Development of the Human Coagulation System in the Healthy Premature Infant

By M. Andrew, B. Paes, R. Milner, M. Johnston, L. Mitchell, D.M. Tollefson, 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) 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, α1-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. In 6 months of age, most components of the coagulation system in premature infants had achieved near adult values.

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Weight (kg) 1.73
Length (cm) 42.3

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 infant 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 at only 1 time point each in the postnatal period, the premature data were amalgamated into one data set. Between 40 and 96 infants were studied at each time point for each assay and were evenly distributed between 30 and 36 weeks of gestational age. Not all infants were studied at each time point owing to transfer to another hospital for a growing period, recruitment after day 1, occasionally clotted samples, missed follow-up appointments, and clinical deterioration in five infants who were subsequently excluded from the study.

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, as are values for premature infants that become indistinguishable from those of adults.

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

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>5</th>
<th>30</th>
<th>90</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>70</td>
<td>49</td>
<td>27</td>
<td>28</td>
<td>24</td>
</tr>
<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>32.8 ± 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>45.6 ± 2.8</td>
<td>47.8 ± 4.2</td>
<td>54.2 ± 4.8</td>
<td>64.2 ± 3.1</td>
</tr>
</tbody>
</table>

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></th>
<th>1</th>
<th>5</th>
<th>30</th>
<th>90</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>67</td>
<td>50</td>
<td>23</td>
<td>26</td>
<td>26</td>
</tr>
<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>28.6 ± 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>
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 distinguished in premature infants during the first 6 months of life. Mean values for factor V, XIIIa, and XIIIb normalized in adults range, whereas values for PC were low, with a mean of 0.28 IU/mL. Mean values for AT-III, HCII, and PS were within normal limits, with plasminogen levels being lower in premature infants compared with adults.

Table 3. Reference Values for Coagulation Tests in Healthy Premature Infants (30 to 36 Weeks Gestation) During First 6 Months of Life

<table>
<thead>
<tr>
<th>Tests</th>
<th>Day 1 (n)</th>
<th>Day 5 (n)</th>
<th>Day 30 (n)</th>
<th>Day 90 (n)</th>
<th>Day 180 (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (s)</td>
<td>13.0 (10.6-16.2)*</td>
<td>12.5 (10.0-15.3)*</td>
<td>11.8 (10.0-15.3)*</td>
<td>12.3 (10.0-14.6)*</td>
<td>12.5 (10.0-15.0)*</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>53.6 (27.5-79.4)*</td>
<td>50.5 (26.9-74.1)*</td>
<td>44.7 (26.9-62.5)*</td>
<td>39.5 (28.3-50.7)*</td>
<td>27.5 (21.7-53.3)*</td>
</tr>
<tr>
<td>TCA (s)</td>
<td>24.8 (19.2-30.4)*</td>
<td>24.1 (18.8-29.4)*</td>
<td>24.4 (18.8-29.4)*</td>
<td>25.1 (19.4-30.6)*</td>
<td>25.5 (18.9-31.5)*</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>2.43 (1.50-3.73)*</td>
<td>2.60 (1.60-4.18)*</td>
<td>2.54 (1.50-4.14)*</td>
<td>2.46 (1.50-3.52)*</td>
<td>2.28 (1.50-3.60)*</td>
</tr>
<tr>
<td>II (U/mL)</td>
<td>0.46 (0.20-0.77)*</td>
<td>0.57 (0.29-0.85)*</td>
<td>0.57 (0.36-0.90)*</td>
<td>0.68 (0.50-0.86)*</td>
<td>0.87 (0.51-1.23)*</td>
</tr>
<tr>
<td>vWF (U/mL)</td>
<td>0.88 (0.41-1.44)*</td>
<td>1.00 (0.48-1.54)*</td>
<td>1.02 (0.48-1.56)*</td>
<td>0.99 (0.59-1.39)*</td>
<td>1.02 (0.58-1.46)*</td>
</tr>
<tr>
<td>VII (U/mL)</td>
<td>0.87 (0.21-1.13)*</td>
<td>0.84 (0.30-1.38)*</td>
<td>0.83 (0.21-1.45)*</td>
<td>0.87 (0.31-1.43)*</td>
<td>0.99 (0.47-1.51)*</td>
</tr>
<tr>
<td>VIII (U/mL)</td>
<td>1.11 (0.50-2.13)*</td>
<td>1.15 (0.52-2.51)*</td>
<td>1.11 (0.51-1.95)*</td>
<td>1.06 (0.58-1.87)*</td>
<td>0.99 (0.50-1.49)*</td>
</tr>
<tr>
<td>uWF (U/mL)</td>
<td>1.38 (0.78-2.10)*</td>
<td>1.33 (0.73-2.19)*</td>
<td>1.36 (0.66-2.18)*</td>
<td>1.12 (0.75-1.84)*</td>
<td>0.98 (0.54-1.58)*</td>
</tr>
<tr>
<td>IX (U/mL)</td>
<td>0.35 (0.19-0.65)*</td>
<td>0.42 (0.14-0.74)*</td>
<td>0.44 (0.13-0.80)*</td>
<td>0.59 (0.25-0.93)*</td>
<td>0.81 (0.50-1.20)*</td>
</tr>
<tr>
<td>X (U/mL)</td>
<td>0.41 (0.11-0.71)*</td>
<td>0.51 (0.19-0.83)*</td>
<td>0.56 (0.20-0.92)*</td>
<td>0.87 (0.35-0.99)*</td>
<td>0.77 (0.35-1.19)*</td>
</tr>
<tr>
<td>XII (U/mL)</td>
<td>0.30 (0.08-0.52)*</td>
<td>0.43 (0.13-0.69)*</td>
<td>0.43 (0.15-0.71)*</td>
<td>0.59 (0.25-0.93)*</td>
<td>0.78 (0.46-1.10)*</td>
</tr>
<tr>
<td>XII (U/mL)</td>
<td>0.38 (0.10-0.66)*</td>
<td>0.39 (0.09-0.69)*</td>
<td>0.43 (0.11-0.76)*</td>
<td>0.61 (0.19-1.07)*</td>
<td>0.82 (0.22-1.42)*</td>
</tr>
<tr>
<td>PK (U/mL)</td>
<td>0.33 (0.09-0.57)*</td>
<td>0.45 (0.28-0.75)*</td>
<td>0.59 (0.31-0.87)*</td>
<td>0.79 (0.37-1.21)*</td>
<td>0.78 (0.40-1.16)*</td>
</tr>
<tr>
<td>HMWK (U/mL)</td>
<td>0.49 (0.09-0.89)*</td>
<td>0.62 (0.24-1.00)*</td>
<td>0.64 (0.16-1.12)*</td>
<td>0.78 (0.32-1.24)*</td>
<td>0.83 (0.41-1.25)*</td>
</tr>
<tr>
<td>XIta (U/mL)</td>
<td>0.70 (0.32-1.08)*</td>
<td>1.01 (0.57-1.45)*</td>
<td>0.99 (0.51-1.47)*</td>
<td>1.13 (0.71-1.55)*</td>
<td>1.06 (0.65-1.55)*</td>
</tr>
<tr>
<td>XIII (U/mL)</td>
<td>0.81 (0.35-1.27)*</td>
<td>1.10 (0.68-1.58)*</td>
<td>1.07 (0.57-1.57)*</td>
<td>1.21 (0.75-1.67)*</td>
<td>1.15 (0.67-1.83)*</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>(ICTA, U/mL)</td>
<td>1.70 (1.12-2.48)*</td>
<td>1.91 (1.21-2.61)*</td>
<td>1.81 (1.09-2.53)*</td>
<td>2.38 (1.58-3.18)*</td>
</tr>
</tbody>
</table>

All values are given as a mean (95% confidence interval) unless otherwise specified. *Values are skewed owing to a disproportionate number of high values. Lower limit which excludes the lower 2.5% of the population is given (B). Values different from those of full-term infants.

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 full-term 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.
sons with adults and fullterm infants provide us with insights for understanding of the development of the coagulation system.

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 α2M, α2AP, 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.3719

The overall pattern of the coagulation system was similar in premature and fullterm infants. The differences between
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.\textsuperscript{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 determined and should be diagnosable at birth.\textsuperscript{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-

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**Fig 5.** Factors XIIIa, XIIIb, 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.

ACKNOWLEDGMENT

We acknowledge the secretarial assistance of Rosemary Phillis, Barbara Lahie, and Janet Butera. In addition, we acknowledge the organization skills of Arlene Lang and Barbara Stewart-Rudolph and the technical support provided by the technologists at the Coagulation Laboratories in St Joseph’s Hospital and McMaster University Medical Centre. We also thank the nursery staff at both St Joseph’s Hospital and McMaster University Medical Centre for invaluable support in the collection of blood samples.

REFERENCES


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