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# Short- and long-term individual variation in NT-proBNP levels in patients with stable coronary artery disease

Anna M. Nordenskjöld [a,*], Håkan Ahlström [b], Kai M. Eggers [c], Ole Fröbert [a], Per Venge [d], Bertil Lindahl [c]

[a] Department of Cardiology, Örebro University Hospital, Sweden
[b] Department of Radiology, Uppsala University, Sweden
[c] Department of Medical Sciences, Cardiology, Uppsala University, Sweden
[d] Department of Medical Sciences, Clinical Chemistry, Uppsala University, Sweden

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ABSTRACT

*Background:* In addition to diagnosis of heart failure (HF) natriuretic peptides (BNP and NT-proBNP) may be used for risk prediction in stable and acute coronary artery disease. The aim of the study was to evaluate the short- and long-term individual variation of NT-proBNP in patients with stable coronary artery disease.
*Methods:* Twenty-four patients with suspected stable coronary artery disease and scheduled for elective coronary angiography were included. Blood samples were drawn at enrolment and, on average 3 weeks later, serially the day prior to coronary angiography. NT-proBNP was determined using Elecsys proBNP sandwich immunoassay (Roche Diagnostics).
*Results:* The individual variation in NT-proBNP over 4 h was 11.8%, over 20 h 12.4% and over 3 weeks 20.4%. The corresponding positive and negative lognormal reference change values (RCV) were +41/−29%, +42/−30% and +76/−43%, respectively. No significant circadian variation was found.
*Conclusions:* Our results suggest that an increase in NT-proBNP levels of >42% or a decrease of >30% is needed to indicate a reliable short-term change; and for a long-term change an increase of >76% or a decrease of >43% is required. This should be considered when interpreting changes in NT-proBNP levels.


## 1. Introduction

The use of natriuretic peptides, B-type natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), for the diagnosis of heart failure (HF) has been evaluated in numerous studies and is recommended in recent guidelines [1]. Particularly, the use of natriuretic peptides to rule out acute or chronic HF has been thoroughly discussed [2]. Natriuretic peptides may also be used for prognostic evaluation in various cardiovascular conditions. Elevated levels of BNP or NT-proBNP are associated with higher morbidity and mortality in patients with HF [3], acute coronary syndromes (ACS) [4], chest pain [5], and stable coronary artery disease [6] and in general community-based populations [7]. Changes in NT-proBNP levels have been shown to provide prognostic importance in ACS [4] and have been evaluated for guidance of medical therapy in HF [8].

NT-proBNP is known to have a large intra-individual variation (CVi) in healthy subjects [9–12], in patients with stable HF [13–19] and in patients with hypertension [20]. However, no study has so far evaluated the individual variation of NT-proBNP in patients with stable coronary artery disease. Moreover, it is not clear whether there is any physiological circadian variation of BNP (and consequently NT-proBNP) affecting the variability in circulating levels. Knowledge on these issues is essential for the correct interpretation of obtained results and their use to guide therapy and predict outcome.

The objectives of the present study were twofold. Firstly, we evaluated the short- and long-term individual variation of NT-proBNP in patients with verified or highly suspected stable coronary artery disease. Secondly, we examined whether any circadian variation of NT-proBNP could be demonstrated.

## 2. Methods

### 2.1. Study population

The present study is a substudy of the ongoing study "Prevalence and prognostic value of Unrecognised Myocardial Injury in stable

*Abbreviations:* BNP, B-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HF, heart failure; ACS, acute coronary syndromes; CVi, intra-individual CV; PUMI, Prevalence and prognostic value of Unrecognised Myocardial Injury in stable coronary disease; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting; GFR, glomerular filtration rate; MRI, magnetic resonance imaging; ECG, electrocardiography; CV, coefficient of variation; CVa, analytical CV; CVt, total CV; RCV, reference change value; SD, standard deviation; IQR, interquartile range; CVg, inter-individual CV; II, index of individuality; CCS, Canadian Cardiovascular Society.

* Corresponding author at: Department of Cardiology, Örebro University Hospital, Södra Grev Rosengatan, 701 85 Örebro, Sweden. Tel.: +46 196021000; fax: +46 196025438.
*E-mail address:* anna.nordenskjold@orebroll.se (A.M. Nordenskjöld).

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coronary artery disease (PUMI)" (clinicaltrials.gov NTC01257282) and the study population has been described previously [21]. Patients with suspected stable coronary artery disease scheduled for coronary angiography were enrolled. Exclusion criteria were previous acute myocardial infarction (AMI), coronary angiography, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), heart failure, renal failure with an estimated glomerular filtration rate (GFR) below 30 ml/min/1.73 $m^2$, or a contraindication to magnetic resonance imaging (MRI). After study inclusion, blood-samples were drawn, and an MRI investigation of the heart was performed prior to coronary angiography.

Twenty-four patients at two study centres were enrolled in this substudy between October 2009 and April 2010. As compared to the main study, there were no changes in inclusion criteria or exclusion criteria. On average 23 (range 4–58) days passed between enrolment and admission to hospital. The patients were admitted to the hospital the day before a scheduled coronary angiography. At admission an electrocardiogram (ECG) was obtained and continuous multi-lead ST-monitoring was performed for 24 h. During this time, blood samples were collected and the blood pressure was measured at six occasions with four-hour intervals. The first blood sample was collected between 8 AM and 10 AM. The patients were nonfasting, had limited physical activity, but were not confined to bed. In the long-term study, blood samples from the time of initial enrolment and the first blood sample at the time of admission were used. In the short-term study both the with-in-day variation (six measurements with 4 h in between) and the day-to-day variation (two measurements with 20 h in between) were analysed. Fig. 1 shows the flow chart for the patients including the timing of blood sampling.

All patients provided written informed consent. The study was approved by the local ethics committee (Uppsala 2007/214) and conformed to the principles of the declaration of Helsinki.

### 2.2. Biochemical analysis

Venous blood was collected in EDTA-containing tubes and immediately centrifuged. The plasma was stored at −70 °C until analysis. NT-proBNP was analysed using the Elecsys proBNP sandwich immunoassay, using two monoclonal antibodies, on an Elecsys 2010 instrument (Roche Diagnostics, Basel, Switzerland). According to the manufacturer, the analytic range extends from 5 to 35,000 ng/L and the recommended cut-off value indicating cardiac dysfunction is 125 ng/L. The upper reference levels (97.5th percentile) in men and women aged 40 to 65 years are 184 and 268 ng/L, respectively, and 269 and 391 ng/L, for ages between 66 and 76 years, respectively [22]. The lowest concentration measurable with a coefficient of variation (CV) <10% is 30 ng/L [23].

The analyses were performed strictly according to the instructions of the manufacturer using a single lot of reagents. Samples were measured with lot no. 162 019. The within-run analytical impression ($CV_a$) was determined internally on duplicate samples and was found to be 3% both at the level of 125 ng/L and at the level of 4440 ng/L.

### 2.3. ECG

The resting 12-lead ECG and continuous multi-lead ST-segment monitoring were analysed by cardiologists blinded to the NT-proBNP results (AMN, BL). ECG changes were classified according to "The Minnesota Code Classification System for Electrocardiographic Findings" [24]. The Minnesota code criteria and the "Sokolow-Lyon voltage system" were used to diagnose left ventricular hypertrophy. Continuous multi-lead ST-segment monitoring was performed for 24 h using the Telegard 6.6 (GE Marquette Medical Systems, Milwaukee, Wisconsin, USA) or CoroNet system (Ortivus Medical AB, Täby, Sweden). An ST vector magnitude or ST change vector magnitude increase or decrease of at least 50 μV from the baseline for at least 1 min was considered indicative of ischemia [25]. A ventricular rate exceeding 120 beats per minute for 1 min or more was considered to be an episode of tachycardia.

### 2.4. Statistical analysis

We calculated the CVi from the total variance (CVt) of NT-proBNP at all time points. The CVa was determined from our internal validation of within-run CV and at NT-proBNP levels corresponding to the mean value. The NT-proBNP values showed a positively (right) skewed distribution and normality of the distributions was rejected using the Kolmogorov–Smirnov test. Therefore, in addition to the normal [26] approach, we determined the reference change value (RCV) with the lognormal approach [27]. Since logtransformed RCV is claimed to have better biological plausibility than RCV [27], both positive and negative logtransformed RCV are reported. For detailed equations please refer to Supplemental Table 1. Outliers were identified by the technique described by Horn et al. [28]. Differences in mean CVt between different subgroups were calculated with a two-way T-test for independent samples. The presence of a statistically significant circadian variation was evaluated with the Kruskal–Wallis test. All data analyses were performed using the Predictive Analytics SoftWare (PASW statistics 17.03) program (SPSS Inc, Chicago, IL, USA).

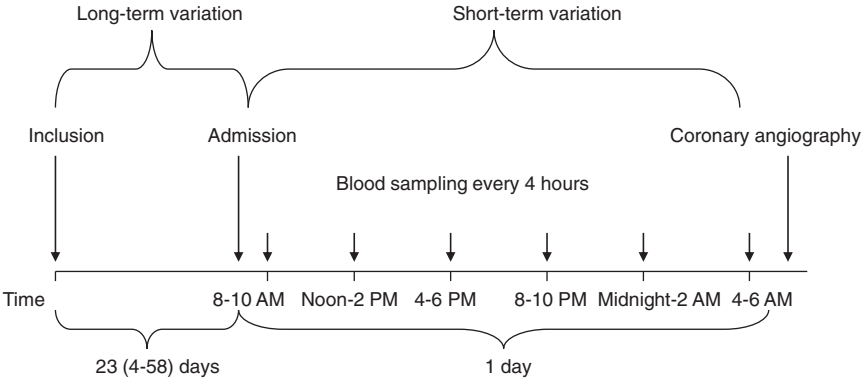


**Fig. 1.** Time line for long- and short-term collection of blood. 23 days (4–58) passed between the first and second blood sample in the long-term study. In the short-term study, the first blood sample was taken between 8 and 10 am and the following blood samples every 4 h.