Reference Change Values for Brain Natriuretic Peptides Revisited

To the Editor:

In 2004, Bruins et al. *(1)* published a report in which they used the approach of Harris and Yasaka *(2)* to determine the reference change values (RCVs) for B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP). Week-to-week RCVs were 113% and 98%, respectively, meaning that concentrations had to increase or decrease by these percentages to be assessed as different from the previous measurement, with a 5% error probability for falsely assessing a change as significant. The clinical relevance of these large RCVs led to a lively discussion *(3–6)* regarding RCV methodology. In response, we have developed some ideas for improving RCV calculation by addressing the skewness of the distribution.

The previous study used for the new approach investigated 43 patients with stable chronic heart failure by within-day, day-to-day, and week-to-week measurements of BNP (Abbott Diagnostics) and NT-proBNP (Elecsys® proBNP, Roche Diagnostics GmbH). For details, see the original report *(1)*.

Natriuretic peptides are secreted in response to increased demand and may not be conceived to fluctuate around a physiologic (homeostatic) set point as do analytes that are regulated by physiologic processes. The assumption of gaussian-distributed measurements may not hold, and indeed, the (interindividual) distribution of natriuretic peptides shows a right-skewed shape. Whereas log-transformation is the recognized remedy for appropriate transformation, its use in the construction of RCVs was not proposed and also requires an intraindividual skewed distribution. Assessing the skewness can be accomplished by comparing the arithmetic mean and median: the mean is greater than the median for right-skewed distributions. The success of log-transformation, based on the raw data from the original report *(1)*, was assessed by determining the percentage deviation from 1 of the week-to-week mean over the week-to-week median, before and after transformation. Mean deviations before and after transformation were 10.6% and −0.1% for BNP and 10.3% and 0.5% for NT-proBNP, respectively. These results suggest that a lognormal approach is preferable over a normal approach for BNP and NT-proBNP. Measurements follow a lognormal distribution if the logarithms of the measurements follow a gaussian distribution.

Mathematically, the lognormal distribution uses 2 parameters, μ and σ^2, the mean and variance of the underlying gaussian distribution. The lognormal mean, SD, and the CV are determined by μ and σ^2:

$$\text{mean} = \exp\{\mu + \tfrac{1}{2}\sigma^2\} \qquad SD = \exp\{\mu + \tfrac{1}{2}\sigma^2\}\sqrt{\exp(\sigma^2) - 1} \qquad CV = \sqrt{\exp(\sigma^2) - 1}$$

Scheme 1.

In the following derivation of RCVs, only the total variation is considered [no split in subcomponents; for a comparison, see Ref. *(7)*]. A change between 2 consecutive (independent) measurements, x and y, is considered significant (with 5% error probability) when the difference,

$$\ln(y) - \ln(x) = \ln\left(\frac{y}{x}\right),$$

exceeds $\pm\sqrt{2} \times z \times \sigma$, where $z = 1.96$. This follows from the gaussian distribution of the log-transformed measurements [compare with Iglesias et al. *(8)*]. By retransforming the difference and the interval borders to the original scale, we see that y/x must be outside of $[\exp\{-1.96 \times \sqrt{2} \times \sigma\}; \exp\{+1.96 \times \sqrt{2} \times \sigma\}]$ to be significant. Because

$$\left(\frac{y}{x} - 1\right) \times 100 = \frac{y - x}{x} \times 100,$$

the RCVs for the relative difference in percentages can be easily derived from the above borders. Note that these RCVs are nonsymmetric—they differ for increases or decreases—and that implausible decreases of 100% or more are not possible, an obvious advantage over the standard calculation.

Turning this approach to practice requires an estimate $\hat{\sigma}$ of the parameter σ. A simple way can be based on aggregate data. Because the lognormal CV depends only on σ (see the scheme), this yields $\sigma = \sqrt{\log(CV^2 + 1)}$. Thus, $\hat{\sigma}$ can be obtained by inserting the untransformed population CV in this expression, and the derivation of RCVs can proceed as delineated above. The results are compiled in Table 1. We note that an estimate of σ can also be based on the single serial measurements given in the original study *(1)*, which lead to very similar RCVs. A statistical article discussing several options for RCVs in skewed distributions is in preparation.

Table 1. Comparison of RCVs calculated according to the lognormal and standard approaches.[a]

	Change, %					
	Within-day		Day-to-day		Week-to-week	
RCV method	Up	Down	Up	Down	Up	Down
BNP						
Lognormal approach	39	−28	109	−52	198	−66
Standard approach	±32		±74		±113	
NT-proBNP						
Lognormal approach	29	−22	73	−42	157	−61
Standard approach	±25		±55		±98	

[a] Data are based on median values from the data of Bruins et al. *(1)*, using a bidirectional probability of 95% ($z = 1.96$) or unidirectional probability of 97.5%.

The lognormal RCVs possess better biological plausibility. Paradoxical values of decreases greater than 100% are eliminated. However, in view of monitoring applications, these RCVs are still rather high. The corrected lognormal RCVs refer to the commonly used 5% bidirectional statistical error. This RCV setup implies that ~5% of clinically stable patients show changes greater than the RCV (false positives). However, for the treatment of heart failure, false negatives present the major risk, and it is imperative that deterioration in a patient's clinical condition is not missed so that appropriate clinical intervention can be initiated *(9)*. The common probability "insurance" of 95% against false positives could be too high, and another value, 80%, would be clinically more appropriate to lower the false-negative rate. When 80% is used in the construction of RCVs, then NT-proBNP week-to-week lognormal RCVs narrow to 85% for increases and to −46% for decreases.

The important message we take from this analysis is that the skewness of the distribution requires adequate methods to deal with it to achieve clinically and biologically valid RCVs.

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Equimolar Ammonia Interference in Potassium Measurement on the Osmetech OPTI Critical Care Analyzer

To the Editor:

Ammonia is a toxic byproduct of amino acid metabolism, and increased blood concentrations of ammonia are associated with severe encephalopathy *(1)*. In mammals, ammonia is detoxified in the liver via formation of urea *(2)*. Hyperammonemia can result from hepatic failure, enzymatic deficiencies of the urea cycle or defects in ornithine transport [e.g., HHH syndrome (hyperornithinemia, hyperammonemia, hyperhomocitrullinuria)], or it may be secondary to other organic acidopathies *(3)*. The hyperammonemia observed in methylmalonic acidemia is thought to arise because accumulated propionyl CoA interferes with formation of *N*-acetylglutamate, an obligatory activator of carbamyl phosphate synthase, the initial step in urea synthesis *(4)*.

The Osmetech (Roswell, GA) OPTI Critical Care Analyzer (CCA) is a point-of-care instrument used to monitor electrolytes and blood gases; at our institution it is used to monitor critically ill patients during transport from outside facilities. The unique potassium (K^+) sensor on this system consists of a macrocyclic ion-selective cryptand covalently coupled to an *o*-alkoxyaniline fluorophore. In the presence of K^+, internal fluorescence quenching is reduced, and fluorescence emission is proportional to the K^+ concentration in the specimen. The sensor displays negligible interference from pH, calcium, or sodium *(5)*.

During the recent transport to our hospital of an infant with methylmalonic acidemia (mut^0 subtype) and plasma ammonia >3000 μmol/L, apparent K^+ concentrations were increased (>8 mmol/L) when measured by the OPTI CCA but were within reference values when measured in plasma by both direct and indirect ion-specific electrodes. We hypothesized that the increased K^+ measurement observed on the OPTI CCA was the result of ammonia interference.

We obtained a plasma pool (endogenous ammonia = 150 μmol/L) and supplemented it with increasing concentrations of NH_4Cl and LiCl. Subsequent K^+ measurements were performed on the OPTI CCA and on the following whole-blood direct ion-selective electrode platforms: ABL 735 (Radiometer), GEM Premier (Instrumentation Laboratories), and i-STAT (Abbott Point of Care). Potassium measurements were also performed with the Vitros 250 (Ortho Clinical Diagnostics). In the presence of increasing concentrations of ammonium chloride, we observed an equimolar increase in apparent K^+ when measured on the OPTI CCA (Fig. 1). Ammonium chloride up to 5000 μmol/L had no effect on K^+ measured with the Vitros 250, ABL 735, GEM, or i-STAT. LiCl had no impact on K^+ measured with the