Long-term clinical variation of NT-proBNP in stable chronic heart failure patients

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Aims Here, the aim is to assess long-term clinical variation (CV) of N-terminal pro-brain natriuretic peptide (NT-proBNP) in stable chronic heart failure (CHF) patients. The proposed use of NT-proBNP for monitoring of CHF patients will require accurate information about long-term CV of the peptide.

Methods and results Medication, biochemical variables, and NYHA class were recorded at 1-year and 2-year follow-up in patients treated in our heart failure clinic. Only patients without changes in medication and the NYHA class who were not hospitalized or died in the period from first follow-up to 12 months after the second follow-up were included. A total of 78 patients fulfilled the criteria, and year-to-year CV was calculated to 30% (median) (range: 0–111%) (% changes range: 287 to 397%). Log transformation of NT-proBNP (skewed to the right) reduced the year-to-year CV to 4.7% (range: 0–22%) (% changes range: 18 to 38%).

Conclusion Long-term CV of plasma concentrations of NT-proBNP in stable CHF patients is 30%, but the variation is substantial. Therefore, high long-term CV of NT-proBNP does not necessarily carry prognostic significance within the subsequent 12 months. Plasma concentrations of NT-proBNP followed a log-normal distribution, and the low CV of log(NT-proBNP) indicate that NT-proBNP levels are constant during stable conditions.

KEYWORDS
Chronic heart failure; Natriuretic peptides; Long-term variation

Introduction
N-terminal pro-brain natriuretic peptide (NT-proBNP) is a promising tool for the diagnosis and prognostication of heart failure patients. It can rule out left ventricular dysfunction in untreated outpatients1 and cardiac causes in patients presenting with acute dyspnoea.2 NT-proBNP carries excellent prognostic information in chronic heart failure (CHF) patients,3 and one rather small study has shown that treatment of CHF patients may be guided by NT-proBNP, which is an intriguing perspective.4

Since the discovery of the peptide in plasma by Hunt et al.5 in 1995, a substantial amount of research has elucidated the usefulness of the peptide for diagnosing heart failure, risk stratification, and monitoring, but only little is known about variation of plasma concentrations over time.6–8 However, such information is crucial if the peptide is to be implemented for monitoring purposes. We suggest that unexplained (biological) and total variation \[=(\text{biological variation}^2 + \text{analytical variation}^2)^{1/2}\] of NT-proBNP should be defined as noise around a homeostatic set point during strict steady-state criteria where acute physiological stimuli for changes are absent.9 Variation in clinically stable patients, where acute physiological stimuli may be present (e.g. changes in sodium intake, changes in exercise habits, angina pectoris, paroxysms of AF, and changes in posture), should be denoted clinical variation (CV). The CV may exceed the total variation.

Bruins et al.6 have shown that within-day, day-to-day, and week-to-week unexplained and total variation (CV according to our suggested definition) of NT-proBNP increases with the interval between peptide measurements and the CV may therefore be time-dependent.10 However long-term, for example, month-to-month or year-to-year, CVs for NT-proBNP are unknown. Furthermore, too short intervals between blood samplings (days, weeks, or a month) may result in autocorrelation of data,11 as only little is known about long-term correlations in biological systems.10–12

The aim of the present study is therefore to assess the long-term (year-to-year) CV of NT-proBNP in stable CHF patients.

Methods
Patients were identified in our heart failure clinic database.13 Regular telephone calls by a heart failure nurse is part of the
routine follow-up for the stable, medically optimized CHF patients who have been discharged from the heart failure clinic and back to the general practitioner. A total of 339 patients (n = 339) were registered in the database as being on optimal medical therapy and educated in heart failure self-care, and these patients had received at least one follow-up telephone call. One-hundred and eighty (n = 180) patients were registered to have received a telephone call at both 1 and 2 years of follow-up. The fact that only 180 patients had received two telephone calls is explained by intercurrent death or <1-year follow-up after discharge from the clinic to the general practitioner. Of these, 88 patients (n = 88) had accepted measurement of NT-proBNP on both occasions and 92 patients had refused to visit the clinic for NT-proBNP measurement. One of the 88 patients had changed heart failure medication, two had been hospitalized and two had changed the NYHA class between 1- and 2-year follow-up, two had died, and three were hospitalized within 12 months after the 2 years of study period. Seventy-eight (n = 78) patients were therefore included in the current study.

Protocol

To determine the CV of NT-proBNP, we defined the following inclusion criteria: unchanged NYHA class, unchanged heart failure medication (ACE-inhibitors/All-blockers, beta-blockers, spironolactone, digoxin, diuretics, and amiodarone), and no hospitalizations between 1 and 2 years of follow-up and no death or hospitalization 12 months after the second year of follow-up. All other patients were excluded. Hence, the effects of changes in heart failure medication on plasma concentrations of NT-proBNP were avoided.14-19 and changes in plasma concentrations of NT-proBNP could be considered without prognostic significance within the subsequent 12 months.

One specialized heart-failure nurse conducted the telephone interview of the patients (NYHA class, weight, medication), and all patients were referred to the hospital laboratory, where basic biochemical variables (haemoglobin, creatinine, potassium, sodium, TSH, lipids, and plasma-glucose) and NT-proBNP were analysed. Analysis of NT-proBNP was approved by the Ethical Committee of Copenhagen (KF 01–019699), and informed consent was obtained according to the Helsinki Declaration, at a previous visit in the heart failure clinic.

After a minimum 8 h of overnight fast and 15 min of rest, venous blood was drawn into heparin tubes. Blood samples were centrifuged within 30 min after sampling at 3000 r.p.m. for 10 min. NT-proBNP was analysed on the same day using the Elecsys 2010 platform20 by a double-antibody sandwich method (radioimmunoassay) with intra- and inter-assay variation coefficients <3%. The analytical range is 5–35 000 pg/mL.

Information about deaths and hospitalizations was obtained from a computerized search using Copenhagen Hospital Corporation’s administrative data system. All deaths are registered immediately in the system.

Statistics and calculation algorithms

Data are presented as median, mean, and range. Per cent-changes are calculated as (Level2-year − Level1-year)/Level1-year *100%. CV is calculated as the year-to-year variation coefficient; SD/mean and variances as (Σ(x_i−x̄)^2)/(n−1), where x_i are the observed values, x̄ are the mean values, and n is the number of observations. All estimates were calculated from NT-proBNP expressed in pg/mL and after log transformation of each observation [log(NT-proBNP)]. As the distribution of NT-proBNP was skewed to the right, parametric (paired t-tests) and non-parametric (Wilcoxon signed rank test) (NT-proBNP, TSH) tests were used to evaluate values at 1 vs. 2 years of follow-up. The Mann–Whitney U-test (non-parametric) was used to compare CV between patients with sinus rhythm and atrial fibrillation (AF). Linear regression analyses were used when appropriate, and standard model control was performed. The Kolmogorov–Smirnov test, probability plots, and histograms were used to assess Gaussian distribution. A P-value <0.05 (two-sided) was considered significant. Analyses were made using Statistical Analysis Software (SAS 9.1, Cary, NC, USA).

Estimated GFR (eGFR) was calculated by the formula of Cockroft and Gault:21 (140 – age) × weight in kilograms/serum concentrations in creatinine measured in μmol/L x a constant (males: 1.25; females: 1.03).

Results

Patient characteristics (n = 78)

Demographic data are presented in Table 1. Visual evaluation of data and Kolmogorov–Smirnov test (P = 0.001) indicated that plasma concentrations of NT-proBNP were skewed to the right, and log transformation was therefore performed. Log(NT-proBNP) was normally distributed, with a tendency towards over-transformation (data not shown), but a probability plot (data not shown) and the Kolmogorov–Smirnov test (P = 0.554) supported normal distribution. One patient accounted for the large range of creatinine (Table 2), and these data were normally distributed.

To further describe the total cohort (n = 339), information on vital status and hospitalization was obtained for all patients. Median follow-up time was 867 days (range: 214–2477 days). During this time, 118 patients died (35%) and 111 patients were hospitalized (33%).

Year-to-year CVs were calculated for all biochemical variables, eGFR, and weight and are presented in Table 2 and Figures 1B and 2B. None of the variables changed significantly except cholesterol (P = 0.01). HDL and LDL did not change significantly, and dosages of statins only changed in two subjects (withdrawal). The variation in cholesterol

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Figures are median and [range] or number and (%). NYHA, New York Heart Association functional class; LVEF, left ventricular ejection fraction.
may therefore merely be significant by chance (mass significance).

Per cent-changes were calculated for all variables (median) (range): haemoglobin, 0% (–23 to 40%); creatinine, –4% (–57 to 105%); Na⁺, 0% (–4 to 10%); K⁺, 0% (–34 to 40%); cholesterol, –3% (–42 to 64%); triglyceride, –7% (–64 to 142%); HDL, 0% (–36 to 111%); LDL, –5% (–61 to 125%); plasma glucose, 2% (–31 to 37%); TSH, 0% (–51 to 350%); weight, 0% (–41 to 60%); NT-proBNP, 0% (–87 to 397%) and log(NT-proBNP), 0% (–18 to 38%); eGFR, –3% (–10 to 134%).

Simple linear regression variation indicated that CV of plasma NT-proBNP concentrations expressed in pg/mL depended on NT-proBNP levels (Model 1) (β = 0.008; 95% CI, 0.004–0.012; intercept = 23; 95% CI, 16–31; P < 0.001; adjusted $R^2 = 0.182$) (Figure 1B), but were independent of log(NT-proBNP) levels after log transformation (Model 2) (β = 0.44; 95% CI, 0.25–1.36; P = 0.2449; adjusted $R^2 = 0.050$) (Figure 2B). Therefore, the consequence of log transformation of a single measurement of NT-proBNP is that the distribution of data becomes Gaussian and more suitable for statistical analyses. Log transformation of serial measurements has further consequences because on the log scale, CV of NT-proBNP is reduced and independent of the NT-proBNP levels.

There was no association between CV of eGFR and CV of NT-proBNP ($R^2 = −0.57$; 95% CI, −39 to 27; $P = 0.73$; adjusted $R^2 = −0.0125$). Patients in AF did not vary more than patients in sinus rhythm [27 (range: 2–97%) vs. 32% (range: 0–111%)] (P = 0.18), and adjustment for AF in Model 1 reduced adjusted $R^2$ (goodness-of-fit), indicating that AF could not explain the relationship between NT-proBNP and CV of NT-proBNP ($R^2 = 0.004$; 95% CI, 0.001–0.008; $β_{AF} = −4.40$; 95% CI, −17 to 9; $P = 0.73$; adjusted $R^2 = 0.0542$).

Discussion

The two main findings of the current study are that (i) the CV of NT-proBNP measured in pg/mL in stable CHF patients is actually stable (5%) in CHF patients without major clinical events during the follow-up period.

To address the issue of CV of plasma concentrations of NT-proBNP, it is necessary to calculate the variation of the peptide both expressed in pg/mL (=CV(NT-proBNP)) and the CV of NT-proBNP after log transformation (=CV(log(NT-proBNP))). It is relevant to calculate CV of NT-proBNP because this is the result reported from the laboratory to the physician in clinical practice, but from a statistical point of view, it is correct to calculate CV(log(NT-proBNP)) because NT-proBNP data follow a distribution skewed to the right.

Year-to-year CV of NT-proBNP amounts to 30% (median) (range of CV(NT-proBNP): 0–111%) in stable CHF patients. CV is defined as the year-to-year variation coefficient (=SD/mean), but this value is difficult to implement in clinical practice. Hence, we also calculated year-to-year % changes (=Level2-year − Level1-year)/Level1-year*100% that would be used in clinical practice. Our data, therefore, suggest that a risk of over-interpretation of peptide changes exists (% changes range without prognostic significance within 12 months: −87 to 397%). Year-to-year CV(log(NT-proBNP)) was only 4.7% (range: 0–22%), demonstrating that NT-proBNP is a stable peptide during stable conditions if it is considered as a logarithmic variable. Year-to-year % changes were also low (range: −18 to 38%). However, monitoring using differences in log(NT-proBNP) may be difficult since the mathematics involved could be time-consuming in clinical practice. Test results given in log(NT-proBNP) instead of pg/mL from the laboratory would simplify monitoring in this way. Small differences on the log scale may, however, reflect biological differences. Therefore, using differences in log(NT-proBNP) for monitoring purposes may carry a risk that real biological changes are overlooked.

Bruins et al. reported within-day, day-to-day, and week-to-week CV of NT-proBNP to be 9, 20, and 35%, respectively. Taking their results and ours together, it could be argued that CV of NT-proBNP may be time-dependent, but the peak of the variation is reached already within a week. It is not known why variation in some biological systems is time-dependent. Possible explanation for the observed year-to-year CV include: (i) increased pre-analytical variation (time, phlebotomist, and posture); (ii) increased time interval between...
measurements; 10–12 (iii) acute physiological stimuli [paroxysms of AF, 23 angina pectoris, 24 changes in sodium intake, 25 recently cured infections, 26 changes in exercise habits, 27 and changes in diurnal rhythm (imbalance in hormonal systems)]. CV of renal function or the presence of AF could not explain the high CV of NT-proBNP in the current study (see results).

The NYHA class did not change, and weight, creatinine, haemoglobin, sodium, and potassium did not vary to the same degree as plasma concentrations of NT-proBNP (Table 2). The patients were not hospitalized nor did they die within 12 month after the second year of follow-up. Therefore, our data indicate that long-term % changes calculated from plasma concentrations of NT-proBNP...
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(%, changes range: −87 to 397%) should be interpreted with caution if traditional biochemical variables, weight, and the NYHA class are stable. From Table 2, it should also be noted that long-term CV of NT-proBNP is much larger than long-term CV of traditional biochemical variables used to monitor patients with chronic diseases including heart failure. However, the year-to-year CV of NT-proBNP is lower than the reported day-to-day variation of microalbuminuria (38–50%).28,29 an implemented diagnostic test and risk marker in endocrinology. Similar to NT-proBNP, microalbuminuria data follow a lognormal distribution. Therefore, high CV does not necessarily hamper the clinical usefulness of a risk marker.

The high year-to-year CV may raise concern for the application of a single measurement of NT-proBNP as a risk marker. However, based on these and other data, it is possible to explain why a single measurement of NT-proBNP carries prognostic information despite the substantial short- and long-term (the present data) CVs. This apparent contradiction seems to be explained by the facts that the distribution skewed to the right is normalized by log transformation and that the high CV of NT-proBNP depending on NT-proBNP levels becomes normally distributed, with a low CV independent of NT-proBNP levels after log transformation. The studies showing that NT-proBNP can predict outcome have relied on using either log-transformed peptide values or have divided patients into groups depending on median, tertiles,30 or quartiles31 of peptide levels. Therefore, if the physician uses medians, tertiles, or quartiles when NT-proBNP is expressed in pg/mL (high CV) and exact values when NT-proBNP is expressed as log(NT-proBNP) (low CV), he or she takes account for the CV, and a single measurement of NT-proBNP can be used safely for risk stratification.

**Limitations**

Follow-up information was obtained by a specialized heart failure nurse through telephone calls, but patients did not undergo a physical examination at follow-up. Hence, minor signs and symptoms may have been missed, and potentially changes in NT-proBNP may, in theory, in some cases have reflected true clinical deterioration or improvement. However, none of the patients changed the NYHA class between the telephone calls, nor did they die or were hospitalized within 12 months after the second year of follow-up, indicating that the patients were in fact stable. Part of the year-to-year CV of NT-proBNP may be explained by ageing.32 Ultimately, more than two observations should be collected for each patient. The number of patients studied does, however, minimize this problem. Collectively, the heart failure patients from our clinic should be denoted a ‘dynamic cohort’, since each year new patients are registered and old patients are lost due to mortality. It is difficult to predict whether this will result in selection bias concerning CV of NT-proBNP in defined stable patients. However, newly registered patients will not likely be more or less stable than the already registered patients were at the time when they entered the cohort, and this would argue against the occurrence of selection bias. Only 88 of 180 patients discharged from the heart failure clinic agreed to go to the laboratory for the measurement of NT-proBNP at both the first and second years, which may result in selection bias. However, it should be noted that 57 of the 92 patients who did not supply both NT-proBNP samples were, in fact, hospitalized or had died within 1 year after the second year of follow-up and four had changed the NYHA class, and as such they would have not met inclusion criteria for this study anyway. Hence, a total of 31 additional patients could have been included in the study. We cannot entirely rule out that inclusion of these patients could have affected our results. Analytical variation (inter- and intra-assay variations) of NT-proBNP is <3%.20 It may, however, be argued that batch-to-batch variation can explain parts of the observed CV. However, if batch-to-batch variation accounted for the variation, the observed median of the % changes would not likely have been zero and the level at years 1 and 2 would be expected to be different because batch-to-batch variation would have changed the level. Therefore, batch-to-batch variation does not appear to explain the findings of the current study.

**Perspectives**

Our data indicate that long-term % changes in NT-proBNP calculated from absolute values must be interpreted with caution if the NYHA class, weight, haemoglobin, creatinine, sodium, and potassium are stable. Whether such variation in peptide levels will hamper NT-proBNP monitoring of CHF failure patients will be determined from ongoing studies.33 If ongoing trials fail to show a positive effect of NT-proBNP-monitoring after % changes calculated from absolute values, monitoring after log(NT-proBNP) is an intriguing alternative because part of the long-term CV of NT-proBNP seems to be explained by the right skewed distribution of the peptide in the cohort. Finally, a single measurement of NT-proBNP can be used safely as risk marker because NT-proBNP levels are constant during stable conditions, but the physicians must familiarize with the distribution skewed to the right and the high long-term CV of NT-proBNP expressed in pg/mL.

**Conclusions**

Long-term CV of plasma concentrations of NT-proBNP amounts to ~30% (range: 0–111%) in stable CHF patients. Our data demonstrate that CV of NT-proBNP can be high without necessarily carrying any prognostic significance within a 12-month period. However, the distribution of plasma concentrations of NT-proBNP is skewed to the right, and the low CV of log(NT-proBNP) indicates that NT-proBNP levels are fairly constant during stable conditions in CHF patients if NT-proBNP is considered as a logarithmic variable.

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