Myeloperoxidase (MPO)\(^1\) 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. 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 \(\chi^2\) 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 \(\sqrt{(1/3)}\), 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 \(\text{CV}_{A}^2 + \text{CV}_{I}^2)/\text{CV}_{G}\), 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-
The interval in which correct classification will not be certain at this concentration can be calculated as

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

where \( MPO_i \) 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 \( \mu \)g/L will not be 95% certain. The reference change value of MPO, calculated as \( 2.77 \times (CV_I^2 + CV_A^2)^{1/2} \), is 102%. In conclusion, the high \( I_i \) of MPO supports the use of a population-based cutoff but the high \( CV_I \) leads to a rather large interval in which correct classification will not be certain.

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**References**


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Mismeasure of C-Type Natriuretic Peptide

To the Editor:

It has been difficult to establish an endocrine role for C-type natriuretic peptide (CNP). Release of CNP from the heart remains to be convincingly demonstrated (1–5), and data concerning CNP in cardiac disease have been conflicting, possibly because of specificity problems in measurement of CNP. All natriuretic peptides (NPs) display strong homology, and antibodies raised against one NP may consequently bind other NPs. With the relatively high plasma concentrations of A- and B-type NPs (ANP and BNP), a CNP assay with even a small degree of ANP or BNP cross-reactivity may produce falsely high CNP concentrations. This is especially relevant in heart failure with augmented cardiac ANP and BNP expression. We examined the selectivity of a widely used CNP RIA.

To evaluate cross-reactivity in the CNP assay, we measured serial dilutions of human CNP-22 (4.5–582 pmol/L), ANP-28 (5–100 000 pmol/L), and BNP-32 (4–81 000 pmol/L) against the calibrators of the CNP assay. We obtained human cardiac tissue samples from a heart failure patient undergoing cardiac transplantation. The local ethics committee approved the use of human tissue, and written informed consent was obtained from the patient before surgery. The extracts were subjected to measurements of CNP, BNP, NT-proBNP, and NT-proCNP using a commercial CNP RIA (Phoenix), a BNP assay (Shionoria), and in-house RIAs directed against the N-terminus of proBNP and proCNP, respectively.

Results are expressed as mean (SE). We used nonlinear regression analysis to fit 4-parameter logistic models to the data from the buffer experiments. Based on the model, we calculated the cross-reactivity as a function of BNP-32 concentration using a 1-phase exponential decay model. Likewise, we calculated cross-reactivity as a function of the BNP concentration by fitting data from the heart extract experiments to a 1-phase exponential decay model. Cross-reactivity was calculated as measured CNP concentration divided by measured BNP concentration.

Serial dilutions of human CNP-22, BNP-32, and ANP-28 in barbital buffer measured with the CNP RIA revealed cross-reactivity for BNP-32 in physiological concentrations (Fig. 1A). The dilution curves for CNP-22 and BNP-32 were not parallel; cross-reactivity increased with decreasing BNP-32 concentrations, being 5.1% at a BNP-32 concentration of 100 pmol/L and 1.6% at 1000 pmol/L (Fig. 1B). As expected, BNP and NT-proBNP concentrations were high in the atria and lower in the ventricles (Fig. 1C). NT-proCNP and particularly CNP concentrations were very low in both atria and ventricles (Fig. 1D). With only trace amounts of NT-proCNP in the extracts, most of the measured CNP represents cross-reacting BNP. Calculating cross-reactivity as CNP concentrations divided by BNP concentrations in the extracts, we fitted a curve parallel to that obtained in the buffer experiment (Fig. 1B). Cross-reactivity was estimated to be 3.2% at a BNP concentration of 100 pmol/L and 1.1% at 1000 pmol/L.

Nearly all reports on CNP measurement in plasma are based on the same RIA. According to the manufacturer, the assay does not exhibit any cross-reactivity with ANP or BNP. Nevertheless, our data indicate substantial BNP cross-reactivity of the CNP assay in buffer with added BNP-32 and in heart extracts (Fig. 1B). Ideally, chromatographic evaluation of the extracts could resolve the BNP and CNP forms, as coelution of CNP and BNP immunoreactivity would have provided further proof of BNP cross-reactivity. However, the CNP concentration in the heart extracts did not exceed 15 pmol/L, rendering chromatography very difficult. The difference in the degree of cross-reactivity between the buffer with added BNP-32 and the unmodified heart extracts may be partly because immunoreactive BNP in the extracts comprises both BNP-32 and intact proBNP, which may have another cross-reactivity profile. Alternatively, BNP measured by the Shionoria assay may partly have been cross-reacting ANP, which is highly expressed in the cardiac atria. Because N-terminal proCNP, proANP, and proBNP have virtually no sequence resemblance, cross-reactivity of the NT-proCNP assay with proANP and proBNP is highly unlikely. If intact proCNP(1–103) is present in the heart, the NT-proCNP assay would probably also measure it.

The CNP assay may still be valuable for CNP measurement in samples with low BNP concentrations; however, measurements done in samples with a high BNP concentration—such as heart tissue extracts or plasma samples from patients with heart failure—should be interpreted cautiously.