Effects of gestational age and prenatal and perinatal events on the coagulation status in premature infants

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Objective: To study prospectively the effects of prematurity and perinatal events on the coagulation status of premature infants.

Patients and main outcome measures: Blood samples from premature infants born before 37 gestational weeks were taken for analysis of coagulation factors II, V, VII, and X and platelet count.

Results: A total of 125 premature infants, 71 boys, were studied at the median postnatal age of 40 minutes (range 12–100). The lowest median activities of coagulation factors II, V, VII, and X and the platelet count were observed, as expected, in infants (n = 21) born at 24–27 weeks gestation. Twin B (n = 14) had lower median activities of coagulation factors II, V, VII, and X than twin A. Infants with evidence of mild asphyxia (Apgar score at 5 minutes < 7 or cord pH < 7.26) had significantly (p < 0.05) lower levels of coagulation factors II, V, VII, and X and platelet counts than infants without asphyxia. Infants who were small for gestational age (SGA) had significantly (p < 0.05) lower levels of coagulation factors V and VII and platelet counts than infants of appropriate size for gestational age. Other prenatal and perinatal variables examined (sex, maternal hypertension and/or pre-eclampsia, antenatal steroid use, mode of delivery, Apgar scores) did not show any significant associations with coagulation status, which may be explained by the small number of infants studied.

Conclusions: The data strongly suggest that there are distinct differences in specific coagulation tests in different patient populations, which could assist in the identification of extremely preterm, SGA, or asphyxiated preterm infants who may be susceptible to haemorrhagic problems perinatally.
are sensitive to hypoxia, we wished to include infants who may have been modestly hypoxaemic. Small for gestational age (SGA) was defined as a standardised birth weight < -2.0 standard deviations of the gestational age adjusted mean birth weight of ethnic growth curves. Ventilation support after birth was recorded.

The charts of the mothers were reviewed for information, including maternal medication and the presence or absence of pre-eclampsia or hypertension. Women who repeatedly had a systolic pressure ≥ 140 mm Hg and a diastolic pressure ≥ 90 mm Hg were defined as having hypertension. Hypertension accompanied by proteinuria of at least 0.3 g per 24 hours was classified as pre-eclampsia.

Antenatal corticosteroid (betamethasone 12 mg intramuscularly) was given to the mother if there was a risk of a premature birth before 35 gestational weeks. If necessary, buphenine hydrochloride infusion was given as a tocolytic agent to delay or prevent premature parturition. Antibiotics were used to treat diagnosed/suspected amnionitis or chorioamnionitis. The most common antihypertensive drugs for mothers were labetalol and nifedipine.

**Laboratory methods**

Tests for the coagulant activity of factors II, V, VII, and X, as well as prothrombin time, activated partial thromboplastin time, and platelet count, were used to evaluate the coagulation status of the preterm baby. As extremely premature babies were included in the study, the maximum volume of blood available for analysis was 3.5 ml, which was enough to determine factors II, V, VII, and X. Factors II, VII, and X are vitamin K dependent.

Factor II has the longest natural half life, and factor VII the shortest half life; thus they effectively reflect synthesis of vitamin K dependent coagulation factors in the liver. Factor V is synthesised very early in pregnancy compared with the other factors. Its synthesis is not dependent on vitamin K, but it is labile and is consumed in disseminated intravascular coagulation, as well as fibrinogen. Analysis of factor IX and fibrinogen would have needed another blood sample.

Samples for the assessment of coagulation status were obtained from the peripheral arterial or venous catheters immediately after placement. Blood samples for assay of coagulation factors flowed directly into Vacuette Greiner tubes (1.8 ml). Samples for determining prothrombin time and activated partial thromboplastin time were drawn into special citrate tubes for infants (CTAD 0.1 ml; Becton Dickinson; 0.9 ml). The amount of anticoagulant was not adjusted for packed cell volume. However, median and range of packed cell volumes in each gestational age group were similar (data not shown). Samples for platelet count were taken into EDTA Microtainer (Becton Dickinson, Franklin Lakes, New Jersey, USA) tubes (0.25 ml).

The blood for the coagulation studies was immediately centrifuged at room temperature at 1400 g for 30 minutes; plasma was removed, divided into aliquots, and kept at −80℃ for future assays. The coagulation factor samples were analysed batchwise in the Department of Hemostasis of the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland. Platelet count, prothrombin time, and activated partial thromboplastin time were measured immediately in the Department of Clinical Chemistry of Kuopio University Hospital, Finland.

The coagulant activity of factors II, V, VII, and X was determined by the one stage method with an STA Compact Analyser from Diagnostica Stago (Asnières, France). Factor deficient plasma and Neoplastine CI Plus were purchased from Diagnostica Stago. A frozen normal plasma pool was used as a standard, and calibrated with the international standard (National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK) for prothrombin and factors VII and X. Activity was expressed as a percentage (100% = 1 IU/ml).

Prothrombin time and activated partial thromboplastin time were measured with a Thrombolyzer (Behnk Elektronik, Norderstedt, Germany) using Thrombotest and Cephotest reagents (Nycomed, Oslo, Norway). Prothrombin time was expressed as the international normalised ratio (INR). Platelet counts were measured with a Coulter STKS analyzer (Coulter Corporation, Miami, Florida, USA).

**Statistical analysis**

Results are expressed as mean (SD) or median (range), as appropriate. Overall differences in the components between the gestational age groups were first tested with the Kruskal-Wallis test. For each component measured, the pairwise comparisons between the gestational age groups were performed using the Mann-Whitney U test. Bonferroni correction was made for multiple comparisons. The Wilcoxon signed ranks test was used to analyse the coagulation factors and platelet count between twins A and B. Linear regression analysis was used to identify the associations between coagulation factors and the following prenatal and perinatal variables: sex, intrauterine growth status, maternal hypertension and/or pre-eclampsia, antenatal steroid use, mode of delivery, gestational age, Apgar scores, and venous cord blood pH. p < 0.05 were considered significant. SPSS (9.0 for Windows) was used for all data analysis.

**RESULTS**

The series comprised 125 children, 71 boys, recruited during 28 months. The median gestational age was 33 weeks (range 24–36), and the median birth weight was 1740 g (range 515–3740). There were 88 singletons, 14 sets of twins, and three sets of triplets. Thirty nine (31%) were SGA. There were 34 (27%) asphyxiated infants; 15 of them were also growth retarded. The newborns were divided into four groups on the basis of gestational age: group 1, 21 (17%) babies with gestational age < 28 weeks; group 2, 25 (20%) babies with gestational age 28–30 weeks; group 3, 34 (27%) babies with gestational age 31–33 weeks; group 4, 45 (36%) babies with gestational age 34–36 weeks. These infants represent 98% of all infants born before 28 weeks, 71% of infants born at 28–30 weeks, 62% of infants born at 31–33 weeks, and 18% of infants born at 34–36 weeks during the study period.

### Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>n</th>
<th>Birth weight (g)*</th>
<th>Male n (%)</th>
<th>Caesarean section n (%)</th>
<th>Apgar score* at 1 min/5 min</th>
<th>Cord vein pH*</th>
<th>SGA† n (%)</th>
<th>Infants with asphyxia‡ n (%)</th>
<th>Ventilation support n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24–27</td>
<td>21</td>
<td>790 (515–1180)</td>
<td>15 (71)</td>
<td>16 (76)</td>
<td>6 [2–9]/8(3–9)</td>
<td>7.31 (7.02–7.43)</td>
<td>9 (43)</td>
<td>8 (38)</td>
<td>21 (100)</td>
</tr>
<tr>
<td>28–30</td>
<td>25</td>
<td>1150 (770–1850)</td>
<td>10 (40)</td>
<td>19 (76)</td>
<td>7 [2–9]/8(2–9)</td>
<td>6.30 (6.50–7.50)</td>
<td>7 (28)</td>
<td>9 (36)</td>
<td>23 (92)</td>
</tr>
<tr>
<td>31–33</td>
<td>34</td>
<td>1820 (1140–2710)</td>
<td>21 (62)</td>
<td>28 (82)</td>
<td>8 [3–9]/9(6–10)</td>
<td>7.31 (7.08–7.48)</td>
<td>10 (30)</td>
<td>7 (20)</td>
<td>17 (50)</td>
</tr>
<tr>
<td>34–36</td>
<td>34</td>
<td>2460 (1670–3740)</td>
<td>25 (73)</td>
<td>29 (84)</td>
<td>9 [0–9]/8(8–14)</td>
<td>7.32 (6.89–7.48)</td>
<td>13 (39)</td>
<td>10 (22)</td>
<td>17 (38)</td>
</tr>
</tbody>
</table>

*Expressed as medians (range); †SGA, small for gestational age (< -2.0 SD gestational age adjusted mean birth weight$^{2}$); ‡asphyxia defined as venous cord blood pH < 7.26 or Apgar score < 7 at 5 minutes.
The median time interval between birth and blood sampling was 40 minutes (range 12–100). The lowest median levels of coagulation factors II, V, VII, and X and platelet count were detected in infants (n = 21) born at 24–27 weeks gestation. The levels increased towards term infant reference data with increasing gestational age. The levels of factors II, V, and VII were significantly different between infants born at 24–27 weeks gestation (n = 21) and those born at 34–36 weeks gestation (n = 45) (table 2). The infants born at 24–27 weeks gestation had a significantly lower median platelet count than those born at 34–36 weeks gestation. No difference was found in activated partial thromboplastin time and INR values between the gestational groups (table 2).

Infants with birth asphyxia had lower mean concentrations of coagulation factors, platelet counts, and INR than infants without asphyxia (table 3). SGA infants had lower mean concentrations of coagulation factors V and VII and platelet count (table 3).

In twin pregnancies (n = 14), twin B had lower median activities of coagulation factors II, V, VII, and X than twin A (fig 1). A–B difference between the twin pairs showed that twin A had consistently higher coagulation factor activities than twin B (table 4). Median platelet count did not differ between the twins (235 × 10^9/l v 240 × 10^9/l). There were no differences in birth weight, Apgar score, or cord blood pH between twins.

Linear regression analysis showed that asphyxiated infants had significantly lower activities of coagulation factors II, V, VII, and X and platelet counts than non-asphyxiated infants. SGA infants had significantly (p<0.05) lower activities of coagulation factors V and VII and platelet counts than appropriate for gestational age infants. Other prenatal and perinatal variables examined (sex, maternal hypertension and/or pre-eclampsia, antenatal steroid used, mode of delivery, Apgar scores) did not show any significant associations with coagulation status. Gestational age, intrauterine growth status, and venous cord blood pH did not affect the haemostasis screening tests in linear regression analysis.

### Table 2 Coagulation status in each gestational age group within two hours of birth

<table>
<thead>
<tr>
<th>Gestational age (weeks)</th>
<th>n</th>
<th>Factor II (%)</th>
<th>Factor V (%)</th>
<th>Factor VII (%)</th>
<th>Factor X (%)</th>
<th>Platelets (× 10^9/l)</th>
<th>APTT† (s)</th>
<th>PT-INR†</th>
</tr>
</thead>
<tbody>
<tr>
<td>24–27</td>
<td>21</td>
<td>31 (9)*</td>
<td>57 (20)*</td>
<td>43 (16)*</td>
<td>35 (10)*</td>
<td>181 (71)*</td>
<td>49 (8)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>30</td>
<td>61</td>
<td>46</td>
<td>34</td>
<td>166</td>
<td>48</td>
<td>1.4</td>
</tr>
<tr>
<td>28–30</td>
<td>25</td>
<td>30 (8)*</td>
<td>67 (25)*</td>
<td>50 (22)*</td>
<td>36 (9)</td>
<td>214 (64)*</td>
<td>46 (10)</td>
<td>1.4 (0.2)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>29</td>
<td>65</td>
<td>51</td>
<td>34</td>
<td>225</td>
<td>45</td>
<td>1.3</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>17–47</td>
<td>29–113</td>
<td>15–103</td>
<td>20–55</td>
<td>59–322</td>
<td>30–72</td>
<td>1.0–2.2</td>
</tr>
<tr>
<td>31–33</td>
<td>34</td>
<td>39 (13)</td>
<td>78 (28)</td>
<td>64 (28)</td>
<td>44 (15)</td>
<td>237 (34)</td>
<td>44 (22)</td>
<td>1.4 (0.7)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>37</td>
<td>73</td>
<td>62</td>
<td>42</td>
<td>238</td>
<td>41</td>
<td>1.3</td>
</tr>
<tr>
<td>34–36</td>
<td>45</td>
<td>38 (10)</td>
<td>76 (26)</td>
<td>63 (23)</td>
<td>40 (10)</td>
<td>255 (65)</td>
<td>46 (28)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td></td>
<td>37</td>
<td>73</td>
<td>63</td>
<td>39</td>
<td>261</td>
<td>41</td>
<td>1.3</td>
</tr>
<tr>
<td>Term infant‡</td>
<td></td>
<td>48 (11)</td>
<td>72 (18)</td>
<td>66 (19)</td>
<td>40 (14)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05 compared with gestational age 34–36 weeks; †Data available for 101 infants; ‡at the age of 1 day.

APTT, Activated partial thromboplastin time; PT, prothrombin time; INR, international normalised ratio.

### Table 3 Coagulation status in asphyxiated versus non-asphyxiated infants and SGA versus AGA infants within two hours of birth

<table>
<thead>
<tr>
<th>Infant group</th>
<th>n</th>
<th>Factor II (%)</th>
<th>Factor V (%)</th>
<th>Factor VII (%)</th>
<th>Factor X (%)</th>
<th>Platelets (× 10^9/l)</th>
<th>APTT† (s)</th>
<th>PT-INR†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asphyxiated</td>
<td>34</td>
<td>30 (10)*</td>
<td>61 (27)*</td>
<td>44 (27)*</td>
<td>32 (10)*</td>
<td>202 (87)*</td>
<td>50 (25)</td>
<td>1.6 (0.6)*</td>
</tr>
<tr>
<td>Non-asphyxiated</td>
<td>91</td>
<td>38 (11)</td>
<td>75 (25)</td>
<td>62 (24)</td>
<td>42 (11)</td>
<td>239 (70)</td>
<td>45 (20)</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>SGA</td>
<td>39</td>
<td>35 (11)</td>
<td>63 (25)*</td>
<td>47 (23)*</td>
<td>38 (12)</td>
<td>182 (60)*</td>
<td>49 (30)</td>
<td>1.5 (0.4)</td>
</tr>
<tr>
<td>AGA</td>
<td>86</td>
<td>36 (11)</td>
<td>76 (26)</td>
<td>62 (23)</td>
<td>40 (11)</td>
<td>250 (74)</td>
<td>45 (17)</td>
<td>1.3 (0.4)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

*p<0.05, compared with non-asphyxiated infants; †p<0.05, compared with AGA; ‡Data available for 101 infants.

APTT, Activated partial thromboplastin time; PT, prothrombin time; INR, international normalised ratio; SGA, small for gestational age; AGA, appropriate size for gestational age.

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The median time interval between birth and blood sampling was 40 minutes (range 12–100). The lowest median levels of coagulation factors II, V, VII, and X and platelet count were detected in infants (n = 21) born at 24–27 weeks gestation. The levels increased towards term infant reference data with increasing gestational age. The levels of factors II, V, and VII were significantly different between infants born at 24–27 weeks gestation (n = 21) and those born at 34–36 weeks gestation (n = 45) (table 2). The infants born at 24–27 weeks gestation had a significantly lower median platelet count than those born at 34–36 weeks gestation. No difference was found in activated partial thromboplastin time and INR values between the gestational groups (table 2).

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The present study confirms previous observations that coagulation factor activity and platelet counts in extremely preterm infants are lower than in those born at 34–36 weeks.

The haemostatic system evolves gradually throughout gestation and early infancy. The newborn physiological status affects the risk for and presence of acquired haemostatic disorders. This may make congenital haemostatic disorders more difficult to diagnose. In particular, prematurity, birth asphyxia, hypoxia, and intrauterine growth retardation have been associated with haemostatic abnormalities.

The present study confirms previous observations that coagulation factor activity and platelet counts in extremely preterm infants are lower than in those born at 34–36 weeks.

Pre-eclampsia is known to be associated with a hypercoagulable state in both maternal and placental circulations. The primary defect is speculated to occur at mid-trimester. Severe pre-eclampsia is one of the most common reasons for intrauterine growth retardation. A hypercoagulable state with consumption may be among the reasons why the coagulation factors and the platelet counts were low in our SGA children. Thrombopoeitin is a potent megakaryocyte colony stimulating factor. In thrombocytopenia associated with intrauterine growth retardation or pregnancy induced hypertension, infants have decreased megakaryocyte mass. Preterm babies may have impaired ability to increase thrombopoietin concentrations in response to thrombocytopenia. However, the exact mechanisms of this regulation are not known, although megakaryocytes are known to be susceptible to asphyxia. The present study confirms that platelet counts in infants with birth asphyxia are low, which may further increase the vulnerability of the haemostatic system.

Not only does the generation of thrombin, a critical enzyme in haemostasis, differ profoundly during fetal and early neonatal life, but also thrombin activity is lower in premature infants than in adults. The altered regulation of thrombin generation is at least partly due to the presence of low levels of the vitamin K dependent procoagulants (factors II, VII, IX, and X) and the contact factors XI and XII. Despite the lower capacity to generate thrombin, there is no clinical evidence that healthy infants have a higher risk of bleeding complications. However, the further reduction in coagulation factor levels seen in sick and premature infants may contribute to the haemorrhagic complications.

Haemostasis of a premature baby is a dynamic system that gradually evolves towards term. However, when prematurity is associated with asphyxia and/or intrauterine growth retardation, alterations in the concentrations of the coagulation factors may cause an imbalance and thus predispose the infant at risk to haemorrhagic or thromboembolic complications.

As the fibrinolytic system was not evaluated in this study, it is difficult to estimate to what extent haemostatic defects are balanced by reduced fibrinolysis or increased thrombotic risk. Preterm babies with intrauterine growth retardation and/or asphyxia may need more careful investigation for haemorrhagic symptoms after birth. These high risk neonates should be properly evaluated during neonatal intensive care. Good perinatal care of preterm twins and triplets should be emphasised. Further research is needed to determine whether these findings have relevance to haemorrhagic problems of extremely preterm infants.

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