ELBW infants for coagulation studies.

Study IV. The retrospective patient series for the thrombosis study comprised 44 infants with CVC inserted within 90 days after birth and treated in the NICU of Kuopio University Hospital between 1990 and 1995. Patient charts of over 600 infants were analysed to identify these infants. Our study represents well the Eastern part of Finland (the Savo area) since our hospital is also the main central hospital and the only hospital in the area with tertiary care NICU.

Asymptomatic infants with CVC and thrombosis were missed from our study because only symptomatic infants were included in the retrospective study. However, our prevalence of thrombosis (23%) is consistent with earlier studies, where thrombotic complications have been observed in 1-22% of neonates with CVCs (Mehta et al. 1992, Andrew et al. 2001).

There are national registries in Canada, Germany and the Netherlands (Andrew et al.1994a, Nowak-Göttl et al.1997b, van Ommen et al. 2001). Data from the Canadian 1-800-NO-CLOTS Consultation Service (Kulhe et al. 2004) contains information from the USA, Europe and Australia. Again, multicentre studies are needed to obtain more data about thrombotic problems in neonates. The collected data and analyses of the results increase our knowledge of the thrombotic problems in children world-wide.

6.2. Methodological discussion

It is known that there are many preanalytical factors that may affect the results of coagulation analyses. Although special care was taken in handling the blood samples, there might have been factors which may have interfered in the results.

Because ELBW infants were included in the study, a maximum volume of 3.5 ml blood was available for the analysis of the coagulation factors. Coagulation factors FII, FV, FVII and FX could be analysed by the same method, but FIX would have needed more

blood. This is why physiological anticoagulants were not measured until the corrected age of six months.

After birth, the blood samples were sent to the Kuopio University Hospital laboratory to be centrifuged, aliquoted and kept frozen (-80°C) until sent to Helsinki for analysis. These samples were sent to Helsinki in dry ice. At the corrected age of six months, all the blood samples for coagulation studies were sent to the haemostasis laboratory in Helsinki at +4°C within 4 to 8 hours. This is the standard method of the laboratory. Thereafter, the samples were separated in the same way as the samples taken at birth.

The Department of Haemostasis of the Finnish Red Cross Blood Service has analysed differently handled coagulation blood samples: immediately handled samples vs. 4 to 12 hours after sampling (personal communication with E.V.). The level of the labile FV especially may fall (0-11%) within hours, but it falls most within 4 to 8 hours. The other coagulation factors (FII, FVII and FX) are considered stable. When the coagulation factor correlations from the same individuals at birth and at the corrected age of six months were analysed (Study III), good correlations between all coagulation factors were observed. There was a statistically significant correlation especially between the levels of the labile FV measured at birth and at the corrected age of six months. Furthermore, the median activity level of FV at the corrected age of six months was at or more than 100 %. Thus, it is seems that the different handling and transportation of the blood samples did not interfere significantly with our results.

Whenever similar coagulation studies are undertaken, care should be taken in the study design to avoid preanalytical errors.

6.3. Coagulation physiology

There is no Finnish reference data of coagulation values for newborn infants. National reference data are needed because the laboratory reference values differ between countries depending on the laboratory methods and reagents used, and national data are affected by the ethnic groups living in each country. Hence, neonatal coagulation data from the USA cannot be compared reliably with Finnish neonatal data. Andrew et al. (1988) studied coagulation data of infants aged from birth to six months, but they did

not follow-up the same infants. In our study the same infants could be followed up to the corrected age of six months. Our coagulation data of preterm infants were similar to those of Andrew et al. (1988). In general, our results confirm earlier coagulation data on preterm infants and may thus be taken as Finnish reference data of the selected coagulation factors studied.

There is a lack of information on the coagulation status of ELBW infants at birth (**Table 3**). The other studies which have included ELBW infants have used cord blood, or the blood tests have been taken from fetuses, and there has not been any follow-up of these infants. Some studies have used arterial or venous blood, but they have only studied screening tests and fibrinogen (Sequin and Topper 1994, Hannam et al. 2003) and physiological anticoagulants (Shah et al. 1992).

Kazzi et al. (1989) studied coagulation factors at birth. They pooled all their coagulation results together (79 preterm infants, GA 22-34 weeks). Infants whose mothers had antenatal vitamin K- treatment (n=40) did not differ from the control infants (n=39) with low levels of the coagulation factors II, VII and X. Barnard et al. (1979) had 11 ELBW infants in their study (n=30): these infants had concentrations of the coagulation factors II, V, VII and X similar to ours. Thorp et al. (1995) studied 188 preterm infants (GA 24-33 weeks) at birth, including 18 preterm infants with GA $\leq$ 26 weeks. Their coagulation results concerning vitamin K-dependent coagulation factors (FII, FVII, FIX and FX) at cord blood were similar to ours.

Neonatal platelets have reduced functional capacity and a hyporeactive response to platelet stimuli. Acquired and inherited neonatal diseases may influence fetal and neonatal platelet physiology (Kühne and Imbach 1998). Platelets are closely linked to the coagulation factors, such as FV, which are stored in platelet α-granules and secreted upon platelet stimulation (Roberts et al. 2001).

We found at birth lower platelet counts and FV in asphyxiated and IVH infants than in infants without asphyxia and IVH. These findings may indicate that preterm infants with pre- and neonatal complications have problems with primary haemostasis and initiation of coagulation.

Table 3. Comparison of this study with other published studies of the specific coagulation data in preterm infants after birth. No studies with fetuses are included.

Authors	Number of infants	Gestational age	Findings in coagulation data F II FVII FX FV activities (%)	Follow-up	Comments
Barnard et al. 1979	30 (three groups: 10+12+8)	24-31	Mean coagulation factor values in each group: FII 31 - 30 - 29; FVII 37 - 42 - 39; FVII-X 37 - 35 - 31; FV 65 - 41 - 44	No	Patients divided into groups: healthy, unwell, and severely ill
Andrew et al. 1988	40-96	30-36	Mean coagulation factor values: d1 and d180: FII 45 - 87; FVII 67 - 99; FX 41 - 77; FV 41 -102	Day 1, 5, 30, 90, 180	healthy infants; number of infants varied in follow-up
Kazzi et al. 1989	79 (two groups: 39 + 40)	22-34	Mean coagulation factor values in two groups: FII 20.5 - 25.5; FVII 45 - 46.5; FX 49 - 55	No	controls and infants in antenatal vitamin K therapy
Pietersma-de Bruyn et al. 1990	two groups: 17	32-34	Mean coagulation factor values: d0 and d28 in two groups: FII 33 - 42; FX 49 - 60	Day 0, 3, 7, 28	Preterm infants (n=4) received 1 mg Konakion i.m. after birth
	4	29-34	FII 27 - 41; FX 39 - 62		
Thorp et al. 1995	188 (two groups: 90 + 98)	24-33	Mean coagulation factor values in two groups: FII 40.3 - 42.0; FVII 67.0 - 66.8; FX 47.0 - 49.2	No	controls and infants in antenatal vitamin K and phenobarbital therapy
Present study	82	24-36	Mean coagulation factor values: FII 37 - 83; FVII 61 - 105; FX 40 - 79; FV 75 - 111	same infants at birth and at corrected age of six months	sick and healthy infants; correlations at birth and 6 months

To our knowledge, this is the only prospective follow-up study of the development of the coagulation status in the same preterm infants from birth to the corrected age of six months, and as far as we know, this is the first study of the selected coagulation factors of ELBW infants at birth.
In future research it would be interesting to discover whether these preterm infants reach the term infant coagulation status earlier than at the corrected age of six months.

6.4 Correlations between coagulation factors and physiological anticoagulants

Recently, van Hylckama Vlieg et al. (2003) found clustering between FVII and FX, and between FII and PC in healthy adults. They showed evidence for inter-relations between the levels of coagulation factors in the clotting cascade.

As far as we know, there are no studies showing the correlations or interactions between coagulation factors and physiological anticoagulants in children. Through our prospective follow-up study it was possible to identify new inter-relations of coagulation factors at birth and during the first six months of development. We found significant correlations between coagulation factors FII, FV, FVII and FX at birth. FII, FVII and FX are vitamin K-dependent coagulation factors, and their correlation is likely to be due to this. Correlation between FV and vitamin K-dependent factors, on the other hand, might be explained by the coagulation value levels: they followed the same pattern at birth. If the values of FII, FVII and FX were low, so were the values of FV.

FII at birth correlated with AT, PC and PS at six months, and FVII correlated with PC and PS at six months. The levels of these factors influence each other both positively and negatively. These inter-relations between pro- and anticoagulant factors suggest that there might be more underlying causes than heredity for high or low levels of coagulation factors.

6.5. Coagulation abnormalities of preterm infants

The main haemorrhagic problem among preterm infants in this study was IVH (Studies II-III). In our study, the incidence of IVH among all infants was 10%. When only the infants with GA 24-32 were included, the incidence of IVH was 22%. If only ELBW

infants were included, the incidence of IVH rose to 48%. Other studies have shown that the incidence of IVH is between 30 and 50% among ELBW infants (Lemons et al 2001, Tommiska et al. 2001). Our results are thus similar to those of earlier studies.

Haemophilia A (FVIII deficiency) and B (FIX deficiency) is associated with IVH in newborns (Chalmers 2004, Revel-Vilk et al. 2004). Newborns with FVIII deficiency may be diagnosed after birth because physiologic FVIII concentrations are the same as physiologic adult plasma concentrations (Andrew et al.1987 and 1988b). Diagnosis of mild forms of FIX deficiency can be difficult after birth because in normal neonates physiologic plasma concentrations of FIX are lower than in adults (Andrew et al.1987 and 1988b). We did not measure FVIII and FIX activities. It could be useful to consider FVIII measurement in male infants with IVH.

The possible contribution of haemostasis defects to the IVH in premature infants has remained controversial. In our study, patients with IVH had a tendency to lower prothrombin levels at birth than those without IVH. Because cranial US could not be performed before the blood samples for the coagulation studies were taken, our results might reflect already on-going haemorrhage. Whether the association between low FII and IVH was incidental or a true causal relation must be further studied.

Beverley et al. (1984) found lower activity of FII, FVII and FX activity in preterm infants with IVH at the age of two days. They found that a comparison of infants with IVH and without IVH showed no significant differences in their haemostatic parameters at birth. However, they noticed a trend for infants with IVH to have a longer PT and APTT at birth and at 48 hours than infants without IVH.

In our study, asphyxiated infants had lower mean concentrations of FII, FVII, FX, FV and platelet counts. These findings are similar to those in other studies (Chessels and Wigglesworth 1979, Barnard et al. 1979). As far as we know, there is no recent study of coagulation factors and hypoxia / asphyxia at birth. Mautone et al. (1997) found no prothrombin stimulation in hypoxic newborns, but hypoxia and acidosis were responsible for high PAI and tPA levels and normal TAT levels, indicating endothelial cell activation and damage.

Peters et al. (1984a) found that the levels of FII, FX, AT, plasminogen and α_2-antiplasmin were significantly lower in preterm infants with RDS than in healthy premature infants. They suggest that low AT level is predictive of a poor outcome.
Cvirn et al. (2001a) increased the α-2-macroglobulin concentration, and did not find any significant influence in the amount of prothrombin activated in cord plasma containing physiological amounts of AT. The same results were obtained in adult plasma. They speculate that AT is the most important inhibitor in both cord and adult plasma.

Brandenberg et al. (1997) gave early AT substation to preterm infants after birth, and found that the incidence of IVH (13%) was lower than in epidemiological studies. Even though low plasma levels of AT have also been observed in RDS (Schmidt et al. 1992b), treatment with AT concentrate did not improve clinical outcomes (Schmidt et al. 1998).

Peters et al. (1984b) report that SGA infants had significantly lower AT values than AGA infants at birth, and the values remained low for several days. They found higher incidence of plascental infarction, polycythemia and thrombocytopenia in these SGA infants than in AGA infants.

We did not measure physiological anticoagulant levels at birth, but there were no differences between SGA infants and AGA infants, or any other perinatal or neonatal complication, detected at the corrected age of six months in our study.

Von Kries et al. (2001) identified foetal thrombophilia (FV G1691A, FII G20210A, elevated lipoprotein (a), PC-, PS-, AT-deficiency) as an additional cause of low birth weight in term infants. On the other hand, Verspyck et al. (2002) did not find any difference in the prevalence of inherited thrombophilia between SGA infants and AGA infants. Frequencies for acquired thrombophila were higher in women with small for gestational age infants compared with controls. Kenet et al. (2003) studied preterm infants with genetic thrombophilia (FV Leiden, FII G20210A, methylenetetrahydrofolate reductase) and did not find any increased risk for neonatal complications.

FV Leiden was diagnosed in five infants, and only one of them was SGA. PS levels were found to have a lower trend in infants with than in infants without IVH at the corrected age of six months, but the number of IVH infants was so small (n=8) that nothing more can be predicted from it.

Prothrombotic risk factors (FV Leiden, FII G20210A, methylenetetrahydrofolate reductase) have also been associated with hydrocephalus and IVH occurring in newborns (Riikonen and Kekomäki 1998, Petäjä et al. 2001, Aronis et al. 2002).

In this study, PVL was found in two infants. They had IVH grade II diagnosed on day 2 and day 3, respectively, and PVL changes were detected a few weeks later. Neither of the mothers had any infection.

Some studies have found an association between premature rupture of the membranes and IVH and PVL (Spinillo et al. 1995b, de Vries et al. 1998).

The pathogenesis of PVL is associated with impaired cerebrovascular autoregulation and fluctuations in cerebral blood flow (Volpe 2001). The same pathogenesis is also related to IVH (Lynch and Nelson 2001, Golomb et al. 2001). We registered the lowest mean arterial blood pressure MABP per day, but no data of the fluctuation of the MABP, which might reflect the cerebral blood flow, were available. In this study, significantly lower MABP was detected in neonates with IVH than in neonates without IVH within the first two days of life. All the infants with IVH needed vasopressors. The Canadian report (Synnes et al. 2001) of NICU-treated infants noted the association between IVH and use of vasopressors and treatment of acidosis on day one. In our study, the treatment of acidosis was not recorded from patient charts. It is not likely that the vasopressors or the treatment of acidosis per se causes IVH, but in future it might be useful to measure the activity of specific coagulation factors and anticoagulants when prevention and/or treatment for hypotension and acidosis in preterm infants is needed.

Beverley et al. (1985) report that FFP appeared to have a beneficial effect in the prevention of IVH. In our study, all infants were studied for coagulation factors before any treatment. Infants who had IVH were treated with FFP, but their haemostatic parameters were not studied thereafter.

Hyytiäinen et al. (2003) measured FV, FVII, PS and AT, and TAT, F1+2 and D-dimer before and 15 min and 2 hours after FFP. There was an increase of PC, AT, FV and FVII during the FFP transfusion, but only modest effects on coagulation activation were observed.

In future, if microtechnique studies or bed-side tests for haemostatic parameters are available, it would be valuable to study detailed coagulation status before and after IVH treatment.

6.6. Central venous catheter-related thrombosis in infancy

Although the haemostasis of premature infants seems to be balanced, it is easily disturbed and become either haemorrhagic (for example, IVH), or thrombotic (for example in association with indwelling catheters). In addition to low levels of vitamin K-dependent procoagulant and anticoagulant factors, defects of some buffering factors may have remained unidentified.

In this study, 23% (10/44) of the infants had symptomatic catheter-related DVT. Other studies have reported thrombotic complications in 1-22% of neonates requiring CVCs (Mehta et al. 1992, Tanke et al. 1994), especially if an indwelling catheter is used in combination with surgery (van Ommen et al. 2003). Van Ommen et al. (2001) found that 94% of neonatal thromboses were CVC-related.

No deficiencies in PS, PC or AT were found in our study. Some studies have reported genetic defects of coagulation inhibitors causing thrombosis in infancy (Nowak-Göttl et al. 1996 and 1997a, van Ommen and Peters 2003). We found one infant with the FV Leiden mutation who had multiple catheter-related DVTs. On the other hand, Kenet et al. (2003) found no increased risk for neonatal complications in preterm infants with thrombophilia. Our findings are consistent with this.

There may be several other predisposing factors, such as surgery, infection, and asphyxia for CVC- related DVT. When these factors are added to the prothrombotic tendency, it may lead to thrombosis (van Ommen and Peters 2003). In our study, all children with CVC-related DVT had undergone abdominal surgery and required CVC for prolonged parenteral nutrition.

Newall et al. (2003) report eight children with short-gut syndrome or small intestinal anomalies who needed long-term total parenteral nutrition. Warfarin therapy was associated with longer mean CVC duration than was no therapy. There were no major bleeding events, nor was clinical thrombosis observed. In our study, no antithrombotic prophylaxis was used, but infants with DVTs had warfarin therapy for six months after DVT.

UFH has been the most commonly used anticoagulant for decades for prophylaxis and treatment of TE (Moll and Roberts 2002). There are few studies of UFH in newborns (Andrew et al. 2001, Monagle et al 2001). The clinically important side effects of UFH include major bleeding (Moritz et al. 2003). LMWHs have been widely used for prophylaxis and treatment of thromboembolic events in recent years (Moll and Roberts 2002).

In future, more studies are needed to discover the most effective and safest prophylaxis and treatment of CVC-related thrombosis in children, especially in infants with intestinal anomalies.

New and evolving methods of diagnosing genetic disorders have made thromboembolic research a vast field, and there are many yet unanswered questions.Well-designed international multi-centre studies are required if we are to obtain accurate evidence-based information concerning neonatal haemostasis.

7. SUMMARY AND CONCLUSIONS

Because of the improvements in antenatal and neonatal intensive care during the last few decades, the mortality of ELBW has declined significantly, and more ELBW infants survive. Bleeding is often the most common problem diagnosed in neonatal intensive care. Acquired disorders most often present in sick term or preterm infants, but many inherited disorders may manifest in otherwise healthy infants. IVH contributes to most of the morbidity and mortality in ELBW infants. The haemostasis of neonates is a developing system, which makes it difficult to set true reference values for newborns. Not only gestational and postnatal ages but also the health status of the infant influence the values.

There were no Finnish reference data of coagulation studies for preterm and newborn infants available before this study. National reference data are needed because the laboratory reference values differ between countries, depending on the laboratory methods and reagents used, and national data are affected by the ethnic groups living in each country. There is a lack of information concerning coagulation status at birth and the natural evolution after birth in ELBW infants especially. Our results confirm the previously reported low coagulation values at birth in preterm infants. In addition, the results concerning the coagulation status of ELBW infants may add to the reference data of the selected coagulation factors available internationally.

Vitamin K-dependent coagulation factors FII, FVII and FX and FV were used in this study. As a central terminal factor of the coagulation cascade regulating the formation of thrombin itself, FII was lower in infants with IVH than in infants without IVH. Labile FV and FVIII are near normal adult values at term birth. In our study, ELBW infants had low activities of FV at birth. Measuring FII, FV and FVIII might help paediatricians to identify the infants who might bleed after birth.
It would be valuable to discover a simple means to analyse the coagulation status of newborn infants.

IVH infants had significantly lower prothrombin activity at birth than infants without IVH. Infants with IVH also had a trend of lower FV and FVII concentrations than

infants without IVH at birth. The sickest infants were in the ELBW group: they had RDS at birth and IVH later. Sick ELBW infants had lower median FII activity than healthy infants at birth and at the corrected age of six months, but the difference was not statistically significant. There were no differences in other coagulation factor concentrations.

Asphyxiated infants had significantly lower mean FII, FV, FVII and FX concentrations and platelet counts than infants without asphyxia at birth. SGA infants had significantly lower mean FV and FVII concentrations and platelet counts than AGA infants. In twin pregnancies, twin B had lower median activities of FII, FV, FVII and FX than twin A. These high risk neonates should be identified, properly evaluated and treated during intensive care.

New inter-relations between selected coagulation factors at birth and physiological anticoagulants during the first six months of development were found. These findings are consistent with those that have been observed earlier in healthy adults. These findings may indicate that some regulatory mechanisms may exist between the coagulation factors and physiological anticoagulants.

Fourty-four infants had central venous catheters inserted within 90 days after birth. The incidence of symptomatic catheter-related DVT was 23%, which is consistent with the incidence reported in other studies.

All the infants with DVT had undergone abdominal surgery, compared with 47% of the infants without DVT. Haemostasis screening tests performed at the time of DVT were unrevealing. Predisposing factors such as surgery, when added to the general prothrombotic tendency in infancy, may lead to thrombosis.

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