Development of the Human Coagulation System in the Healthy Premature Infant

By M. Andrew, B. Paes, R. Milner, M. Johnston, L. Mitchell, D.M. Tollefsen, V. Castle, and P. Powers

This study was designed to determine the postnatal development of the human coagulation system in the healthy premature infant. Consecutive mothers of healthy premature infants born at either St Joseph’s Hospital or McMaster University Medical Centre in Hamilton were asked for consent. One hundred thirty-seven premature infants (30 to 36 weeks of gestational age) born at St Joseph’s Hospital or McMaster University entered the study. The premature infants did not have any major health problems and did not require ventilation or supplemental oxygen. Demographic information and a 20-ml blood sample were obtained in the postnatal period on days 1, 5, 30, 90, and 180. Between 40 and 90 premature infants were studied on each day for each of the following tests: prothrombin time; activated partial thromboplastin time; thrombin clotting time; plasminogen; 13 factor assays [fibrinogen, II, V, VII, VIII, IX, X, XI, XII, XIII, high-mol-wt kininogen (HMWK), prekallikrein (PK), von Willebrand factor (vWF)] and eight inhibitors [antithrombin III (AT-III), heparin cofactor II, α2-antiplasmin, α2-macroglobulin, α2-antitrypsin, C1 esterase inhibitor, protein C (PC), and protein S (PS)]. A combination of biologic and immunologic assays were used. Between 30 to 36 weeks there was a minimal effect of gestational age for levels of AT-III, PC, and factors II and X only; therefore, the entire data set was used to generate reference ranges for these components of the coagulation system for premature infants. Next, the results for the premature infants were compared with those of a previously published study in 118 fullterm infants and with those for adults. An effect of gestational age was shown for plasminogen, fibrinogen, factors II, V, VIII, IX, XI, XII, HMWK, and all eight inhibitors. In general, the postnatal maturation towards adult levels was accelerated in premature infants as compared with the fullterm infants. By 6 months of age, most components of the coagulation system in premature infants had achieved near adult values.

RECENTLY, we published reference ranges for the human coagulation system in the fullterm infant during the first 6 months of life.1 A similar study of the healthy premature infant was undertaken because of considerable evidence in the literature that levels of the components of the coagulation system differ in the premature infant compared to the fullterm infant.2-5 All previously identified problems contributing to the difficulty of establishing reference values in the fullterm infant apply equally to the premature infant. These include the need for a large sample size, the need for data beyond the first week of life, and the use of plasma from the infant rather than the cord for day 1 values. In addition, many premature infants are ill, which should exclude them from a study directed at determining normal values. Thus, there are no complete reference values as yet for the components of the coagulation system in the healthy premature infant throughout the postnatal period. The purpose of the following study was to determine such a reference range and to compare the premature infant with our previous data for both the fullterm infant and the adult.

MATERIALS AND METHODS

Subjects. Healthy premature infants (30 to 36 weeks of gestational age) born at St Joseph’s Hospital or McMaster University Medical Centre in Hamilton, between December 1, 1983 and February 1, 1987 were eligible for this study. The gestational age was based on a combination of maternal dates and the Dubowitz assessment, with the latter used in cases of disagreement. The premature population was carefully screened to exclude infants who had any of the following: perinatal asphyxia, respiratory distress syndrome, oxygen support, sepsis, ventilation, or any other significant postnatal problem. In addition, infants who were small for gestational age were not recruited for this study. All infants received 1 mg vitamin K intramuscularly (IM) at birth, and the Apgar score and mode of delivery were recorded. On each study day, information regarding head circumference, crown to heel length and weight, milk formula, and medications was recorded. This study was approved by the Ethics Committee both at St Joseph’s Hospital and at McMaster University Medical Centre, and informed consent was obtained for all infants. The information from the premature infants was compared with the previously published normal values for 118 healthy fullterm infants and 29 healthy adults.1

Laboratory. The techniques for obtaining blood samples, handling blood samples, and measuring the factor assays have been described in detail for the fullterm infant.1 In brief, a 2-ml blood sample was collected in the postnatal period on days 1, 5, 30, 90, and 180. Platelet-poor plasma (PPP) was fractionated and frozen for future coagulation studies. A combination of biologic and immunologic assays was performed using previously published micro techniques.6 The screening tests consisted of a prothrombin time (PT; Dade C rabbit thromboplastin with an international sensitivity index of 2.5), an activated partial thromboplastin time (APTT; Dade Actin PS) and a 2-U thrombin clotting time (TCT). The measured components of the fibrinolytic and coagulation systems included plasminogen, fibrinogen, factors II, V, VII, VIII, IX, X, XI, XII, prekallikrein (PK), high-mol-wt kininogen (HMWK), XIIa, XIIIb, and von Willebrand factor (vWF). The inhibitors measured included antithrombin III (AT-III), heparin cofactor II (HCII), α2-antitrypsin, C1 esterase inhibitor, protein C (PC), and protein S (PS). A combination of biologic and immunologic assays were used. Between 30 to 36 weeks there was a minimal effect of gestational age for levels of AT-III, PC, and factors II and X only; therefore, the entire data set was used to generate reference ranges for these components of the coagulation system for premature infants. Next, the results for the premature infants were compared with those of a previously published study in 118 fullterm infants and with those for adults. An effect of gestational age was shown for plasminogen, fibrinogen, factors II, V, VIII, IX, XI, XII, HMWK, and all eight inhibitors. In general, the postnatal maturation towards adult levels was accelerated in premature infants as compared with the fullterm infants. By 6 months of age, most components of the coagulation system in premature infants had achieved near adult values.

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**Table 1. Demographic Data of Study Population: Postnatal Age of Infants Born at 34-36 Weeks of Gestational Age (Days)**

<table>
<thead>
<tr>
<th>No.</th>
<th>1</th>
<th>5</th>
<th>30</th>
<th>90</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>2.16 ± 0.35</td>
<td>2.03 ± 0.29</td>
<td>3.06 ± 0.73</td>
<td>5.10 ± 1.03</td>
<td>7.03 ± 0.89</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>32.6 ± 1.4</td>
<td>31.4 ± 1.4</td>
<td>34.5 ± 2.1</td>
<td>39.5 ± 3.5</td>
<td>42.5 ± 1.0</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>45.4 ± 3.0</td>
<td>46.5 ± 2.8</td>
<td>47.8 ± 4.2</td>
<td>54.2 ± 4.8</td>
<td>64.2 ± 3.1</td>
</tr>
</tbody>
</table>

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**Table 2. Demographic Data of Study Population: Postnatal Age of Infants Born at 30 to 33 Weeks of Gestational Age (Days)**

<table>
<thead>
<tr>
<th>No.</th>
<th>67</th>
<th>50</th>
<th>23</th>
<th>26</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>1.73 ± 0.35</td>
<td>1.64 ± 0.29</td>
<td>2.47 ± 0.64</td>
<td>4.40 ± 0.55</td>
<td>6.40 ± 0.78</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>29.6 ± 2.9</td>
<td>29.3 ± 1.9</td>
<td>32.3 ± 2.4</td>
<td>37.7 ± 1.1</td>
<td>43.1 ± 4.1</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>42.3 ± 3.2</td>
<td>43.7 ± 1.8</td>
<td>46.5 ± 1.8</td>
<td>52.6 ± 3.4</td>
<td>61.6 ± 3.4</td>
</tr>
</tbody>
</table>

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For each component measured, change due to gestational age between 30 and 36 weeks was assessed using both a linear analysis and a two-way analysis of variance (ANOVA) with repeated measures comparing mean values of infants from 30 to 33 weeks to those of 34 to 36 weeks at all postnatal time points. The premature data were then amalgamated into one data set, and each component measured in the premature infant was compared with previously published data for the fullterm infant by measuring the change in mean values over time in the postnatal period using a two-way ANOVA with repeated measures. Specific differences between the premature and fullterm infants were then tested using Student's t-test. For the latter, because of the use of up to five comparisons, P values <0.01 were considered significant. Finally, specific differences between the premature and adult data were tested using Dunnett's test.

**RESULTS**

**Subjects.** One hundred thirty-seven infants entered the study, 67 between 30 and 33 weeks of gestational age and 70 between 34 and 36 weeks of gestational age. Specific demographic information for the two groups of premature infants is given in Tables 1 and 2. The mode of delivery was vaginal in 75% of infants in the 30- to 33-week group and 64% for infants between 34 and 36 weeks. All infants had a five-minute Apgar score >7. The infant population was largely white, with an equal proportion of boys and girls. Because only four components (AT-III, PC, factor II, and X) differed significantly from those of the fullterm infant, they are identified in Tables 3 and 4, and as values for premature infants that become indistinguishable from those of adults.

**Laboratory.** The results of the screening tests, coagulation factors, and inhibitors for the entire premature population are given in Tables 3 and 4. Figures 1 through 7 give the mean values, the 95% confidence interval for the mean values and the upper and lower limit encompassing 95% of the population (±2 SD) for each of the tests performed. The confidence intervals are small (less than ±0.06%), which facilitates accurate detection of changes in mean values for specific coagulation tests over time as well as comparisons between premature infants and either adults or fullterm infants. Values that show a skewed distribution are identified in Tables 3 and 4. The upper and/or lower limit for these values were adjusted to exclude 2.5% of the population, and the adjusted values are given in Figs 1 through 7 and Tables 3 and 4. When the values for the premature infant are statistically different from those of the fullterm infant, they are identified in Tables 3 and 4, and as values for premature infants that become indistinguishable from those of adults.

Table 3 and Fig 1 give the values for the coagulation screening tests in premature infants during the first 6 months of life. The mean values for PT were comparable with those of adults throughout the postnatal period; however, there is a wider range of normal for PT in the infant with a value of 15 seconds, which is still considered normal at 6 months of age. The mean values and upper limit for the APTT were prolonged in the premature infant as compared with the adult throughout the postnatal period. The fibrinogen level and TCT were always in the adult range; however, the fibrinogen level showed a significant rise on day 5 of life.

Table 3 and Fig 2 give the values for the vitamin K-dependent factors (II, VII, IX, X) in premature infants during the first 6 months of life. On day 1 of life, factors II, IX, and X were <50% of adult values whereas factor VII levels were 67% of adult values. Although the mean values for all four vitamin K-dependent factors were in the adult range by 6 months of life, only factor VII showed a mean value that was not statistically different from that of adults. The normal range for the premature infant was frequently narrower than for the adult.

Table 3 and Fig 3 give the values for the four contact factors, XI, XII, PK, and HMWK, in the premature infant during the first 6 months of life. On day 1 of life, values for all four factors were <50% of adult values. By 6 months of life, the mean values for all four contact factors were in the adult range, but were statistically lower than those of the adult.

Table 3 and Figs 4 and 5 give the postnatal values for factors V, VIII, vWF, plasminogen, and factor XIII with its subunits A and B in premature infants during the first 6 months of life. Values for factor VIII and vWF were near or above adult values throughout the postnatal period. Both
factor VIII and vWF showed a marked, persistently skewed distribution with many high values. The lower limit of normal was adjusted in Fig 4 to accurately reflect the range of values. Mild, moderate, and severe hemophilia A could be confidently diagnosed in premature infants as early as day 1 of life. Mean values for factor V, XIIIa, and XIIIb normalized to adult levels by day 5 of life. Throughout the postnatal period, plasminogen levels were lower in premature infants as compared with adults.

Table 4 and Fig 6 show postnatal values for the inhibitors AT-III, HCII, PC, and PS in premature infants during the first 6 months of life. On day 1 of life, mean values for all four inhibitors were <50% of adult values. By 6 months, mean values for AT-III, HCII, and PS were within normal adult range, whereas values for PC were low, with a mean value of 0.57 U/mL.

Table 4 and Fig 7 show postnatal values for the inhibitors a2M, C1INH, a2AP, and a1AT in premature infants during the first 6 months of life. a2M values were above adult values at birth and continued to rise to twice adult values by 6 months of age. C1INH and a2AP levels were low at birth but also rose to levels above adult level by 6 months.

**DISCUSSION**

Premature infants may be affected by serious health problems. Not infrequently, hemorrhagic and/or thrombotic complications contribute to these health problems and thus to morbidity and mortality in this age group. To detect, classify correctly, and treat the coagulopathies present, reference ranges for the components of the coagulation system are essential. Apart from the many identified difficulties in obtaining such a data set for the fullterm infant, the major limitation in generating reference values for the premature infant is the need to study healthy infants. In previous small-scale studies, we and other investigators showed that even mild degrees of hypoxia or respiratory distress syndrome can affect levels of coagulation factors and inhibitors. In this study, only infants of an appropriate weight for age, with good Apgar scores, with no requirement for oxygen or ventilatory support, and without any other major illnesses were enrolled. Only five infants became ill during their postnatal course and were excluded from further study. Therefore, the reference ranges generated in this study reflect values from healthy premature infants evenly distributed in age between 30 and 36 weeks.
In the early postnatal period, the coagulation system in the premature infant differed markedly from the adult but not in a uniform pattern; eg, the mean values in premature infants for the vitamin K-dependent factors, contact factors, and the inhibitors AT-III, HCII, PC, and PS were between 25% to 70% of adult values. In contrast, fibrinogen, factors V, VIII, vWF, XIII, and the inhibitors a2M, a2AP, C1INH, and α1AT were relatively spared, with premature infants showing values between 70% and 140% of adult values. This observation supports the concept of a highly selective pattern of maturation for the coagulation system. Because healthy premature infants do not develop spontaneous hemorrhagic or thrombotic complications, this unusual balance between procoagulants and inhibitors should be considered hemostatic. The reasons for the differences observed in the coagulation system between the premature infant and the adult are still uncertain but likely reflect differences in rates of protein synthesis, secretion, and turnover. Some data have been provided for the latter concept.

The overall pattern of the coagulation system was similar in premature and fullterm infants. The differences between

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**Fig 1.** APTT, PT, fibrinogen level, and thrombin clotting time in 137 healthy premature infants during first 6 months of life. Inner line represents mean values, inner clear area is 95% confidence interval, and shaded area includes 95% of all values (±2 SD). Adult values: ––––.

**Fig 2.** Vitamin K-dependent factors (II, VII, VIII, IX) in 137 healthy premature infants during first 6 months of life. Set-up of Fig 2 is as described in legend to Fig 1.

**Fig 3.** Contact factors (XII, XI, PK, HMWK) in 137 healthy premature infants during first 6 months of life. Setup of Fig 3 is as described in legend to Fig 1.

**Fig 4.** Factors V, VIII, and vWF in 137 healthy premature infants during first 6 months of life. Setup of Fig 4 is as described in legend to Fig 1.
premature and fullterm infants, although frequent, were minor as compared with the large differences between premature infants and adults. For example, although differences in mean values between premature and fullterm infants existed for eight coagulation factors and eight inhibitors at some time in the postnatal period, the magnitude of the differences ranged only from 0.06 to 0.27 U/mL (mean ± SD 0.15 ± 0.06). Specific differences between premature and fullterm infants are shown in Tables 3 and 4. Thus, the period between 30 and 40 weeks of life does not appear to be a time of rapid change in the coagulation system. Based on in utero studies of healthy fetuses, it is likely that there are important changes that occur earlier in life.6,7 We were unable to accrue a sufficient number of healthy infants aged less than 30 weeks of gestation to test this hypothesis further. The postnatal maturation toward adult levels was generally accelerated in premature infants as compared with fullterm infants. Thus, by 6 months of life, fullterm and premature infants showed equivalent levels for all but four components of the coagulation system, with most mean values well within normal adult range.

One of the more important purposes for a reference range is as an aid to the correct diagnosis of specific congenital or acquired coagulopathies. Of the common congenital hemostatic deficiencies, severe forms of both hemophilia A and B, as well as moderate and mild forms of hemophilia A, can be confidently diagnosed in premature infants. In contrast, diagnoses of the more common forms of von Willebrand's disease are difficult in the first months of life because vWF behaves as an acute phase reactant and is markedly elevated in the early postnatal period. Other difficulties complicate accurate diagnosis of the heterozygote state for specific inhibitors. For example, the lower limits of normal for AT-III, PC, and PS are as low as 0.24, 0.12, and 0.14 U/mL, respectively. In contrast, the homozygote form for these inhibitors has been and should be diagnosable at birth.40-43 Acquired disorders such as disseminated intravascular coagulation and liver disease in their moderate to severe forms are likely to be easily diagnosed in view of the adult levels for fibrinogen and factors V and VIII that exist in premature infants. Accurate diagnosis of Vitamin K deficiency is difficult in newborns because they have very low levels of the four Vitamin K-dependent factors. Assays that measure discrepancies between the amount of existing protein and its activity may be more helpful.

In summary, we determined the postnatal development of the human coagulation system in healthy premature infants. These reference ranges should provide the basis for a system-

Fig 5. Factors Xilla, XIllb, and plasminogen in 137 healthy premature infants during first 6 months of life. Setup of Fig 5 is as described in legend to Fig 1.

Fig 6. Inhibitors antithrombin III (AT-III), heparin cofactor II (HCII), protein C (PC), and protein S (PS) in 137 healthy premature infants during first 6 months of life. Setup of Fig 6 is as described in legend to Fig 1.

Fig 7. Inhibitors C1INH, α2M, α2AP, and α1AT in 137 healthy premature infants during first 6 months of life. Setup of Fig 7 is as described in legend to Fig 1.
atic approach to our understanding of hemorrhagic and thrombotic complications in the premature infant.

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REFERENCES


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