Biological Variation of Myoglobin in Serum

To the Editor:

Measurement of myoglobin in serum provides essential information that aids in both early diagnosis and treatment of patients with myocardial infarction or skeletal muscle damage [1, 2]. However, information on the biological variability of myoglobin is lacking, a fact that may seem surprising when one considers the many reports on myoglobin as an indicator of muscular damage.

To investigate the biological variation of this analyte, we took four blood specimens from each of 10 apparently healthy laboratory workers (5 men and 5 women, ages 25–50 years) on the same day once a week for 4 weeks. In accordance with the Helsinki II Declaration, the design and execution of the experiment were explained thoroughly to the subjects, and informed consent was obtained. They were urged to continue their dietary habits or activities, and their weight remained stable within ±1.0 kg. Furthermore, none took any medication or consumed substantial quantities of alcohol.

After the volunteers fasted for 12–14 h and refrained from any morning exercises or smoking, venous blood was obtained while they were in the sitting position between 0800 and 0900 for 1–5 min with minimal stasis by the same phlebotomist with the use of reduced-pressure blood collection tubes (Sarstedt). Serum specimens, separated by centrifugation (4000g for 15 min), were aliquoted and stored at −25°C until analysis. When all the specimens were available, they were thawed, mixed, and centrifuged for analysis in a single run in duplicate, in random order. Myoglobin was measured with an automated fluorogenic ELISA (Opus myoglobin assay, Behringwerke AG) as described previously [2].

Biological within-subject variance was estimated from the total within-subject variance minus within-run analytical variance. The latter was estimated from replicate analyses of specimens from the subjects themselves. Biological between-subject variance was estimated from the total variance of the set of duplicate data from the assay performed on each subject minus analytical and within-subject components [3]. All of the components of variation were then transformed to the relevant CV with the use of the overall mean.

The mean values of myoglobin and estimated components of variation [analytical variation (CV\(_A\)), intrasubject variation (CV\(_I\)), and interindividual biological variation (CV\(_B\))] are shown in Table 1.

The means and the intrasubject variations did not differ significantly (P = 0.55 and P = 0.76, respectively) between sexes. The data on analytical and biological variation allowed the calculation of the analytical goal for imprecision (≤1/2 CV\(_A\), i.e., ≤5.6%) and inaccuracy [≤0.25(CV\(_A^2 + CV\(_B^2\)\)^1/2, i.e., ≤4.4%], the critical difference required for serial results from an individual to change significantly [2.77(CV\(_A^2 + CV\(_B^2\)\)^1/2, i.e., 35%], the number of specimens that should be collected to estimate the homeostatic set point of an individual to within 5% [1.96(CV\(_A^2 + CV\(_B^2\)\)/25, i.e., 25] [4], and the index of individuality (CV\(_I\)/CV\(_B\), i.e., 0.8).

This last calculation shows that population-based reference intervals can be of some value in assessing patients’ results, with the caveat that results for some individuals may be unusual for them even if they lie within the population reference interval [5]. In confirmation of this, Tucker et al. [6] recently described a substantial number of patients whose myocardial infarction can be diagnosed early by observing significant changes in serial myoglobin results, with the concentrations of this marker still within the reference interval.

Among other things, this study shows that the Opus precision assay does not meet the goal based on biological variation. Improvement in the precision of the measurement is therefore required if this assay is to be offered on a routine basis. However, the performance of at least two replicate analyses on the same spec-

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**Table 1. Mean values of myoglobin and estimated components of variation.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean conc., µg/L</th>
<th>CV(_A) %</th>
<th>CV(_I) %</th>
<th>CV(_B) %</th>
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<tr>
<td>All</td>
<td>17.0</td>
<td>6.0</td>
<td>11.1</td>
<td>13.8</td>
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<tr>
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<td>17.5</td>
<td>6.0</td>
<td>10.3</td>
<td>12.8</td>
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<tr>
<td>Female</td>
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<td>12.0</td>
<td>16.0</td>
<td></td>
</tr>
</tbody>
</table>

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Interference with Nephelometric Assay of C-Reactive Protein and Antistreptolysin-O by Monoclonal IgM-κ from a Myeloma Patient

To the Editor:

The serum concentrations of C-reactive protein (CRP) and antistreptolysin-O (ASO) are measured by any of several rapid and reliable nephelometric and immunoturbidimetric methods. However, the presence of pathological concentrations of mono-

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**References**


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