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Advantages of the lognormal approach to determining reference change values for N-terminal propeptide B-type natriuretic peptide

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ABSTRACT

Background: Serial measurement of NT-proBNP is performed routinely in the monitoring and assessment of the effectiveness of therapy in patients being treated for chronic heart failure (CHF). Intra-individual changes in NT-proBNP levels over time are compared typically to a reference change value (RCV) determined using either a standard [i.e., nested analysis of variance (nANOVA)] or a lognormal approach. The RCV defines the minimum percent change in serial analyte values that exceeds the percent change expected due to biological variation alone. Currently, there is no consensus on which approach (nANOVA or lognormal) to determining RCV is better.
Aims: Based on these considerations, we aimed to illustrate the impact of data transformation on the calculation of the biological variation of NT-proBNP and discuss the utility of logarithmic transformation in monitoring patients with heart failure.
Methods: 15 healthy subjects were enrolled after informed consent; 5 blood specimens were collected twice a week. Nested ANOVA from replicate analyses was applied to obtain components of biological variation, on the raw data and after data transformation.
Results: NT-proBNP distribution being highly skewed required data transformation. Natural log transformation yielded normalization. An example demonstrates that for untransformed values the RCV was overestimated for low concentrations of NT-proBNP and underestimated for higher concentrations.
Conclusions: Log-transformed data are often used in the establishment of reference intervals for evaluating laboratory tests results in clinical practice, especially when the reference interval data are not Gaussian distributed. As log-normal approach is the best approach to determining RCV values we encourage its use assessing the clinical utility of NT-proBNP serial testing. We propose that the log-normal approach becomes the standard approach to determining RCV and replaces the use of nANOVA.

1. Background

Several clinical trials have demonstrated that modifications throughout a follow-up greater than 30% of BNP/NT-proBNP concentrations have a relevant prognostic value [1–4]. The large biological variability of B-type natriuretic peptides, may limit their usefulness in serial measurements in individual patients, and one study reports that only large reductions of concentration of natriuretic peptides are associated with improved outcome in New York Heart Association class III–IV outpatients [5]. However, despite the large biological variation of these biomarkers, early studies showed that N-BNP-guided treatment of heart failure reduced total cardiovascular events, and delayed time to first event compared with intensive clinically guided treatment [6]. Furthermore, several meta-analyses showed better outcomes of the biomarkers-guided therapy in chronic heart failure in respect to the standard clinical approaches [7,8], especially in patients younger than 75 years [9,10]. Indeed, the results of a prospective randomized study, recently published, showed that compared to multidisciplinary care alone, the addition of NT-proBNP-guided intensive patient management improves clinical outcome in patients following hospitalization due to heart failure.

Intra-individual changes in NT-proBNP levels over time are compared typically to a reference change value [RCV; formerly, critical difference (CD)] determined using either a standard [i.e., nested analysis of variance (nANOVA)] or a lognormal approach. The RCV defines

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the minimum percent change in serial analyte values that exceeds the percent change expected due to biological variation alone [11,12]. Currently, there is no consensus on which approach (nANOVA or lognormal) to determining RCV is better. Data on biological variation of NT-proBNP have been published with markedly differing results (reviewed in [13]), mainly related to the use of data transformation, particularly in patients with chronic heart failure [14,15]. As component of variation is computed based on nANOVA, the distributional assumptions underlying the use of this method should be satisfied, which is not the case for skewed distributions, as NT-proBNP's. This aspect has been underlined in the literature not only for NTproBNP, but also for other biomarkers in oncologic research [16,17]. Particularly, Fokkema et al. [18] showed that the deviation of the mean from the median of week to week measurements was much larger (by a factor of 20) before log-transformation of NT-proBNP concentrations. Moreover they underlined that leaving untransformed data could yield implausible 0 or negative values for downward changes of the metabolite if the RCV was 100% or more. Also, in the lognormal case RCVs are nonsymmetric, with clinical implications when assessing downward and upward changes, and obtaining 0 or negative concentrations is not possible any more.

2. Methods

The design of the study and the analytical procedures were described in details elsewhere.

Briefly, five blood specimens were collected from each of 16 apparently healthy laboratory workers (5 M, 11 F) twice a week (Tuesdays and Fridays) over a 17-day period. Blood was collected under standardized conditions to minimize sources of preanalytic variation. Sera were separated and stored at −70 °C until analysis.

Nested analysis of variance from replicate analyses was applied to obtain components of biological variation [11,12]: the mean analytical variation (CV_A), the intraindividual variation (CV_I), the interindividual variation (CV_G), and the index of individuality (CV_I/CV_G) for assessing utility of conventional population based reference intervals. Conventionally, if the index of individuality is <0.6 population-based reference values are of limited value for detecting unusual results for a particular individual; the opposite applies if >1.4. The RCV, the minimal significant difference ($p<0.05$) for 2 consecutive measurements and, finally, the number of specimen required to estimate the homeostatic setpoint of an individual (with ±10%, with a confidence of 95%) were calculated. Also approximated values for the components of variation for the analyte in its original scale were computed from the standard deviation of its ln-transformed value (which is itself a CV). It is to underline that this approximation (based on Tchebycheff inequality) holds for small values of CV, say between 30 and 40%. Finally the calculation of the up and down RCVs in the lognormal approach was computed according to Fokkema [18].

Distributional assumptions for applying F test were assessed graphically by histograms and rankit plots [19], and by the Shapiro–Wilk test on the observations as a whole and for each individual. A modified D'Agostino test for skewness was also performed [20] to determine the source of non-normality. Homogeneity of variances between subjects was assessed by means of Barlett test.

Stata 11 (StataCorp, College Station, TX, USA) was used for computation.

3. Results

Normality assumption was violated for the whole data both graphically (Fig. 1) and at significance test ($p<0.001$) with marked right skewness ($p<0.001$). The assumption was questionable in half of the individuals graphically and in 5 at significance test ($p<0.015$). Moreover, variance dishomogeneity was present ($p<0.001$). Therefore normalizing transformations were advisable before assessing biological variability. A series of usual transformations was used: natural logarithmic, Box–Cox with zero skewness, inverse and square root (Table 1). Natural logarithm (ln) and Box–Cox functions only produced normality (Fig. 1). The analytical, intraindividual and interindividual components of variation were all lower in the case of normalizing transformations. The index of individuality was close to 0.6 for transformed variables only. Two consecutive measures must change by 16 to 34% before significance can be claimed when a normalizing transformation is applied, and by 62 to 159% for the raw data.

Finally the number of specimen to be collected to estimate the homeostatic set point of an individual was comparably lower in the case of normalizing transformations.

The consequences of using or not a normalizing transformation are illustrated in the numerical example of Table 2. We report hypothetical values of two consecutive measures. The first measurement ranges from 10 to 500. The follow-up measurement was calculated so to be significantly different from the first one, based on the RCV of 160% (from the standard approach) and 26% (from the lognormal approach). Concentrations are reported for both an upward (up) and a downward (down) change of the metabolite. In the lognormal approach, these changes are nonsymmetric; for illustration, up and down concentrations according to the mean RCV are also reported. As shown in the table, changes computed with the standard approach are larger than those computed with the lognormal approach for low concentrations of NT-proBNP and lower for high concentrations of NT-proBNP. Moreover, the down RCV yields unplausible negative concentrations.

4. Discussion

These results show that the biological variation and the RCV are greatly affected by the distribution of the original data. Ignoring the data distribution results in distorted estimates, with overestimation of the RCV for lower concentrations and underestimation for higher concentration of the metabolite.

A review by Clerico [21] discusses on how the RCV reported in the literature (calculated on untransformed values) might be excessive in regard to the clinical relevance for monitoring changes over time. Similar considerations are presented by Fokkema [18] who suggest to adopt a 80% "insurance level" against false positives when computing RCVs, in replacement of the traditional 95% level. Larger values will hamper the potential clinical usefulness of the marker, while low indices of individuality are important in a monitoring situation were small changes from the set point of the individual need to be detected in serial measurements. Similarly, the usefulness for monitoring will increase for smaller critical differences. The transformed variables achieved these two goals in our study. As shown by the numerical example, ignoring the skewed distribution of the data results in higher uncertainty when judging the relevance of the difference between 2 consecutive measurements.

Our results for the index of individuality on untransformed data are in keeping with Wu and Smith [22] who report an index of individuality of 0.92; however, RCV in our series is much higher than the reported 92%. Conversely, normalizing transformations yield much smaller estimates on the transformed scale (for ln transformation: 0.64 and +30 and −23% for the index of individuality and the up and down RCV, respectively). These findings support the previous work of Fokkema [18] who reported up and down RCVs of 29 and −22%, while the mean RCV of 26% found here is comparable to that obtained by Bruins [14] in a modified standard approach, based on median values of CV_T and CV_I to overcome the skewness of data. The values of these indexes are of particular relevance for concluding on the diagnostic or prognostic ability of NT-proBNP and on its use for patient monitoring over time, as underlying also by O'Hanlon [23] in a different population of stable heart failure patients. These results may be extended to other settings, where the lognormal approach was

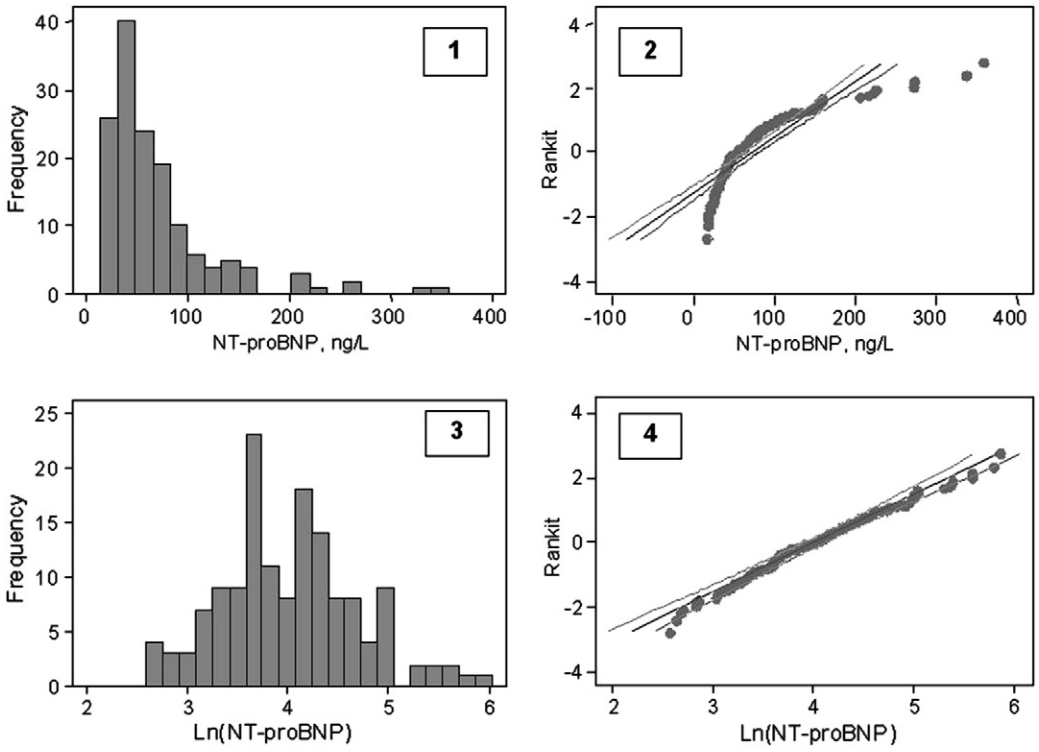

Fig. 1. Graphical assessment of the departure from normality. Panels 1 and 3 report the distribution of NT-proBNP (ng/L) and of ln(NT-proBNP), respectively, over all observations (146 measures in 15 subjects). Panels 2 and 4 show the corresponding rankit plots, including 95% confidence intervals. Both the histogram and the rankit plot illustrate the presence of skewness of the untransformed NT-proBNP and its disappearance after ln transformation.

recognized to make a better use of data distribution when assessing biological variation. This was shown by Tuxen et al. [16] for cancer antigen 125 (CA 125), carcinoembryonic antigen (CEA), and tissue polypeptide antigen (TPA) in the monitoring of ovarian cancer patients or by van Eyben et al. [17] for serum lactate dehydrogenase isoenzyme1 (S-LD-1) in the monitoring of patients with testicular germ cell tumors.

5. Limits of the study

Physicians are used to express results of laboratory tests using conventional or SI units and rely on biological variability computed on this scale and log-transformation might appear counter-intuitive. However, when assessing biological variation this practice may be potentially misleading if data are not Gaussian distributed. The RCV will generally be overestimated for lower concentrations of the metabolite and underestimated for higher concentrations, as in the NT-proBNP case shown here (but also for any metabolite with nonsymmetric distribution) and misinterpretation of the clinical relevance of changes in NT-proBNP in patients with chronic heart failure or other cardiologic conditions might ensue. The lognormal approach will provide estimates of the RCV more consistent with the distribution of the data, and thus we encourage its use. In addition, the back-transformation to the original scale will allow for nonsymmetrical values of the up/down RCVs, with larger changes needed to claim clinical relevance for an increase in NT-proBNP and lower changes for a decrease. Nonsymmetrical values will also prevent the calculation of implausible (negative) values.

Assessing the RCV is not sufficient for claiming clinical relevance. The RCV defines the minimum percent change in serial analyte values that exceeds the percent change expected due to biological variation alone. Clinician, when making decision regarding clinical significance

Table 1
Estimates of biological variation for original and transformed data.

Transformation	Normalizing	Mean (SD)	Min–max	CV_A%	CV_I%	CV_G%	Index of individuality	Desired analytical imprecision	Reference change value (RCV)%	Number of specimens for homeostatic point ±10%
Original scale[a]	–	70.99 (58.5)	13.5–356.0	5.8	57.2	61.1	0.94	28.6	159.38	127.18
Log_n[b]	Yes	4.02 (0.67)	2.60–5.87	2.7	9.1	14.2	0.64	4.6	26.33 (−23; +30)[c]	3.47
Box–Cox with zero skewness[d]	Yes	2.64 (0.27)	1.98–3.29	2.0	5.4	8.8	0.62	2.7	16.05	1.29
Inverse[e]	No	0.02 (0.01)	0.00–0.07	24.5	36.4	56.9	0.64	18.2	121.53	73.95
Square root[f]	No	7.92 (2.90)	3.67–18.87	3.6	22.3	29.7	0.65	11.2	62.59	19.61

Notes to Table 1.
[a] Approximated values (based on Tchebycheff inequality) for CVA = 10.9, CV_I = 36.6, CV_G = 57.1, Index of Individuality = 0.64 and critical difference = 105.58.
[b] ln(NTproBNP).
[c] RCV mean, RCV for downward change and RCV upward change are reported.
[d] $(NTproBNP^{(-0.22-1)})/-0.22$.
[e] 1/NTproBNP.
[f] $NTproBNP^{0.5}$.

Table 2
Numerical example to illustrate the minimal change in NT-proBNP needed to claim that the difference is above the value expected due to biological variation alone. Hypothetical measured baseline values for NT-proBNP are reported in column 2; the value to be reached to claim a difference above the expected one, based on biological variation is reported in the next columns: Column 3 makes use of the standard approach for computing RCV (untransformed data). Column 4 makes use of the lognormal approach, ignoring the asymmetric boundaries. Columns 5 and 6 make use of the lognormal approach and apply the asymmetric boundaries for down and up changes in NT-proBNP.

	Baseline measure	Standard approach	Lognormal approach	Lognormal approach	Lognormal approach
[1]	[2]	[3]	[4]	[5]	[6]
Patient no.	NT-proBNP, ng/l	NT-proBNP, ng/l [up and down: RCV ±160%]	NT-proBNP, ng/l [up and down: mean RCV ±26%]	NT-proBNP, ng/l [down: RCV −23%]	NT-proBNP, ng/l [up: RCV +30%]
1	10	−6 and 26	5 and 18	6	20
2	25	−15 and 65	11 and 58	12	66
3	50	−30 and 130	18 and 138	20	162
4	75	−45 and 195	24 and 240	28	274
5	100	−60 and 260	30 and 331	35	398
6	150	−90 and 390	41 and 552	47	674
7	500	−300 and 1300	99 and 2516	120	3226

of a serial change of a parameter, will consider whether the change is greater than what expected on not only the basis of the biological variation but also a variety of other patient-specific factors.

Moreover, we used the mean of the within subject biological variation in apparently healthy people to calculate RCV but this approach has a theoretical disadvantage. In fact in certain pathologies CV_I is higher than in healthy states for certain analytes. In consequence, the real RCV in these patients would be higher than in the healthy. However, a compilation of known data on CV_I in diseases [24] and comparison with relevant data in the healthy suggests that, in general, CV_I are indeed similar in health and chronic stable disease. In conclusion, for most quantities, data on health can be used appropriately in monitoring of disease [25].

6. Conclusions

Based on the observed results along with similar findings reported in the literature, we suggest that the lognormal approach become the standard for determining RCV and replace the use of nested ANOVA on untransformed data, when distributions are nonsymmetric, as is the case for NT-proBNP. This choice has clinical implications when assessing the clinical relevance of a change in the metabolite against its background biological variation.

Conflict of interest

None.

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