3. Binkley N, Krueger D, Cowgill CS, Plum L, Lake E, Hansen KE, et al. Assay variation confounds the diagnosis of hypovitaminosis D: a call for standardization. J Clin Endocrinol Metab 2004;89: 3152–7.

4. Souberbielle JC, Fayol V, Sault C, Lawson-Body E, Kahan A, Cormier C. Assay-specific decision limits for two new automated parathyroid hormone and 25-hydroxyvitamin D assays. Clin Chem 2005;51:395–400.

5. Singh RJ, Taylor RL, Reddy GS, Hollis BW, Grebe SK. C-3 epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status. J Clin Endocrinol Metab 2006;91:3055–61.

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Biological Variation of Myeloperoxidase

To the Editor:

Myeloperoxidase (MPO)[1] has been shown to play a role in the pathogenesis of coronary artery disease. It is released from activated neutrophils at sites of vascular damage and has been shown to increase in atherosclerotic plaques before the onset of myocardial injury (1). Elevations in MPO occur independently of C-reactive protein (CRP) and other markers of inflammation, and it has been suggested as a marker of risk in patients who have high as well as low cardiac troponin T concentrations and who present with acute coronary syndrome (2).

We recruited 12 apparently healthy individuals, 10 women and 2 men with a mean age of 33 years (range 27–43) for estimation of the biological variation of MPO. None had a history of heart disease or any clinically apparent inflammatory processes. Serum samples were collected between 0800 and 1000 on the same day of the week, weekly for 5 weeks as described by Fraser and Harris (3). Venous blood was collected in plastic serum separator tubes. All samples were centrifuged after clotting and were stored at −70 °C before analysis. All samples were analyzed with the MPO ELISA kit from Immundiagnostik and the model 3550 microplate reader from Bio-Rad.

The statistical methodology of Fraser and Harris was used unless otherwise stated (3). Except for the analytical assessment, all assessments were done on both untransformed and logarithmically transformed (natural logarithm) data. The data, which are presented in Fig. 1, provided 60 estimates of analytical variance and 12 estimates of within-subject variance. The analytical variance plotted against the cumulative percentile ranking followed the expected χ^2 distribution within good proximity. Cochran's test of maximum variance identified 1 analytical outlier. The residuals of a 1-way ANOVA of the means of duplicates were used for assessment of within-subject distribution (a deviation from the methodology of Fraser and Harris). Cochran's test detected 1 within-subject outlier that was not present after log-transformation. The outlier was in a sample from subject 1, from whom the analytical outlier was also obtained. In order not to be biased toward 1 distribution, all values of subject 1 were excluded from further analysis. No other outliers were detected after this exclusion. Nonnormality of the untransformed and log-transformed data (after exclusion of subject 1) was not apparent by Shapiro-Wilk tests ($P = 0.58$ and $P = 0.26$, respectively). The within-subject index of heterogeneity was calculated as the ratio of the CV of the individual variances to the theoretical CV calculated as $[2/(n - 1)]^{1/2}$, where n is the average number of specimens from each subject (3). The absolute difference of this ratio from unity was less than the theoretical SD calculated as $1/(2n)^{1/2}$, suggesting that the within-subject variances were homogeneous. Nonnormality of the between-subject distribution as assessed using subject averages and the Shapiro-Wilk test could not be shown for either the untransformed or log-transformed data ($P = 0.10$ and $P = 0.09$, respectively). No outliers were found in either of the 2 distributions using a Reed-Dixon ratio criterion of 1/3. Given these statistics, the analytical (CV_A), within-subject (CV_I), and between-subject (CV_G) CVs were estimated with nested ANOVA of the untransformed data only.

The CV_A, CV_I, and CV_G were 4%, 36%, and 30%, respectively. The design of the biological variation study led to an unrealistically low CV_A, which is desirable for accurate estimates of the other variance components (3). A more realistic CV_A of 8.4%, obtained from the literature, was used in further calculations (2). The index of individuality (Ii), calculated as $(CV_A + CV_I)/CV_G$, was 1.5, which is satisfactory for comparison of an observed value to a reference value (3). Contrary to CRP, which has a low Ii, the high Ii of MPO supports the use of a population-based cutoff for risk stratification (4). A cutoff of 350 μg/L has been proposed for risk stratification and is used here only as an example because the assay has not been stan-

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[1] Nonstandard abbreviations: MPO, myeloperoxidase; CRP, C-reactive protein.
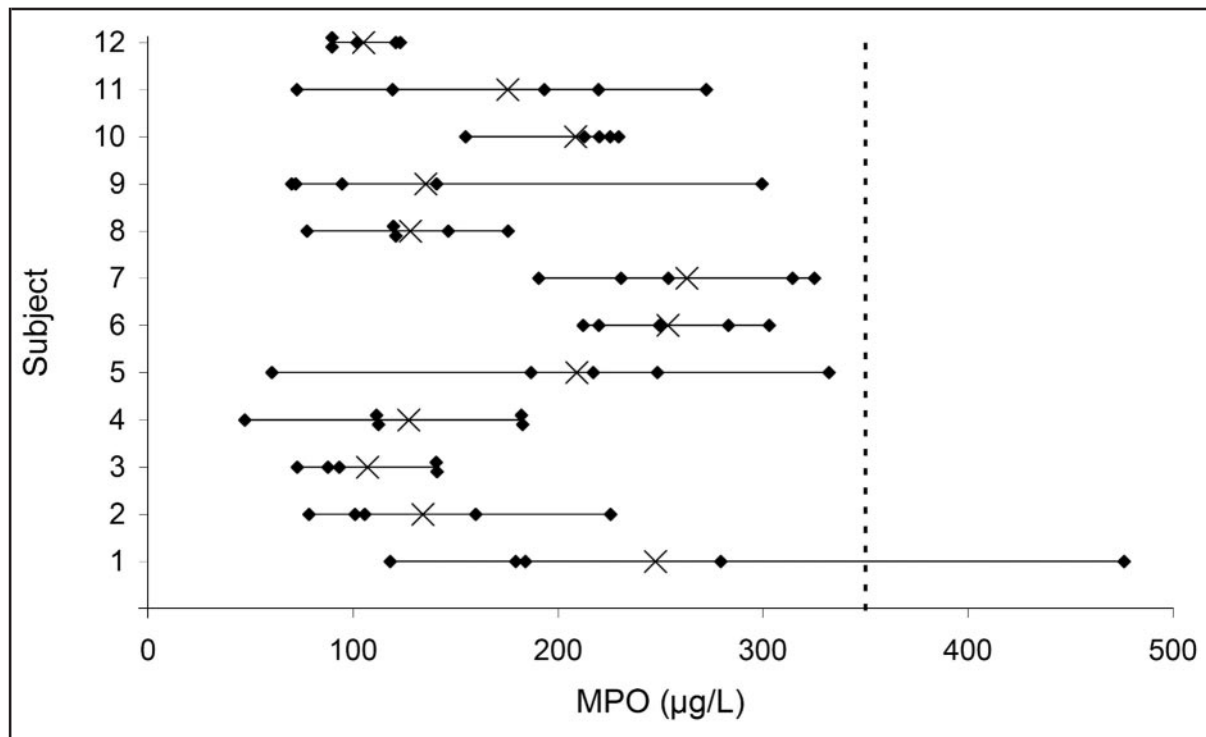
Fig. 1. Average of the duplicate MPO measurements over a 5-week period.
The mean concentration of each subject is indicated by ×, and a published population-based risk cutoff (350 μg/L) is indicated by the dotted line.

dardized (2). The interval in which correct classification will not be certain at this concentration can be calculated as

$$MPOi =$$
$$350 \pm \left(z * \sqrt{\frac{CV_A^2 + CV_I^2}{n}} * 350 \right)$$

where *MPOi* is the value to be calculated, 350 is the suggested concentration in ug/L, *n* is the number of replicate samples, and *z* is 1.64 for a 1-sided 95% confidence interval (5). With a single-sampling strategy, correct classification of patients with concentrations between 138 and 562 μg/L will not be 95% certain. The reference change value of MPO, calculated as $2.77 * (CV_I^2 + CV_A^2)^{1/2}$, is 102%. In conclusion, the high Ii of MPO supports the use of a popula-

tion-based cutoff but the high CV_I leads to a rather large interval in which correct classification will not be certain.

References

1. Zhang R, Brennan M, Fu X, Aviles RJ, Pearce GL, Penn MS, et al. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001;286: 2136–42.
2. Baldus S, Heeschen C, Meinertz T, Zeiher AM, Eiserich JP, Munzel T, et al. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 2003;108: 1440–5.
3. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27: 409–37.
4. Macy EM, Hayes TE, Tracy RP. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 1997; 43:52–8.
5. Sölétormos G, Semjonow A, Sibley PEC, Lamerz R, Hyltoft Petersen P, Albrecht W, et al. Biological variation of total prostate specific antigen: a survey of published estimates and consequences for clinical practice. Clin Chem 2005;51: 1342–51.

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