evaluated in our small study sample is also a potential limitation.

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References

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Pediatic Reference Intervals for Seven Common Coagulation Assays, Michele M. Flanders,1 Ronda A. Crist,2 William L. Roberts,1,3 and George M. Rodgers1,3,4* (1 ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT; 2 ARUP Laboratories, Salt Lake City, UT; 3 Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, UT; 4 Department of Medicine, University of Utah Health Sciences Center, Salt Lake City, UT; address correspondence to this author at: Division of Hematology, University of Utah Health Sciences Center, 50 North Medical Dr., Salt Lake City, UT 84132; fax 801-858-5469, e-mail george.rogers@hsc.utah.edu)

Accurate interpretation of pediatric coagulation tests is complicated by the fact that reference intervals for many assays differ from those for adults. In 1992, Andrew et al. (1) reported childhood coagulation reference intervals for ages 1–5, 6–10, and 11–16 years with a minimum of 4 and maximum of 7 individuals at each age, with 20–50 per age group. These results served as the basis for most pediatric coagulation reference intervals for over a decade. However, with the development of newer reagents, methodologies, and instruments to measure coagulation analytes, results from this study, which was limited by its small size, may be less relevant.

In 2002, we initiated a project to collect blood and urine samples from healthy children 7–17 years of age, with the goal of establishing pediatric reference intervals for many laboratory tests (2). The purpose of this study was to determine pediatric reference intervals for 7 coagulation tests associated with common bleeding disorders.

Samples were drawn for reference interval studies from 902 healthy children, ages 7–17 years, all of whom had no history of bleeding or thrombotic disorders, and were taking no medications for at least 2 weeks before specimen collection. Informed consent was obtained from parents, and the study was approved by the University of Utah Institutional Review Board. Samples used to establish adult reference intervals were purchased from George...
King Bio-Medical or Precision Biologic, or were collected from local volunteers.

Blood was obtained by clean venipuncture; a pilot tube was drawn first. An exact ratio of 9 volumes of blood to 1 volume of anticoagulant (32 g/L citrate) was maintained. Specimens were centrifuged immediately at 3000 g for 20 min at room temperature, aliquoted, and frozen at −80 °C.

Prothrombin time (PT); partial thromboplastin time (PTT); factors VIII, IX, and XI; and von Willebrand factor (vWF) antigen were assayed on the STA-R coagulation analyzer (Diagnostica Stago). Ristocetin cofactor activity (RCF) assays were performed on the BCS (Dade Behring). All coagulation assays were done according to manufacturer specifications and laboratory standards. Coagulation screening tests included in the study were the PT (Neoplastin Cl+; Diagnostica Stago) and PTT (STA-PTTa; Diagnostica Stago) assays, and results were measured in seconds with a clot-based methodology. Intrinsic factors VIII, IX, and XI [STA-PTTa (Diagnostica Stago) and factor-deficient plasma (HRF Inc.)] were measured by a modified activated PTT (APTT) (3). A calibration curve was generated by use of dilutions of a commercial calibrator and Owrens buffer, and the results were calculated from this curve. RCF (BC vonWillebrand reagent; Dade Behring) was assayed by use of stabilized platelets added to a sample containing vWF (ristocetin cofactor), which causes platelet agglutination and decreased absorbance in the presence of ristocetin (4). The vWF antigen (STA LIATEST vWF; Diagnostica Stago) was measured by microlatex particle-mediated immunoassay (5).

For each assay described, calibration and control sam-

<table>
<thead>
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<th>Table 1. Summary of pediatric reference intervals.</th>
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<tr>
<td><strong>Age, years</strong></td>
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<td><strong>n</strong></td>
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<tr>
<td>PT, s</td>
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<td>Lower limit</td>
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<td>PTT, s</td>
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<td>Factor VIII, %</td>
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<td>Factor IX, %</td>
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<td>Factor XI, %</td>
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<td>RCF, %</td>
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<td>Mean</td>
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<td>vWF antigen, %</td>
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<td>Mean</td>
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* The 90% confidence interval for each reference limit is given in parentheses.

*b Adult reference intervals for RCF and vWF antigen were calculated from samples drawn from Utah donors only as described in the text (n = 78).

*c The Z-test of the mean, the ratio test for the SD, or both are statistically different from the adult reference intervals.
Fig. 1. Data from pediatric populations for 7 coagulation assays. 

*Shaded boxes* indicate the pediatric reference intervals (central 95% interval) for each age group. Within the box is a *solid line* that represents the median of the group. *Dashed lines* represent the median and central 95% interval of the adult reference data compared with the pediatric intervals. In panel B, the median is the same for children ages 7–15 years and adults and is indicated by a *dashed line only.*
amples were assayed daily. Testing was performed in batches of 20 specimens. For each batch, a total of 20 (10 male/10 female) randomly chosen samples were thawed for 7 min in a 37 °C water bath and run concurrently. Data from the 902 samples were grouped by age: 7–9, 10–11, 12–13, 14–15, and 16–17 years. Data were grouped with a minimum of 164 individuals in each age group, allowing 12–13, 14–15, and 16–17 years. Data were grouped with a minimum of 164 individuals in each age group, allowing the establishment of reference intervals by a nonparametric method (NCCLS C28-A) (6). Mean (SD) values of each age group were compared with those for adults; if the Z-test of the means (calculated Z is greater than critical Z), or if the SD ratio (adult SD/pediatric SD) was >1.5, separate reference intervals were warranted (6). We used 120 normal adult samples to establish the adult reference intervals (6). Of these, 78 were ARUP (Utah) donors. The mean adult ages for ARUP donors and commercial donors were 32 and 36 years, respectively. The adult age range for local donors was 20–55 years, and that for purchased plasmas was 18–55 years. Statistical calculations were performed with EP Evaluator, release 5 (David G. Rhoads Associates).

Pediatric reference intervals for the central 95% (age group 7–9, n = 245; all other age groups, n = 164) and 90% confidence intervals are summarized in Table 1. Confidence intervals were calculated by EP Evaluator from Table 8 of NCCLS C28-A. Individual data for each analyte are shown in Fig. 1 (panels A–G), and both pediatric and adult reference intervals and median values are indicated. For RCF and vWF antigen, mean values are also included because the median value most likely includes data from a blood type O donor, which caused the value to be skewed low.

The median PT for pediatric samples was 14.0 s, nearly 1 s longer than the adult mean of 13.2 s. Although the median PT for pediatric samples decreased slightly at age 16–17 to 13.9 s, the intervals for all pediatric age groups were statistically significantly different from that for adults for this analyte. However, pediatric PTT intervals did not differ from that for adults (Table 1).

For factor VIII, RCF, and vWF antigen, all pediatric age groups had higher median values than those of adults. The lower limits of the reference intervals for factor VIII and vWF antigen were markedly higher at younger ages. However, by ages 16–17 years, reference limits were comparable to those for adults. vWF antigen was slightly lower (2%). RCF lower limits were slightly higher (1%) at age 7–9 years and significantly higher (9%) at age 10–11 years, whereas the limits for ages 12–17 were slightly lower than that for adults. For all pediatric age groups, the upper limits for RCF and vWF antigen were considerably lower than that for adults; however, the upper limits for factor VIII were higher than for adults. The median pediatric factor XI concentrations were similar to that for adults, but the reference intervals had a higher lower limit. Vitamin K-dependent coagulation factor IX concentrations were significantly different in the younger years of life. Pediatric concentrations of factor IX trended upward until ages 16–17, when adult values were reached.

In this study, 0.44% of the presumed “normal” samples were found to be abnormal. Three children had factor XI deficiency (concentrations <34%), and 1 had von Willebrand disease (RCF <29% and vWF antigen <35%). These values were well outside established reference intervals and were excluded when we calculated the current pediatric reference intervals.

Most coagulation laboratories rely on the published pediatric reference intervals reported by Andrew et al. (1) over a decade ago. That study reported results for a maximum of 50 children per age group, and each age group spanned 5 years (1–5, 6–10, and 11–16 years). Their study did not identify significant differences between children of any age and the adult population for the PT, PTT, factor VIII, and vWF antigen assays. Factor IX concentrations did show age dependence, as did factor XI in the older pediatric age group (11–16 years). RCF was not reported in the study by Andrew et al. (1).

In our study, we found age-dependent differences between children and adults for the PT assay; factors VIII, IX, and XI; RCF; and vWF antigen (Table 1). The major reason we detected these differences and the earlier study did not is probably the larger number of individuals and the more restrictive age groups in our study.

Our results have implications for the interpretation of pediatric coagulation assays. For example, a PT that is prolonged by 1 s in a child, based on an adult reference interval, is probably physiologic and does not indicate a disease state. Similarly, our new findings for the vWF panel component reference intervals (factor VIII, RCF, and vWF antigen) could lead to more accurate identification of von Willebrand disease in childhood.

One genetic modifier of plasma vWF concentrations is the ABO blood group; individuals with type O have ~25% lower vWF concentrations than do those with a non-O blood type (7). Although our pediatric samples were not analyzed for ABO blood type, blood bank data from Utah blood donors indicates that a similar but slightly lower percentage have blood type O compared with the overall US blood donor pool (35% vs 45%). Therefore, to calculate the vWF and the RCF adult reference intervals, we omitted out-of-state donor plasma samples and relied on Utah adult donors to match Utah pediatric donors. Our adult reference interval values for vWF and RCF are slightly higher than the corresponding pediatric intervals, a finding similar to that reported by Andrew et al. (1).

In conclusion, we found several significant differences between pediatric and adult coagulation reference intervals, supporting the need to establish new pediatric reference intervals. Our study has established reference intervals based on the use of newer reagents, methodologies, and instrumentation currently used in the clinical laboratory. Future analysis of our stored plasma samples will focus on the remaining clinically relevant coagulation factors [fibrinogen, prothrombin (factor II), and factors V, VII, and X] and the natural anticoagulants (protein C, protein S, and antithrombin). These studies should improve the diagnosis of childhood bleeding and thrombotic disorders.
ARUP Institute for Clinical and Experimental Pathology provided support for this project. Reagents and instrumentation were provided in part by Diagnostica Stago. We would like to thank John Simmons, LaRayne Burrows, and Ashley Widdison for their recruitment of participants and collection of samples.

References

Purine Metabolites in Gout and Asymptomatic Hyperuricemia: Analysis by HPLC-Electrospray Tandem Mass Spectrometry, Jyuan Zhao, Qioglin Liang, Guan Luo, Yiming Wang, Yanzhu Zuo, Ming Jiang, Guilan Yu, and Ting Zhang (Analysis Center, Institute of Biomedicine, Tsinghua University, Beijing, China; XieHe Hospital, Beijing, China; Capital Institute of Pediatrics, Beijing, China; address correspondence to this author at: Analysis Center, Institute of Biomedicine Tsinghua University, Beijing 100084, People’s Republic of China; fax 86-10-62781688, e-mail luoga@mail.tsinghua.edu.cn)

Hyperuricemia, a serum urate concentration >0.45 mmol/L (7.0 mg/dL) in men and 0.36 mmol/L (6.0 mg/dL) in women, is the biochemical hallmark of gout (1–3), but many individuals with life-long hyperuricemia do not develop gouty arthritis (4). Conversely, serum urate concentrations may be within reference values in some patients with acute gout, particularly during the early phases of the disorder. We hypothesized that gout patients may have a different profile of purine precursors than do asymptomatic people with hyperuricemia and that concentrations of these compounds may reflect disorders of purine metabolism. To our knowledge, purine metabolites have not been studied and compared in gout and asymptomatic hyperuricemia.

With established methods for measuring purine compounds in blood and urine (5–9), retention time is not always adequate for identification of every peak because urine and blood usually contain many interfering compounds. Quantification by tandem mass spectrometry (MS/MS) (10, 11) may require an internal standard, which often is hard to obtain.

In this study of 10 purine compounds in the blood of gout patients and hyperuricemia patients with no gout symptoms, we used HPLC for serum sample separation, MS/MS for peak identification, and ultraviolet (UV) detection for quantification.

Purines were purchased from Fluka and Sigma. The HPLC-MS experiment was performed on an Agilent 1100 series liquid chromatograph with a mass spectrometric detector trap. Separation was carried out on a Supelcosil™ LC-18-DB column [25 cm × 4.6 mm (i.d.); 5-µm film thickness]. The column temperature was maintained at 25 °C. The mobile phases were as follows: 10 mmol/L ammonium acetate, adjusted to pH 6.5 with glacial acetic acid (eluant A), and methanol (eluant B). The elution gradient was as follows (flow rate, 1 mL/min): 0 to 10 min, 100% A; 10 to 16 min, 100% A to 92% A; 16 to 30 min, 92% A to 80% A; 30 to 45 min, 80% A to 0% A; 45 to 50 min, 0% A to 100% A; 50 to 60 min, equilibration with 100% A. All gradient steps were linear, and the total analysis time, including equilibration, was 60 min. The column eluate was monitored at 254 nm. A splitter was used between the HPLC column and the mass spectrometer, and 100–200 µL/min of eluate was introduced into the mass spectrometer. Negative electrospray ionization mode was used. Nitrogen was used as both nebulizing and collision gas, and capillary voltage was maintained at 3.5 kV. Other MS conditions were as follows: nebulizer, 25.0 psi; dry gas, 8.0 L/min; dry temperature, 325 °C; target mass, 200 m/z; compound stability, 60%; trap drive level, 50%; ICC target, 20,000. Auto-MS/MS was performed on molecular ions of all peaks to obtain their fragment information. Compound peaks were identified by their retention times and auto-MS/MS. Identification results for the molecular ions and the most abundant ions of 10 compounds are listed in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue9/. Quantification was based on UV detection at 254 nm.

Blood samples were obtained from 49 untreated gout patients, 12 treated gout patients, 20 patients with untreated asymptomatic hyperuricemia, 13 patients with treated asymptomatic hyperuricemia, and 35 healthy controls. All patients and controls were males with no renal dysfunction. All treated patients were receiving 0.1 g/day allopurinol. All blood samples were centrifuged to obtain serum in the hospital and sent to our laboratory, where they were analyzed within 2 h or stored at −80 °C. Before analysis, 100 μL of serum was mixed with 500 μL of methanol for deproteinization, centrifuged at 14,800g for 10 min, dried with gentle nitrogen at 50 °C, and then mixed with 100 μL of water for HPLC-UV-MS/MS analysis.

The limits of detection and the calibration equations for 10 compounds are listed in Table 2 of the online Data Supplement. We determined the intrain assay variation of the method (see Table 3 of the online Data Supplement) by measuring a serum sample 7 times and a serum sample enriched with synthetic compounds at low (100 μg/L),...