Indirect Estimation of Pediatric Between-Individual Biological Variation Data for 22 Common Serum Biochemistries

Tze Ping Loh, MBBChBAO, and Michael Patrick Metz, MD

From the Department of Laboratory Medicine, National University Hospital, Singapore; Division of Chemical Pathology, SA Pathology, Women’s and Children’s Hospital, South Australia, Australia; and School of Paediatrics and Reproductive Health, University of Adelaide, South Australia, Australia.

Key Words: Children; Biological variation; Data mining; Indirect sampling; Reference; Between-individual

ABSTRACT

Objectives: Derivation of between-individual biological variation (CV_g) data requires repeat sampling of the same subject, which is undesirable and challenging in children. We describe an indirect sampling (data mining) approach to obtain these data in children.

Methods: Twenty-two serum biochemistry results from 6,989 children, who visited their primary care physician in Queensland, Australia, and were tested only twice within a year were included. The CV_g and index of individuality of the boys and girls were estimated by year of age, according to the procedures recommended by Fraser and Harris.

Results: The CV_g was generally higher during the first year of life and declined to reach a constant level by age 4 to 6 years, except for aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, and phosphate. The CV_g for these tended to increase after age 10 years. Most of the serum biochemistries examined in this study had indices of individuality 0.6 or less, except sodium, anion gap, bicarbonate, and chloride, which ranged from 0.6 to 1.4. The indices of individuality were very stable across all ages.

Conclusions: These data are comparable to those reported by the Canadian Laboratory Initiative on Pediatric Reference Intervals study and the Ricos database for adults. This study reports the CV_g trends and data for boys and girls by year of age, which have not been described previously.

Between-individual biological variation (CV_g) describes the amount of variance in the homeostatic set point of a biological parameter among different individuals. On the other hand, within-individual biological variation (CV_i) describes the amount of variance around the homeostatic set point in the same individual. These two parameters can be combined and expressed as a ratio, termed the index of individuality, where CV_i and analytical variation (CV_a) are the numerator, and CV_g is the denominator.

These data are highly useful in various aspects of laboratory medicine. For example, CV_i can be used to determine the analytical quality requirement for assay imprecision, reference change value, and specimen of choice for a particular test. On the other hand, CV_g can be used to set the analytical quality requirement for between-method bias. Combined, the index of individuality can be used to assess the distribution of variance of individual participants within reference intervals and determine the need to stratify participants to improve the utility of a test result.

Pediatric biological variation data are inherently difficult to generate due to the requirement of repeat phlebotomy in the same child. We recently described an indirect data-mining approach to derive CV_i trend and data for 22 common biochemistry tests in a large cohort of a pediatric population. Here, we extend the work by describing the CV_g trend and data for the same panel of serum biochemistries in children aged between 0 and 19 years.

Materials and Methods

This study did not require institutional review board approval where it was performed. The deidentified results
of 22 common biochemistry tests requested by primary care physicians for a large cohort of children, aged between 0 and 19 years, were extracted from a large network of 31 laboratories in Queensland, Australia. The laboratory tests were performed during a 1-year period, ending September 30, 2013.

All laboratory tests were performed on serum samples using Cobas Integra instruments (Roche Diagnostics, Basel, Switzerland). The creatinine and albumin were measured using the modified Jaffé and bromocresol green methods, respectively. The analytical imprecision (CV<sub>a</sub>) data were obtained from the running coefficient of variation of the quality control for the most recent 6 months of the study period.

The anion gap was calculated by (sodium − chloride – bicarbonate) (all in mmol/L). The globulin concentration was calculated by (total protein – albumin) (both in g/L), while the osmolality was calculated by \((1.86 \times \text{sodium} + \text{glucose} + \text{urea} + 9)\) (all in mmol/L).

Children who were tested on only two separate occasions during the 12-month study period were included. The results of the children were grouped according to their sex and analyzed separately. Results derived from clinical samples with hemolysis or icteric indices above the analytical thresholds recommended by the instrument manufacturer were excluded from further analysis. None of the samples had a lipemic index that exceeded the analytical threshold recommended by the instrument manufacturer. Outlying values were examined at three levels, as recommended by Fraser and Harris.<sup>1</sup>

First, outlying individual laboratory results were identified by Tukey’s criteria, defined as any value lying below the first quartile value minus three times the interquartile range, or above the third quartile value plus three times the interquartile range.<sup>5</sup> Subsequently, the same criteria were applied to the average of the two results of the children to identify outlying individuals. Finally, Cochran’s criteria were used to identify abnormally large variances between the two laboratory results belonging to an individual child.<sup>1</sup> These procedures were performed at single-year intervals. All outlying values, individuals, and variances were removed.

The remaining results were subjected to statistical analysis to derive the CV<sub>g</sub> and CV<sub>i</sub>. The CV<sub>a</sub> data were derived from smoothed median total within-individual variation; that is, the median CV<sub>g</sub> using the λ-μ-σ approach<sup>6-8</sup> as described in detail previously.<sup>2-4</sup> The median CV<sub>g</sub> was then subtracted by the CV<sub>a</sub> to derive the median CV<sub>i</sub>, which is considered the physiologic CV<sub>i</sub>.

Since all children had the same number of repeat testing performed (ie, two), the CV<sub>g</sub> was derived using the following equation, as recommended by Fraser and Harris:<sup>1</sup> \[ CV_{g}^{2} = \frac{(kr-1)}{k(r-1)}[CV_{i}^{2} - CV_{a}^{2} - \frac{(N-2)}{(N-1)}CV_{a}^{2}]. \]

where \(k\) = number of specimen, \(r\) = number of subjects, \(N\) = total number of measurements, and \(CV_{i}^{2} = \) the total variance of all \(N\) measurements. This procedure was performed at single-year intervals.

The index of individuality was calculated as the ratio of \(CV_{g}^{2}/CV_{i}^{2}\), with values of less than 0.6 and more than 1.4 considered low and high, respectively. The analytical quality specifications were calculated as follows: imprecision: optimal = 0.25 × CV<sub>i</sub>, desirable = 0.50 × CV<sub>i</sub>, and minimal = 0.75 × CV<sub>i</sub>; bias: optimal = 0.125 × \((CV_{i}^{2} + CV_{g}^{2})^{0.5}\), desirable = 0.25 × \((CV_{i}^{2} + CV_{g}^{2})^{0.5}\), and minimal = 0.375 × \((CV_{i}^{2} + CV_{g}^{2})^{0.5}\), and total allowable error = \((1.65 \times \text{imprecision}) + \text{bias}\).

The biological variation and analytical quality specification data from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) study and Ricos database were extracted and compared with those derived from this study. A difference in CV<sub>g</sub> data of more than 50% is considered significantly different. This arbitrary threshold was used by the CALIPER group and was adopted in this study for consistency. The statistical analyses were performed using Microsoft Excel 2010 (Microsoft, Richmond, WA), SPSS version 17.0 (SPSS, Chicago, IL), and LMS ChartMaker Light software (Medical Research Council, London, UK).

**Results**

This study included 6,989 children who had laboratory measurements performed on two separate occasions during the study period. Of these children, 61.4% were girls. The overall number of outliers removed and the final number of children included in the analysis for each laboratory test are summarized in **Table 1**. Details of the number of outliers removed for each year of age for the boys and girls are summarized in Supplemental Tables 1 and 2, respectively (all supplemental materials can be found at [http://www.ascp.org/docs/default-source/pdf/press/lohmay15.pdf](http://www.ascp.org/docs/default-source/pdf/press/lohmay15.pdf)).

The trends of the CV<sub>g</sub> and CV<sub>i</sub> for each biochemistry test by age for the boys and girls are shown in **Figure 1**. The biological variation data from the CALIPER study and Ricos database (https://www.westgard.com/biodatabase1.htm) were also included in the same figure for comparison. In general, the CV<sub>i</sub> derived from this study tended to decline with advancing age. The data agreed well with the CALIPER study as well as the Ricos database for children 18 years old.

The CV<sub>g</sub> was generally higher during the first year of life and declined to reach a constant level by the fourth year (Figure 1). The exceptions to this trend were aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase,
Table 1

Original Number of Children, Number of Children Removed After Application of the Outlier Detection Procedures, and Final Number of Children Subjected to Statistical Analysis for the Derivation of Biological Variation Data

<table>
<thead>
<tr>
<th>Test</th>
<th>Boys Original</th>
<th>Boys Removed</th>
<th>Boys Final</th>
<th>Girls Original</th>
<th>Girls Removed</th>
<th>Girls Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>2,011</td>
<td>113</td>
<td>1,898</td>
<td>2,989</td>
<td>99</td>
<td>2,890</td>
</tr>
<tr>
<td>ALP</td>
<td>2,009</td>
<td>59</td>
<td>1,950</td>
<td>2,978</td>
<td>80</td>
<td>2,898</td>
</tr>
<tr>
<td>AST</td>
<td>2,005</td>
<td>199</td>
<td>1,806</td>
<td>2,928</td>
<td>185</td>
<td>2,743</td>
</tr>
<tr>
<td>ALT</td>
<td>2,007</td>
<td>281</td>
<td>1,726</td>
<td>2,906</td>
<td>265</td>
<td>2,641</td>
</tr>
<tr>
<td>GGT</td>
<td>1,986</td>
<td>211</td>
<td>1,775</td>
<td>2,890</td>
<td>264</td>
<td>2,626</td>
</tr>
<tr>
<td>LDH</td>
<td>1,812</td>
<td>104</td>
<td>1,708</td>
<td>2,762</td>
<td>106</td>
<td>2,656</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1,970</td>
<td>39</td>
<td>1,931</td>
<td>2,924</td>
<td>23</td>
<td>2,901</td>
</tr>
<tr>
<td>Sodium</td>
<td>1,906</td>
<td>28</td>
<td>1,878</td>
<td>2,916</td>
<td>20</td>
<td>2,896</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,904</td>
<td>15</td>
<td>1,889</td>
<td>2,913</td>
<td>12</td>
<td>2,901</td>
</tr>
<tr>
<td>Osmolality</td>
<td>1,800</td>
<td>25</td>
<td>1,775</td>
<td>2,789</td>
<td>17</td>
<td>2,772</td>
</tr>
<tr>
<td>Chloride</td>
<td>1,905</td>
<td>23</td>
<td>1,882</td>
<td>2,914</td>
<td>13</td>
<td>2,901</td>
</tr>
<tr>
<td>Anion gap</td>
<td>1,885</td>
<td>11</td>
<td>1,874</td>
<td>2,895</td>
<td>19</td>
<td>2,876</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>1,933</td>
<td>11</td>
<td>1,882</td>
<td>2,904</td>
<td>8</td>
<td>2,896</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1,933</td>
<td>23</td>
<td>1,860</td>
<td>2,896</td>
<td>40</td>
<td>2,856</td>
</tr>
<tr>
<td>Urea</td>
<td>1,903</td>
<td>29</td>
<td>1,874</td>
<td>2,907</td>
<td>20</td>
<td>2,887</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1,817</td>
<td>30</td>
<td>1,787</td>
<td>2,803</td>
<td>27</td>
<td>2,776</td>
</tr>
<tr>
<td>Glucose</td>
<td>1,046</td>
<td>42</td>
<td>1,004</td>
<td>1,845</td>
<td>34</td>
<td>1,811</td>
</tr>
<tr>
<td>Total protein</td>
<td>2,019</td>
<td>18</td>
<td>2,001</td>
<td>3,016</td>
<td>15</td>
<td>3,001</td>
</tr>
<tr>
<td>Albumin</td>
<td>2,045</td>
<td>20</td>
<td>2,025</td>
<td>3,046</td>
<td>16</td>
<td>3,030</td>
</tr>
<tr>
<td>Globulin</td>
<td>2,011</td>
<td>11</td>
<td>2,000</td>
<td>3,014</td>
<td>8</td>
<td>3,006</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1,822</td>
<td>28</td>
<td>1,794</td>
<td>2,810</td>
<td>18</td>
<td>2,792</td>
</tr>
<tr>
<td>Calcium</td>
<td>1,828</td>
<td>11</td>
<td>1,817</td>
<td>2,822</td>
<td>21</td>
<td>2,801</td>
</tr>
</tbody>
</table>

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; LDH, lactate dehydrogenase.

Figure 1

Comparison of the CV<sub>g</sub> (gray box) and CV<sub>i</sub> (open triangle) derived from this study with those derived from the Canadian Laboratory Initiative on Pediatric Reference Intervals study (CV<sub>g</sub> is represented by a dotted line; CV<sub>i</sub> is represented by a dashed line) for boys (left) and girls (right). The CV<sub>g</sub> (red box) and the CV<sub>i</sub> (red triangle) for adult population, extracted from the Ricos database, were annotated at age 18 years. The x-axis represents age in years, while the y-axis represents biological variation in percentage coefficients of variation.
Aspartate Aminotransferase (U/L)

Alanine Aminotransferase (U/L)

γ-Glutamyltransferase (U/L)

Lactate Dehydrogenase (U/L)

Total Cholesterol (mmol/L)

Figure 1 (cont)
Figure 1 (cont)
Figure 1 (cont)
Figure 1 (cont)
and phosphate. These tests showed an increasing \( CV_g \) after age 10 years. The \( CV_g \) of alkaline phosphatase showed a peak at 14 to 15 years for boys and 12 to 14 years for girls. The \( CV_g \) of the children in this study tended to be higher than that of the CALIPER study and those provided in the Ricos database (Figure 1). **Table 2**. Details of the \( CV_g \) and \( CV_i \) for boys and girls by age are provided in Supplemental Tables 2 to 6. The analytical quality specifications derived from the biological variation data from this study are summarized in **Table 3** and **Table 4**.

The indices of individuality of the children’s analytes are shown in Figure 1. Most of the biochemistry tests examined in this study had indices of individuality equal to or less than 0.6 for both sexes. The exceptions to this were sodium, anion gap, bicarbonate, and chloride, where the indices of individuality were between 0.6 and 1.4. The indices of individuality were very stable across all ages and between the sexes. Details of the indices of individuality for the boys and girls by age are provided in Supplemental Tables 7 and 8.

**Discussion**

The direct approach to obtaining biological variation data requires repeat blood sampling of the same healthy individuals over a period of time. This is difficult to achieve in children due to ethical concerns relating to unnecessary invasive procedures being performed on healthy children, more so in younger children, who have low blood volume. Furthermore, parents may be reluctant to provide consent. Such an exercise can be time and resource demanding. It is unsurprising that to date, there are very limited published biological variation data for the pediatric population.

The recent publication of a comprehensive set of biological variation data for 38 biochemistry tests by the CALIPER group filled a large knowledge gap for this population. The use of an indirect data-mining approach is a pragmatic way to obtain representative estimates of \( CV_g \) data in the pediatric population. By careful application of the procedures recommended by Fraser and Harris, we have derived the \( CV_g \) data for children aged between 0 and 19 years. The relatively large number of children in this cohort made it possible to stratify them by sex and age in years. It allowed the examination of the yearly changes and trends in \( CV_g \) for boys and girls as they grow and develop. These attributes have not been described in the literature for children. It also increases the reliability of the estimations.

The stratification of sex is particularly relevant in children since boys and girls grow and mature at different rates during childhood and adolescence. A classic example of this is the rise in \( CV_g \) in alkaline phosphatase in adolescence. This occurs earlier in girls (Figure 1). This study also...
extends the study population to children younger than age 4 years, who are otherwise difficult to recruit for such studies using the direct sampling approach. Children of this age group were not included in the CALIPER study.

The $CV_g$ data generated in this study compared well with existing data. The average $CV_g$ data were largely similar to the CALIPER study and the Ricos database. Crucially, the $CV_g$ data of children who were 18 years old were very close to the Ricos database for adults. Only the $CV_g$ data for total bilirubin, anion gap, bicarbonate, and random glucose in this study were higher by more than 50% compared with the Ricos database. This lends confidence that the indirect approach can provide reasonable estimates of physiologic $CV_g$ data, as has been shown to be the case for the $CV_i$ data.

The higher number of significantly different $CV_g$ data between this and the CALIPER study may be related to the several factors, including the differences in the interval between the repeat sampling. The CALIPER study collected four samples within the same day, with an average time between the repeat sampling. The CALIPER study collected four samples within the same day, with an average time between the repeat sampling. The CALIPER study included children who visited their primary care physician, whereas this study included children who visited their primary care physician in Australia. It is possible that geographical and racial factors may partly contribute to the differences seen in the derived biological variation data. This is supported by the finding of differences in reference intervals derived from healthy individuals, which are composites of $CV_i$ and $CV_g$, among different geographical regions and racial populations in Asia.4,15

Of note, in Australia, children with minor illnesses will visit their primary care physician, whereas those with more severe conditions are attended to in the hospitals. Hence, after application of stringent outlier criteria, the remaining children are likely to be reasonably healthy surrogates, although the inclusion of children with minor pathology cannot be fully excluded. Furthermore, detailed examination of published data by Ricós and colleagues16 suggests $CV_i$ in healthy and diseased states is mostly similar, except for tumor markers, creatinine, and glycated hemoglobin A1c.17 Such comparison is not available for $CV_g$.

The index of individuality for most of the biochemistry tests was low, being close to or less than 0.6. There has been
some confusion with regard to the significance of a low index of individuality and the utility of population-based reference intervals. Traditionally, a low index of individuality suggests that the CV$_{i}$ is relatively small compared with the CV$_{g}$, and population-based reference intervals are of limited use for diagnostic purposes since the result of a person may lie far away from his or her homeostatic set point yet be within the reference interval.

More recently, several studies have shown that a low index of individuality does not adversely affect the use of population-based reference intervals for detecting pathology. The index does not affect the positive and false-positive rates for disease detection for a given magnitude of deviation from a homeostatic set point, provided that only one sample is analyzed. However, when repeat testing is performed to confirm the finding of an initial abnormal value, a low index has a limited impact on the false-positive results since the repeat test is likely to be similar to the original result. When the index is high, a repeat result is likely to be significantly different from the original result and will provide new information to reclassify the patient. Other factors that influence the use of reference intervals and cutoffs in relation to the index of individuality under different clinical scenarios are discussed elsewhere.

The other use of an index of individuality is for determining the need for stratification of the study population according to certain characteristics, such as sex or age. The index of individuality in this study was very stable across age for all tests. This may be related to the well-stratified study population used to derive the CV$_{i}$ and CV$_{g}$ data. By contrast, the index of individuality for certain biochemistry reported by the CALIPER group varied greatly (eg, aspartate aminotransferase, where the index of individuality ranged from 0.21 to 8.38). This suggests that further age or sex partitioning may be required for that study population.

In summary, we have described the use of a data-mining (indirect sampling) approach to estimate the CV$_{g}$ data for children aged between 0 and 19 years. These data are comparable to those derived by the direct sampling approach. This study reported the yearly CV$_{g}$ trends and data for boys and girls, which have not been described previously.

### References


