Short-Term Biological Variation of Plasma Analytes in Renal Disease

Callum G. Fraser and Phillip Williams

Analytical, intra-individual, and inter-individual components of variation were estimated from duplicate analyses of 11 plasma analytes in an average of 13 specimens obtained, over a two-day period, from nine patients with impaired renal function. Analytical variance was 17.3% of the total variance for sodium; <5% of the total variance for potassium, chloride, bicarbonate, albumin, and calcium; and <1% for urea, creatinine, glucose, creatine kinase (EC 2.7.3.2), and alanine aminotransferase (EC 2.6.1.2). Average intra-individual variances were of the same order as those found in healthy individuals. All analytes except glucose showed strong individuality. We postulate that, in nonacute pathological processes where new homeostatic steady states are reached, biological variations around the new set points are of the same order as those found in healthy individuals.

Additional Keyphrases: intra- and inter-individual variation · reference interval

The biological variation of certain commonly assayed analytes of serum has been investigated by several groups (1–6). As is also true for biological variation of analytes in urine (7), these have been studies of ostensibly healthy individuals. Much useful information has been generated, but the clinical chemist is usually dealing with diseased persons in hospital. It has therefore been suggested that the data of most value would be on the biological variation in specific disease states (8).

We have described the short-term biological variation in eight analytes in sera from a group of 20 patients who had uncomplicated myocardial infarction (9). The average intra-individual biological variation for these analytes was larger, but of the same order, as for healthy individuals. This was expected, because pre-analytical variance was not minimized and the particular analytes were not likely to have been disturbed by the disease process. We concluded that the extensive published data on biological variation could be validly used more often in routine laboratory practice. To assess whether these findings were unique to patients with myocardial infarction, and in an attempt—for the first time, to our knowledge—to assess the components of variation of certain plasma analytes significantly affected by a pathological process, we investigated the short-term biological variation of 11 commonly assayed analytes in nine patients with various degrees of renal failure. They were inpatients of the Repatriation General Hospital, Daw Park, South Australia 5041. Three of the patients were said to have had mild renal impairment, three moderate impairment, and three severe impairment. The subjects were adults who had given informed consent to participate in a trial to determine the bioavailability of ranitidine, an H₂ antagonist. Patients were given a single 150-mg dose of ranitidine, orally.

Procedures

Samples were collected during 48 h via an intravenous catheter (31.8 mm, 20 gauge; Jelco Laboratories, Raritan, NJ 08869) at 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, and 48 h after the administration of ranitidine. Each sample was immediately transferred to a 10-mL heparinized plastic container and promptly centrifuged. An aliquot was frozen at −20 °C, in a screw-capped 2.5-mL container. The samples were then stored at −70 °C until analysis. On the day of analysis, all samples from one patient were allowed to thaw at room temperature, mixed thoroughly, and centrifuged briefly in a Sorvall centrifuge (Du Pont Instruments, Wilmington, DE 19898) to precipitate fibrin. Two aliquots of each sample were pipetted into 0.5-mL sample cups and one aliquot into a 0.8-mL container.

Analyses for Na⁺, K⁺, Cl⁻, HCO₃⁻, urea, creatinine, glucose, Ca²⁺, and albumin were performed in an Astra 8 discrete analyzer (Beckman Instruments, Inc., Fullerton, CA 92634). Assays of creatine kinase (CK; EC 2.7.3.2) and alanine aminotransferase (ALT; EC 2.6.1.2) activity were...
done in a Cobas Bio centrifugal analyzer (Roche Analytical Instruments, Inc., Nutley, NJ 07110). The Astra was calibrated twice with each of the Beckman serum-based multi-analyte calibrators 1 and 2. The first aliquot of all samples from a single subject were placed in random order and analyzed in a single batch that, in addition, contained one sample of each of two quality-control materials ("Wellcome Unassayed," Wellcome Reagents Ltd., Beckenham, England; and "Monitrol ILX Chemistry Control," Dade Division, Am. Hosp. Supply Corp., Miami, FL33152). The Astra was recalibrated and the second aliquot of all samples from a single subject was analyzed in the above manner. The third aliquot of all samples from a single subject and the two samples of the quality-control materials were assayed twice for each enzyme activity in the Cobas Bio analyzer; the samples were randomized between each analytical run.

All analyses were done by a single operator. Analyses for all samples from each subject were completed in a single working day. The same lots of calibrators and reagents, all supplied by Beckman, and quality-control materials were used throughout the study.

Results and Discussion

From each subject we obtained, on average, 13 specimens for analysis. Table 1 shows the mean, standard deviation, and coefficient of variation for each analyte, combining all results for the first sets of specimens from each subject. No results were excluded, because the results of analyses of the quality-control materials were satisfactory and the values for imprecision were similar to those obtained in routine laboratory practice. By analysis of variance techniques, the total variance was divided into analytical variance (\(V_A\)), intra-individual variance (\(V_I\)), and inter-individual variance (\(V_O\)); analytical variance was calculated from results of the duplicate analyses performed on each specimen. The component variances and the percentages of each of the components of the total variances are shown in Table 2.

Several factors may have affected the estimates of the components of variance. However, use of random duplicate analyses minimized the effect of drift. Day-to-day analytical variance was minimized by such strategies as use of a single operator; use of single lots of calibrators, reagents, and quality-control materials; and recalibration between all analytical batches. Storage of specimens at \(-70\,^\circ\text{C}\) was unlikely to have caused any significant changes (3).

Analytical variance exceeded 5% of the total variance only for sodium analyses. Most previous studies have highlighted this finding (1–6, 9). This implies that analyses for sodium should be performed with better precision than is currently provided by laboratories if patient care is to be optimum.

Our estimates of the average intra-individual variation of each analyte were, in general, of the same order as those documented in previous studies (1–6, 9). This finding demonstrates that, in patients with impaired renal function, individuals generally have biological fluctuations around their own homeostatic "set points" that are of the same order as those that occur in healthy individuals.

It has been suggested that the square root of the ratio \(V_I/V_O\) can provide an index of the degree of individuality of analytes (10). For all analytes except glucose, this ratio was <1.0, and for all analytes except potassium and creatinine kinase it was <0.5. These findings show that, in patients with impaired renal function, analytical results for most analytes have a high degree of individuality. Thus, for each patient, the best guide to changes in the severity of disease processes will indeed be comparison of test results with results of recent analyses performed on specimens from that patient. We therefore postulate that, in patients with impaired renal function, the analytes that are affected by the disease process, irrespective of whether they are outside the conventional population-based reference intervals (for example, urea and creatinine) or within conventional reference intervals (for example, sodium and chloride) as shown in Table 1, do have—in the short-term at least—biological fluctuations around set points exactly like healthy individuals. It is only the set points that vary.

It is attractive to consider that this finding may be general. We advocate the hypothesis that, in the short-term, all diseases that cause abnormal or normal biochemical results but in which a new homeostatic steady-state is achieved (for example, primary hyperparathyroidism) the biological intra-individual variations in plasma analytes are similar to those found for healthy peers. It is therefore

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Units</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
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<tr>
<td>Na⁺</td>
<td>mmol/L</td>
<td>142.0</td>
<td>3.1</td>
<td>2.2</td>
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<tr>
<td>K⁺</td>
<td>mmol/L</td>
<td>4.5</td>
<td>0.9</td>
<td>2.1</td>
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<tr>
<td>Cl⁻</td>
<td>mmol/L</td>
<td>104.3</td>
<td>5.2</td>
<td>5.0</td>
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<tr>
<td>HCO₃⁻</td>
<td>mmol/L</td>
<td>23.4</td>
<td>4.3</td>
<td>18.3</td>
</tr>
<tr>
<td>Urea</td>
<td>mmol/L</td>
<td>13.2</td>
<td>7.5</td>
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<tr>
<td>Creatinine</td>
<td>μmol/L</td>
<td>190.4</td>
<td>117.6</td>
<td>61.9</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>39.1</td>
<td>4.2</td>
<td>10.7</td>
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<tr>
<td>Ca²⁺</td>
<td>mmol/L</td>
<td>2.4</td>
<td>0.2</td>
<td>6.4</td>
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<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>5.1</td>
<td>1.5</td>
<td>29.1</td>
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<tr>
<td>CK</td>
<td>U/L</td>
<td>75.2</td>
<td>52.5</td>
<td>70.0</td>
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<tr>
<td>ALT</td>
<td>U/L</td>
<td>12.4</td>
<td>9.2</td>
<td>74.4</td>
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*Figures in parentheses are percentages of total variance.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>(V_A)</th>
<th>(V_I)</th>
<th>(V_O)</th>
</tr>
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<td>Na⁺</td>
<td>1.69</td>
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<td>0.36</td>
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<tr>
<td>Cl⁻</td>
<td>0.75</td>
<td>1.26</td>
<td>24.63</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>0.19</td>
<td>1.72</td>
<td>16.52</td>
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<tr>
<td>Urea</td>
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<td>0.73</td>
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<tr>
<td>Creatinine</td>
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<td>151.27</td>
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<tr>
<td>Albumin</td>
<td>0.28</td>
<td>2.84</td>
<td>14.56</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>0.92 × 10⁻³</td>
<td>2.57 × 10⁻³</td>
<td>18.9 × 10⁻³</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>CK</td>
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<td>1061.39</td>
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<td>ALT</td>
<td>0.43</td>
<td>10.02</td>
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believed that the extensive published data on biological
variation, generated for the most part from adults of work-
ing age, should be and validly can be much more widely
used in the interpretation of routine clinical biochemistry
laboratory results. We suggest that it is only in acute sit-
uations (for example, plasma enzyme activity assays after
myocardial infarction) or in the long-term in chronic con-
tions, when deterioration or amelioration is occurring, that
currently available data on biological variation cannot be
used.

It would be of much interest to test this hypothesis
further, in particular to assess components of biological
variation in patients with specific diseases over longer time
periods.

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Suitability of Control Materials in the Differential Inhibition Assay for Human
Pancreatic and Salivary Amylase

Mary D. O’Donnell and K. F. McGeeney

We investigated the behavior of 26 quality-control sera with
the inhibitor method for differential amylase (EC 3.2.1.1) assay. We also studied the sensitivity to the wheat-derived
inhibitor of pancreatic amylases from 10 different animals in
comparison with human pancreatic and salivary amylase.
The results indicate that only control materials containing
human amylases can be measured accurately. The animal
amylases (bovine, equine, porcine) used in many quality
control sera are relatively insensitive to the inhibitor as
compared with human pancreatic and salivary amylase.

Additional Keyphrases: isoenzymes • animal vs human
sources of enzymes used in quality control

The suitability of control materials for determination of
total amylase (EC 3.2.1.1) activity has been studied (1). The
authors used 11 common techniques (five different princi-
ple processes) and processed the results by statistical methods. How-
ever, the analytical techniques they used did not measure
human pancreatic in the presence of human salivary amyl-
ase, or vice versa. The inhibitor method (2) can distinguish
between human pancreatic and human salivary amylase, which
has some clinical advantages. The method is based on
different degrees of inhibition of the two isoenzymes by the
protein inhibitor from wheat (3). In contrast to the two
human amylases, porcine pancreatic amylase is relatively
resistant to the inhibitor (3). Because the amylase in many
quality-control sera is of porcine or other animal origin, or is
human serum with unspecified additions, the use of some of
these products with the inhibitor method might give errone-
ous results. We examined the use of various commercially
available quality-control sera in the measurement of human
pancreatic and salivary amylase by the inhibitor method and
report our findings here.

Materials and Methods

Quality-control sera: We studied 26 control sera from 11
suppliers, as follows: Ortho I and II (Ortho Diagnostics,
Raritan, NJ 08869); Phadebas reference serum (Pharmac Dia-
gnostics, Upsala, Sweden); Target, normal and abnor-
mal (SmithKline Instruments, Sunnyvale, CA 94086); Du
Pont, normal and elevated (Du Pont UK Ltd., Stevenage,
Hertfordshire, U.K.); Monitrol I-E, II-E and Enza-Trol-E
(Merz & Dade AG, 3186 Dudingen, Switzerland); Monitrol
IX and ILX (Dade Division, American Hospital Supply
Corp., Miami, FL 33152); Precinorm U, Precipath U, Preci-
norm E, Precipath E, PreciFlo (Boehringer, Mannheim,
F.R.G.); Q-Pak I and II (Travenol Laboratories S.A., B-7860
Lessines, Belgium); Biotrol-33 Plus (Laboratoires Biotrol,
Rue du Foin, 75140 Paris, France); Armtrol, medium and
high (Purce Associates, Antrim, Northern Ireland); Well-
control one, two, and QC (Wellcome Reagents Ltd., Becken-