differences from the clinical results respectively obtained with NT-proBNP and BNP assays, thus suggesting that the performance of the immunoassays used may a very crucial point in determining the results of a clinical study comparing different CNH assays.

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Biological Variation of N-Terminal Pro-Brain Natriuretic Peptide in Healthy Individuals

To the Editor:

Brain natriuretic peptide (BNP) and its N-terminal prohormone (NT-proBNP) fragment have been shown to be effective in diagnosing left ventricular dysfunction *(1, 2)*, and in particular, they have a strong negative predictive value *(3)*. NT-proBNP and the hormone are secreted on an equimolar basis, but NT-proBNP lacks a clearance receptor. It therefore has a longer half-life in serum than the active hormone does, and its circulating concentration is believed to be less influenced by the conditions under which the blood sample is taken.

Information on the biological variation of NT-proBNP is not available; this is limiting because the clinical utility of laboratory data can be affected by physiologic variation *(4)*. Here we report the results of a study to determine the biological variability of NT-proBNP.

Five blood specimens were collected from each of 16 apparently healthy laboratory workers (5 men and 11 women; age range, 43–62 years) twice a week (Tuesdays and Fridays) over a 17-day period. None of the workers smoked, took any medication, or consumed substantial quantities of alcohol. In accordance with Helsinki Declaration II, the design and execution of the experiment were explained thoroughly to the participants, and informed consent was obtained. Blood was collected under standardized conditions to minimize sources of preanalytic variation. After an overnight fast, a blood specimen was taken by conventional venipuncture between 0800 and 0900 with the volunteers in the sitting position, avoiding venous stasis. All samples were drawn by the same phlebotomist, allowed to clot, and then centrifuged at 3000*g* for 15 min at room temperature within 1 h of collection. Sera were separated and stored at −70 °C until analysis. It has been documented that the N-terminal peptide can be safely stored frozen at −20 and −80 °C for at least 3 months *(5)*.

At the end of the collection period, all frozen samples were thawed, mixed, and centrifuged for analysis in a single run in duplicate. NT-proBNP concentrations were determined by an electrochemiluminescence sandwich immunoassay (Roche Diagnostics). The assay was performed on an Elecsys System 2010 by the same analyst, who followed the assay manufacturer's recommendations. After exclusion of one outlier and logarithmic transformation of the data (required because of the skewed distributions of the NT-proBNP data), the analytical (CV_A) and intra- (CV_I) and interindividual (CV_G) components of variation were calculated by nested ANOVA. We also calculated the critical difference for significant changes in serial results ($P < 0.05$), the index of individuality, the number of specimens required to estimate the homeostatic setpoint of an individual (within ± 10% with a confidence of 95%), and the desirable quality specifications for imprecision (I), bias (B), and total error (TE), which were calculated using the formulas: $I < 0.5CV_I$; $B < 0.25\ (CV_I^2 + CV_G^2)^{1/2}$; and $TE < 1.65\ I + B$ ($\alpha < 0.05$). The results are reported in Table 1.

Minor, not statistically significant differences ($P = 0.87$), were observed between genders and were attribut-

Table 1. Mean values; estimated mean analytical (CV_A), intraindividual (CV_I), and interindividual (CV_G) variation; and derived indices for serum NT-proBNP.

						Desirable quality specifications				
Group	Mean, pmol/L	CV_A, %	CV_I, %	CV_G, %	II[a]	Imprecision, %	Bias, %	Total error, %	CD, %	No. of specimens
All	8.37	2.7	9.1	14	0.64	4.6	4.22	11.72	26.33	3
Men	9.42	1.1	6.5	16	0.41	3.2	4.29	9.65	18.18	2
Women	7.98	3.1	10	14	0.71	5.0	4.32	12.57	29.04	4

[a] II, index of individuality; CD, critical difference.

able to the lower CV_I in the men. The quality specifications for assay imprecision ($CV_A < 0.5CV_I$) were widely fulfilled. The individuality index was close to 0.6, indicating that an individual's results are more useful as reference values than are population-based data when the results are used in monitoring. Finally, serial results for pro-BNP must change by >26% before significance can be claimed.

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PCR-based Detection of *CYP21* Deletions

To the Editor:

We read with interest the Technical Brief by Lee et al. *(1)*, in which the authors describe a novel method to detect *C4-CYP21* deletions in patients with steroid 21-hydroxylase deficiency. Such deletions result from an unequal crossover in the RCCX module (*RP-C4-CYP21-TNX*) on chromosome 6. In most cases, chromosome 6 carries two RCCX modules, one with a *CYP21P* (*CYP21A1P*) pseudogene and a truncated *XA* pseudogene, and one with a functional *CYP21* (*CYP21A2*) gene (encoding steroid 21-hydroxylase) and a functional *TNXB* gene (encoding tenascin-X). Meiotic misalignment and recombination may occur at several locations and create a chromosome with a single chimeric RCCX module. The PCR described by Lee et al. uses one primer in the 5′ flanking sequence of *CYP21* and *CYP21P (2)*, whereas the other primer is positioned in a 120-bp sequence of *TNXB* that is not present in the *XA* pseudogene *(3)*. Although this PCR is indeed suitable for the detection of chimeric *CYP21P/CYP21* genes, it would fail to detect any RCCX chimera in which the pseudogene-like region includes the 120-bp deletion of *XA (4)*, as illustrated in Fig. 1.

Lee et al. *(1)* successfully characterized 18 patients by this method, finding three categories of *CYP21P/CYP21* chimeras. Therefore, *XA/TNXB* chimeras may be rare in the Chinese population they studied. In The Netherlands, however, such hybrids are common *(5, 6)*: in our patient group, the PCR would have yielded no product in four of nine chimeric RCCX modules on bimodular chromosomes *(6)* as well as in a recently described de novo deletion *(7)*. Thus, this method fails to detect all *CYP21* deletions.

To amend this problem, we recommend that the *TNXB*-specific primer be positioned beyond the RCCX duplication boundary, in the nonduplicated area of *TNXB* (see Fig. 1). This will produce three additional *Taq*I fragments, but these are smaller than 1 kb and should not interfere with the agarose gel separation shown in Fig. 1D of the Technical Brief by Lee et al. *(1)*. In addition, *CYP21P/CYP21* chimeras would then produce a 2.5-kb *Taq*I fragment and could be readily distinguished from *XA/TNXB* chimeras, which would produce a 2.4-kb *Taq*I fragment.

Southern blotting remains the established approach for comprehensive analysis of this highly complex and variable region of the human genome. Genomic *Taq*I digestion coupled with cohybridization with *CYP21*, *TNX*, and *C4* probes provides direct information about these three genes and alerts the investigator to uncommon configurations that require further analysis by long-range restriction mapping *(8, 9)*. Although we recognize the benefits of rapid nonradioactive detection methods, especially for diagnostic purposes, careful evaluation of such methods is necessary, notably if the method is recommended for general use and not limited to the population for which it was originally designed.

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