Biological Variability Of 25-hydroxy Vitamin D And HbA1c In Healthy Subjects.

Niketa Vasan1,2, Sarah Vossoughi2, Biren Patel3, Shashi Mehta1, Alex J. Rai2
1Rutgers-SHRP, MS in Medical Laboratory Science Program, Newark, NJ
2New York Presbyterian Hospital – Columbia University Irving Medical Center, New York, NY
3Saint Michael’s Medical Center, Newark, NJ

Abstract
The aim of this study was to generate data on the components of biological variation, including within-subject variation (CVI) and between-subject variation (CVG) for 25-OH vitamin D and HbA1c from a presumed healthy population, in addition to defining quality specifications for laboratory analysis. Serum and whole blood samples were collected from 24 healthy volunteers (9 male, 15 female) once weekly for four weeks. Serum samples were used for the measurement of 25-OH vitamin D using the Architect i2000SR automated analyzer. EDTA whole blood samples were used for the measurement of HbA1c levels using the Dacie-Calco 520 analyzer. From the data generated, the CVI and CVG biological variation values were established using nested analysis of variance (ANOVA). Quality specifications, index of individuality (II), and reference change value (RCV) were calculated from CVI and CVG. CVI was calculated to be 5.2% and CVG was 50.5% for 25-OH Vitamin D. CVI was 1.5% and CVG was 11.49% for HbA1c. The II was 0.10 and the RCV (95% probability) was 14.5% for 25-OH Vitamin D. The II was 0.13 and the RCV (95% probability) was 4.4% for HbA1c. These results suggest that population-based reference ranges for HbA1c and 25-OH Vitamin D may be of limited value in diagnosis. In addition to reference ranges, consideration of RCV and II will help with accurate interpretation of patient results and assessment of the variation in total measurements. Moreover, analytical quality goals were derived at three levels (optimal, desirable, minimal) for precision, bias, and total error and our current methodology for HbA1c and 25-OH Vitamin D was assessed using these generated quality goals.

Table 1. Study results

<table>
<thead>
<tr>
<th>Variable</th>
<th>CV%</th>
<th>RCV%</th>
<th>Bias%</th>
<th>Total Error%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>1.13</td>
<td>3.89%</td>
<td>0.13</td>
<td>4.40%</td>
</tr>
<tr>
<td>25-OH</td>
<td>0.90</td>
<td>11.49%</td>
<td>0.10</td>
<td>11.56%</td>
</tr>
</tbody>
</table>

Results

Towards achieving desired goals for HbA1c and 25-OH Vitamin D, we were able to generate the analytical quality (precision/accuracy/total error) goals based on generated biological variation data using the formula suggested by Fraser.

Conclusion

- Between-subject biological variation value far exceeds the within-subject biological variation value for both 25-OH vitamin D and HbA1c. The mean values were 23.82ng/mL (range 14.20 to 39.60) for 25-OH Vitamin D, 5.51% (range 4.8 to 6.2) for HbA1c.
- We were able to generate the analytical quality (precision/accuracy/total error) goals based on generated biological variation data using the formula suggested by Fraser. We were also able to assess the quality of current methodology for 25-OH vitamin D and HbA1c.
- The index of individuality for both analytes is <0.6, which suggests that population-based reference intervals for HbA1c and 25-OH Vitamin D are of limited value in demonstrating deficiency and excess. In addition to reference intervals, consideration of RCV and II may help with the accurate interpretation of patient results and assessment of the variation in serial measurements.

Table 2. Analytical goals for 25-OH vitamin D and HbA1c measurements derived from biological variation data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Imprecision%</th>
<th>Bias%</th>
<th>Total error%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>0.10</td>
<td>0.13</td>
<td>0.26</td>
</tr>
<tr>
<td>25-OH</td>
<td>0.9</td>
<td>0.10</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Materials and Methods

Analytical Variation

- Within-subject Variations
- Between-subject Variations
- Pre-analytical
- Post-analytical

Analytical, Pre-analytical

- Technical validation
- Applying reference methods
- Determining bias and imprecision

Within-subject Variation

- Intra-subject variation (3 replicates)
- Inter-subject variation (3 replicates)
- Same subject, same day

Near Analytical Errors

- Non-compliance
- Data analysis

Analytical Variation

- Total Variation
- Biological Variation

Methods

- Analyte selection
- Health questionnaire and consent
- Sample collection, storage and analysis

Data Analysis

- Outlier & Normality check
- ANOVA

Results (con’t)

Future Directions

- Biological variation data generated in this study may help in building a biological variation database and may help to set the performance quality specification for laboratories and EQA programs.
- Application of biological variation data from laboratory-specific population and laboratory-specific method should increase the usefulness of these tools and may be considered a recommended practice.

References

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Acknowledgments

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