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Short Communication

Biological variation of secretoneurin; a novel cardiovascular biomarker implicated in arrhythmogenesis

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ABSTRACT

Background: Secretoneurin is a novel prognostic biomarker that may predict mortality in heart failure and the occurrence of ventricular arrhythmias. This study reports the within subject variation (CV_I), between subject variation (CV_G), reference change values (RCV) and index of individuality (II) of secretoneurin.
Methods: Thirty healthy volunteers were included. Non-fasting samples were obtained between 8 and 10 am once a week for ten weeks. Secretoneurin was analyzed in duplicate using ELISA. No outliers were present according to Burnett and Reeds' criteria. Simple linear regression did not identify significant trends. Variance homogeneity in the analytical variance and CV_I were tested using Cochrane's and Bartlett's tests and four participants were excluded. Calculation of CV_I, CV_G and RCV were done on ln transformed data as described by Fokkema, the II was calculated using retransformed data.
Results: The median age of the participants was 36 years and 53% were female. Non-fasting glucose, $eGFR_{(CKD-EPI)}$, cTnT and NT-proBNP concentrations were within the normal range. Median secretoneurin concentrations were 38 pmol/L (women) and 33 pmol/L (men), p-value < 0.001. CV_I and CV_G were 9.8% (CI 8.7% to 11.0%) and 20.0 (CI 15.4% to 28.0%), respectively. RCV were 38.7% (CI 35.5% to 42.7%) and −27.9 (CI −29.9 to −26.2) and the II were 0.60 (CI 0.42–0.78). No gender differences were present.
Conclusion: Secretoneurin has a fairly low CV_I, CV_G, RCV and II, indicating that it could be suitable as a diagnostic or prognostic biomarker and that delta values in serial samplings may be preferable for identifying clinical changes.

1. Introduction

Secretoneurin is a 33-amino acid peptide derived from neuroendocrine and myocardial tissue that belongs to the granin protein family. Secretoneurin may play a role in regulating processes in the myocardium, including cardiomyocyte Ca^{2+} handling, and has been implicated in arrhythmogenesis [1–3]. Used as a novel circulating cardiovascular biomarker, secretoneurin is associated with mortality risk in acute heart failure, as well as in other critical illness conditions, including post-cardiac arrest and severe sepsis/septic shock [3–6].

For all novel biomarkers it is useful to have data on the within and between subject variation (CV_I and CV_G, respectively), the reference change values (RCV) and the index of individuality (II). These measures may be used for deciding analytical quality specifications in the

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Table 1
Biological variation, RCV and II of secretoneurin.

Biological variation, RCV and II			
	Total (n = 26)*	**Women (=13)**	**Men (n = 14)**
Number of samples	257	124	138
Median concentrations, pmol/L	34.62	37.53	32.80
CV_A, mean (95% CI), %	6.7 (6.1–7.3)	5.8 (5.2–6.6)	6.6 (5.6–7.5)
CV_I, mean (95% CI), %	9.8 (8.7–11.0)	9.6 (8.2–11.3)	10.6 (9.1–12.4)
CV_G, mean (95% CI), %	20.0 (15.4–28.0)	22.1 (15.6–37.6)	16.2 (11.4–26.8)
Positive RCV, mean (95% CI), %	38. 7 (35.5–42.7)	36.5 (32.3–42.2)	41.2 (36.6–47.4)
Negative RCV, mean (95% CI), %	−27.9 (-29.9 to −26.2)	−26.7 (−29.7 to −24.4)	−29.2 (−32.2 to −26.8)
Index of individuality, mean (95% CI)	0.60 (0.42–0.78)	0.51 (0.30–0.74)	0.77 (0.45–1.11)
Exclusion of outliers*			
Analytical outliers	None	None	None
Exclusion due to a significant 10 week trend (p-value < 0.01)	None	None	None
Exclusion due to Reeds criterion	None	None	None
Exclusion due to within-subject non-homogeneity according to Bartlett or Cochrans test	4 subjects (ID 9, 16, 24, 28)	3 subjects (ID 9, 24, 28)	None
Exclusion due to analytical non-homogeneity according to Bartlett or Cochrans test	None	4 samples (1 sample from ID 5 and 8, 2 samples from ID 10)	None

* Note that 4 subjects were excluded due to inhomogeneity in the total cohort, whilst only three subjects (women) were excluded due to this reason in the gender specific subgroups.

laboratory and for clinical interpretation of test results. A large CV_I will increase the number of measurements required to achieve sufficient diagnostic and prognostic accuracy as several consecutive measurements will be needed to establish the homeostatic set point of the individual, which may be compared to the relevant reference interval or clinical cut-off, as applicable [7]. RCV data is useful when serial measurements are interpreted, as this measure gives information on the expected magnitudes (including statistical uncertainty) of changes in concentration that could be observed due to analytical and biological variation, in clinical stable individuals [7]. Finally, the II provides information on whether clinical change is best assessed with population-based reference intervals (II > 1.4) or with serial changes in concentration in the individual patient (II < 0.6) [7].

The current study aims to describe the CV_I, CV_G, RCV and II of secretoneurin. Furthermore, we calculate desired analytical quality specifications for secretoneurin that may be used when implemented in routine practice.

2. Methods

The study was conducted in accordance with the European Federation Laboratory Medicine checklist for biological variation studies [8]. The protocol was approved by the South Central - Berkshire Research Ethics Committee (London), and the Regional Committee for Medical and Health Research Ethics in Bergen. Thirty healthy volunteers from three different study centers (i.e. Haukeland University Hospital (Norway), Akershus University Hospital (Norway) and Kings College London (United Kingdom) were included after signing informed consents [9]. Inclusion criteria were subjects 18–75 years old who reported being generally healthy, with no history of diabetes, cardiovascular disease, renal disease, chronic lung disease or cancer. Glucose, $eGFR_{(CKD\text{-}EPIcreat)}$, cardiac troponin (cTn) and NT-proBNP were measured on the first visit and all subjects showed results below the pre-specified concentrations defined as healthy: Non fasting glucose < 7.8 mmol/L, $eGFR_{(CKD\text{-}EPIcreat)}$ > 60 ml/min/1.73 m2, cTn < 99th percentile for the assay and NT-proBNP < the local reference limit. Non-fasting samples were obtained between 8 and 10 am once a week for ten weeks. Sampling was done on the same weekday +/-1 day. Participants rested in a sitting position for at least 15 min until blood was collected in serum-separating Vacutainer SST II Advance gel tubes (Becton Dickinson), centrifuged after 30 min and frozen at −80 °C within 1 h. Secretoneurin was measured in duplicate using a research-use-only ELISA (CardiNor AS, Oslo, Norway) [10].

Total and gender stratified median (10 and 90 percentiles) secretonurin concentrations were calculated. Gender stratified concentrations were compared using the Mann-Whitney *U* test. For the duplicate measurements, results were identified as analytical outliers if the difference between duplicates were larger than k * SD, and k was defined according to Burnett [11]. No samples were excluded as analytical outliers. Simple linear regression was used to identify trends that could indicate a non-steady-state clinical situation, which was not observed (p value > 0.01). Outliers in individual mean secretoneurin concentrations were defined according to Reeds' criteria [12], and none of the participants needed to be excluded. The data were not Gaussian distributed, hence natural logarithm (ln) transformation was undertaken. As suggested by Fraser and Harris [7], homogeneity of the analytical and within-person variances was tested using Cochrane's and Bartlett's tests. Four participants were excluded for homogeneity (within-subject) to be achieved (Table 1).

Calculations of σ_A, σ_I, σ_G were done (ln transformed data) using nested ANOVA. The σ was thereafter re-transformed into CV_A, CV_I, and CV_G using:

$$CV_{ln} = \sqrt{(exp\sigma^2 - 1)} \times 100$$

in which σ is the estimated standard deviation for the ln-transformed data and CV_{ln} is the adjoining re-transformed CV.

The RCV (with 95% confidence intervals) were calculated according to Fokkema et al. [13]:

$$\mathrm{RCV}pos = \left[\exp\left(1.96x2^{\frac{1}{2}} \times \left(\sigma_A{}^2 + \sigma_I{}^2\right)^{\frac{1}{2}}\right) - 1\right] \times 100$$

$$\mathrm{RCV}neg = \left[\exp\left(-1.96x2^{\frac{1}{2}} \times \left(\sigma_A{}^2 + \sigma_I{}^2\right)^{\frac{1}{2}}\right) - 1\right] \times 100$$

in which σ_A is the analytic standard deviation and σ_i is the within-person standard deviation of the ln transformed data.

The II was calculated using the retransformed data as follows:

$$II = \frac{\sqrt{CV_A^2 + CV_I^2}}{CV_G}$$

The search for outliers and calculations were repeated in separate dataset for women and men. Three women and four samples needed to be excluded for the female dataset to achieve within-subject and analytical homogeneity, respectively. No individuals or samples were excluded from the male dataset.

Analytical quality specifications were calculated as [14]:

Desirable analytical variation: CV_A < ½ CV_I

Desirable analytical bias: Bias < ¼ $\sqrt{CV_I{}^2 + CV_G^2}$

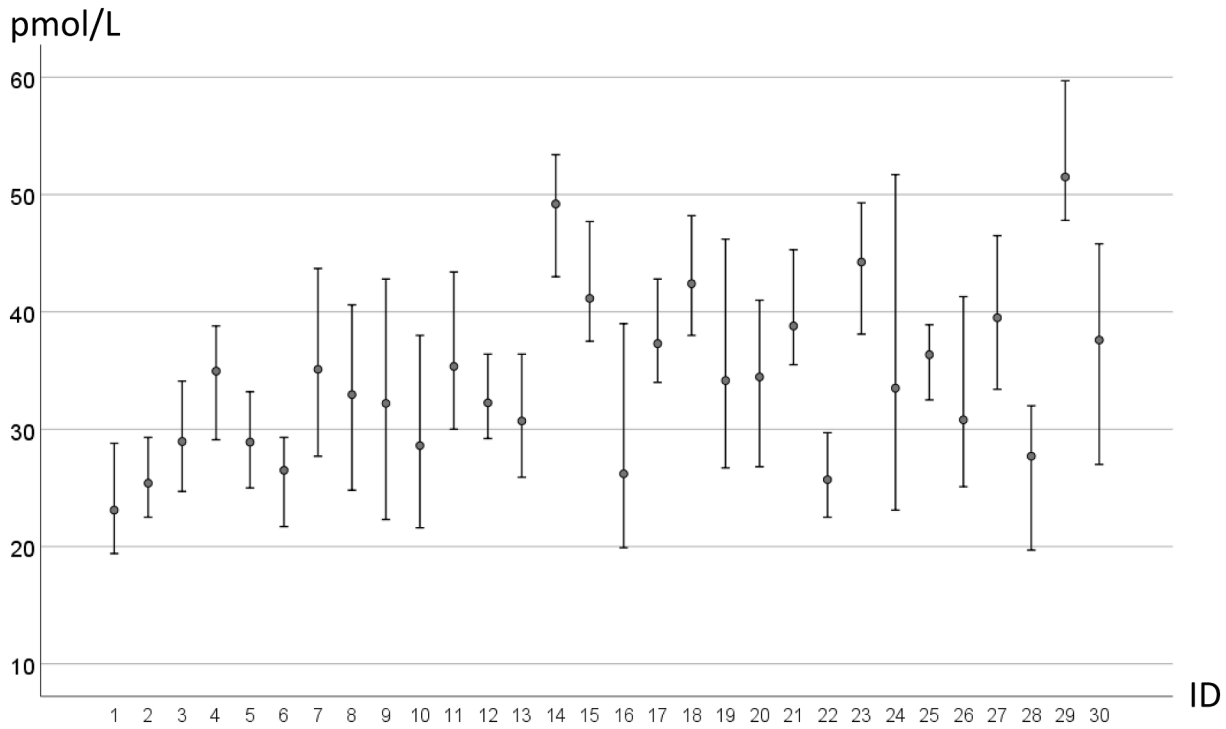

Fig. 1. Median and range of secretoneurin concentrations (pmol/L) in the 30 participants.

3. Results

Participants' median age was 36 years (10th–90th percentile, 24–59 years), 16 were women and 14 were men, all were healthy. Median (10th–90th percentile range) values for BMI were 22.6 kg/m^2 (18.9–25.3 kg/m^2), p-glucose 5.1 mmol/L (4.2–6.0 mmol/L), $eGFR_{(CKD\text{-}EPIcreat)}$ 99.0 ml/min/1.73 m^2 (78.1–115.7 ml/min/1.73 m^2), cardiac troponin T 2.0 ng/L (1.5–6.9 ng/L) and NT-proBNP 43.0 ng/L (19.1–83.3 ng/L). Median (10th–90th percentile range) of secretoneurin concentration for the group was 34.0 pmol/L (25.1–46.1 pmol/L), with individual medians ranging from 23.1 to 51.5 pmol/L, see Fig. 1. Women had significantly higher secretoneurin values compared to men (median: 37.5 pmol/L vs. 32.8 pmol/L, respectively; p-value < 0.001). The biological variation, RCV and II data are shown in Table 1. Values were generally low and no significant gender differences were observed. The II were close to the borderline value of 0.6, in the total cohort and for both genders.

Desirable CV_A and bias were 4.9% and 5.6%, respectively.

4. Discussion

The main findings in this study are that the biological variation and reference change value data for secretoneurin are low, indicating a biomarker suited to diagnose and monitor disease. Data were similar in both genders. The index of individuality was also fairly low suggesting that clinicians should monitor differences in consecutive results rather than applying absolute cut-offs to identify clinical changes [7].

Circulating secretoneurin levels are raised in critical illness and provide prognostic information in acute heart failure [15], after cardiac surgery [6] and after cardiac arrest [2], as well as in severe sepsis and septic shock [5]. Although limited data is available concerning the value of repeated secretoneurin measurements, it is conceivable that serial measurements could provide valuable information concerning disease progression and corresponding change in risk.

The low biological variation indicates that the analytical performance criteria for the assay needs to be rather strict when implemented in routine practice. The calculated values are lower than what is typically seen for routine immunoassay, especially the specifications for bias may be difficult to achieve as lot variations of +/- 10% or larger are common. However, based on the clinical use of the test, less stringent analytical quality specifications may be suggested. This should be accounted for in recommendations for clinical use and interpretation of results.

The study has several strengths: It is the a multi-center study, standard operating procedure was followed to minimize pre-analytical variability, the study included a relatively large number of participants who were prescreened to ensure healthiness, systematic outlier exclusion was performed as recommended by EFLM [8], and gender-specific results were reported. The limitation of the study is that measures were only calculated for healthy subjects, potentially limiting the clinical validity in patients with chronic cardiac disease.

In summary, the current study is the first to report biological variation, RCV, II and analytical quality specifications for secretoneurin. Overall low values were found indicating that secretoneurin has characteristics suitable for a biomarker that could be useful for diagnosing and monitoring disease.

Disclosures

B.A, M.S.S, J.T and M.M has no conflict of interest.

K.M.A. has served on advisory boards for Roche Diagnostics and received personal fees from Siemens Healthineers.

T.O. has served on advisory boards for Abbott Diagnostics, Roche Diagnostics, and Novartis, and has received research support from AstraZenica, Abbott Dioagnostics, Roche Diagnostics, ThermoFisher, Singulex and Biomedica via Akershus University Hospital, and speaker's honoraria from Roche Diagnostics and Novartis.

A.L.F. has received personal fees from CardiNor AS and has financial interests in CardiNor AS.

HR have received personal fees from Novartis and Thermo Fisher BRAHMS.

G.C., H.R., and T.O. are partners in a patent regarding the use of secretoneurin as a biomarker in cardiovascular disease and in patients with critical illness.

A.H.O, A.L.F., G.C., H.R., and T.O. are stock owners in CardiNor AS, which holds the license to commercialize secretoneurin.

A.H.O., A.L.F, H.R., and T.O. have also received personal payments from CardiNor AS.

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