Letter to the Editor

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Week-to-week within-subject and between-subject biological variation of copeptin

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To the Editor,

Copeptin is used as a sensitive surrogate marker of ADH (antidiuretic hormone) when investigating fluid imbalance such as in diabetes insipidus, SIADH (syndrome of inappropriate antidiuretic hormone secretion) and psychogenic polydipsia [1]. Unlike ADH, Copeptin concentration is stable in plasma [2]. Copeptin and ADH originate from the molecule prepro-vasopressin and are co-secreted in equimolar amounts. Plasma osmolality, hypovolemia and stress determine the secretion of both peptides from the neurohypophysis. Before implementation of high sensitivity cardiac troponin copeptin was suggested as an acute chest pain biomarker [2], and has recently evolved as a promising stroke marker [3].

Understanding the biological variation (BV) of copeptin and its components within-subject (CV I) and between-subject (CVG) variation has several applications, including the interpretation of test results, suggesting analytical performance specifications, determining sample size for steady-state concentration, calculating the reference change value (RCV) and the index of individuality (II). RCV is used to assess the significance of a change in serial measurements, whereas II is useful for determining whether the change in serial measurements rather than the reference intervals should be used to evaluate if the patient could be in a physiological vs. pathological status.

The European Federation of Laboratory Medicine (EFLM) variation database provides BV data and a checklist for studies estimating BV [4]. EFLM recommend BV estimations using ANOVA analysis with outlier exclusion [4], and the Bayesian approach for the estimation of BV was recently suggested [5]. This study aims to establish the week-to-week biological variation of copeptin using both ANOVA and the Bayesian methods.

This study was performed in accordance with the Declaration of Helsinki (REC ID number 2018/92 for Bergen and Oslo and South Central – Berkshire Research Ethics Committee for London) and EFLM checklist for biological variation studies (BIVAC) [4] and has been described earlier [6, 7]. A total of 30 presumably healthy volunteers were recruited from three different centers. The age range of the participants were 21–64 years (mean 38 years), and 8 of 16 women were presumed fertile. Weekly venous blood samples were collected for ten consecutive weeks, plasma was
frozen at \(-80\, ^\circ\text{C}\) and later analyzed for copeptin in one run at
the Hormone Laboratory at Oslo University Hospital using a
compact PLUS Copeptin proAVP Kryptor Kit (Brahms Krypt-
or, Thermo Fisher Scientific). The limit of detection (LoD)
was 0.69 pmol/L, limit of quantification (LoQ) 1.1 pmol/L
and long-term analytical variation (CV,\(_A\)) was 7 % at 5.3 pmol/L
and 4 % at 99 pmol/L. The method is accredited according to
NS-EN ISO/IEC 17025:2017, and used for analyzing copeptin
concentrations reported to clinical care at Oslo University
Hospital.

Statistical analysis including the detection and exclusion
of outliers, checking for trends in the concentra-
tion, transforming skewed data, checking for homo-
geneity, and AVOVA were performed as per the BIVAC
recommendations [4], and were described in detail in our
previous work [6, 7].

The Bayesian model was applied as described in
detail by Røraas et al. [5] which assumes Student
t-distributions and accommodates extreme observations
and non-homogeneous variances. In brief, the model
infers a posterior distribution of CV\(_I\) (CV\(_{P(i)}\)) with esti-
mates of the mean (μCV\(_{P(i)}\)) and SD (σCV\(_{P(i)}\)). Based on these
parameters the model provides a predicted distribution
(dCV\(_{P(i)}\)) based on randomly generated CV\(_{P(i)}\). The model
also allows for assessment of heterogeneity through the
Harris–Brown ratio [5, 8, 9], for both the estimated and
predicted distribution of CV\(_I\). Ratios <100 %/\sqrt{2S},
where S is the average number of samples per individual,
would signal a homogenous population, and for our study
S would be 10, so <22.4 %. The prior assumptions used
in this model were based on our ANOVA results, where
we applied the following prior distributions and hyper-
parameters, N-truncated indicates that only the positive
part of the normal distribution is used in the estimation
routine, and SDs are defined as positive, the 10 % (0.1) of
the SD has been applied to the hyperparameters:

\[
S\text{D}_{P(i)} \sim N_{\text{truncated}}[\mu(S\text{D}_{P(i)}), \sigma(S\text{D}_{P(i)})]
\]

\[
\sigma[S\text{D}_{P(i)}] \sim N_{\text{truncated}}(0, 0.1)
\]

\[
\mu[S\text{D}_{P(i)}] \sim N_{\text{truncated}}(S\text{D}_I, 0.1 \times S\text{D}_I)
\]

\[
S\text{D}_G \sim N_{\text{truncated}}(S\text{D}_A, 0.1 \times S\text{D}_G)
\]

\[
S\text{D}_A \sim N_{\text{truncated}}(S\text{D}_A, 0.1 \times S\text{D}_A)
\]

The asymmetrical RCV values (with 95 % confidence
intervals) were calculated according to Fokkema et al. [8]:

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

in which \(\sigma_A\) is the analytic standard deviation and \(\sigma_I\) is
the within-person standard deviation of the logarithmic
data.

The II was calculated using the retransformed data as
follows [2]:

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

Desirable analytical performance specifications were
calculated as [2]:

\[
CV_A < \frac{1}{2} CV_I
\]

\[
\text{Bias} < \frac{1}{4} \sqrt{CV_I^2 + CV_G^2}
\]

Two specialists in endocrine biochemistry (KMA and
KV) classified females into fertile and peri/postmenopausal
groups based on the concentration and covariation observed
in FHS, LH, estradiol and progesterone during the 10-week
data collection period (weekly samples during 2.5 menstrual
cycles) of the study.

The distribution of the concentrations of the 30 partici-
pants are shown in Figure 1. Three subjects were identified
as outliers (Table 1) (based on trend, Reeds criterion and non-
homogeneity) and were not included in the ANOVA analysis.
For the Bayesian approach estimates, no outliers needed to
be excluded.

Median copeptin concentration for all participants (30
individuals) was 3.9 (25 and 75 percentile, 2.7–5.8) pmol/L
(Table 1). Analytical and biological variation, RCV and II for
the total cohort and fertility-stratified sub-groups, using both
methods are reported in Table 1. The estimation of the CV\(_I\) by
the ANOVA and Bayesian approaches (μCV\(_{P(i)}\)) produced
similar results, with 2 percentage points differences or less.
The RCV ranged (mean values) from approximately \(-40\ %\)
(deteriorating values) to 80 % (increasing values). The II was
generally low (≤0.5). There were no major differences in
the estimated parameters between the sub-groups, except a
slightly higher CV\(_G\) and a slightly lower II in the fertile
females. Both the estimated (reflecting the actual study data)
and predicted (reflecting the simulated distribution) Harris–
Brown ratios were generally high, indicating a within-
subject heterogeneity in the distribution of copeptin con-
centrations in healthy individuals. Based on the data the
following analytical performance specifications could be
calculated; 9.9 % as desirable CV\(_A\) and 12 % as desirable
analytical bias.
The main limitation in our study is the relatively low number of included subjects, however all estimates are given with confidence intervals indicating the level of uncertainty. The subgroups were small, resulting in larger uncertainty for the specific estimates, and these need confirmation in other studies. The fertility status were determined based on expert consensus. We did not collect data on menstrual cycles so we could not determine if copeptin varied according to the menstrual status. Also, our study only included healthy subjects, so the biological variation data are not applicable for interpreting clinically relevant changes in patients with chronic disease.

To our knowledge weekly biological variation of copeptin has not been reported in previous studies. Our data indicate that important sex differences in the biological variation of copeptin are unlikely. Further, no definitive differences between fertile women and post-menopausal women or men were detected. Based on the low II (<0.6), delta values should be preferred for identifying a possible clinical change as compared to reference intervals. During physiological conditions delta values between two serial measurements could range from −40 to 80 %. As the Harris–Brown ratio indicated heterogeneity for all subgroups, a strategy of using different dCVp(i) percentiles as an alternative to the mean CV may be adopted to set analytical performance specifications or calculate RCV, as suggested by Aarsand et al. [9], depending on the local clinical needs. Finally, the results indicate that routine laboratories have the potential to achieve satisfactory analytical performance when copeptin is measured, given the significant disparity between biological and analytical variation.

Figure 1: Median, 25 to 75 percentile and total range of copeptin concentrations by participants. ID 8, 16, 23 were excluded as outliers before the CV-ANOVA analysis. M, male; F, peri/postmenopausal female; FF, fertile female.
Table 1: Biological variation, RCV and II are presented in the table. The top rows show the number of outliers subjects and samples included in the analysis of analytical (CV_a), within (CV_i) and between subject biological-variation (CV_b). Uncertainty is estimated as 95 % confidence/ credibility-intervals (CRI) as applicable.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>CV-ANOVA</th>
<th></th>
<th></th>
<th>Bayesian approach</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
</tr>
<tr>
<td>Outliers (samples): analytical</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Outliers (individ): trend</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outliers (individ): Reed test</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outliers (individ): homogeneity</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Samples/participants</td>
<td>268/27</td>
<td>130/13</td>
<td>139/14</td>
<td>69/7</td>
</tr>
<tr>
<td>Median concentration</td>
<td>3.7 (2.6–5.4)</td>
<td>4.1 (3.2–5.6)</td>
<td>3.3 (2.2–5.0)</td>
<td>3.5 (2.3–5.5)</td>
</tr>
<tr>
<td>(25–75 percentiles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CV_a (95 % CI/CR)</td>
<td>6.9 (6.3–7.5)</td>
<td>7.6 (6.8–8.6)</td>
<td>6.6 (5.7–8.0)</td>
<td>6.2 (5.5–7.1)</td>
</tr>
<tr>
<td>CV_i (95 % CI/CR)</td>
<td>19.8 (17.9–21.8)</td>
<td>19.3 (16.9–22.2)</td>
<td>21.0 (17.4–25.6)</td>
<td>20.2 (17.7–23.4)</td>
</tr>
<tr>
<td>dCV(i) median (20–80 percentile)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>CV_b (95 % CI/CR)</td>
<td>44.9 (34.3–64.3)</td>
<td>47.3 (33.1–83.4)</td>
<td>54.7 (33.1–159.7)</td>
<td>40.9 (28.3–73.0)</td>
</tr>
<tr>
<td>RCV pop. mean (95 % CI)</td>
<td>77.7 (69.7–87.2)</td>
<td>78.6 (67.7–93.8)</td>
<td>76.9 (66.4–90.4)</td>
<td>83.0 (67.1–106.0)</td>
</tr>
<tr>
<td>RCV reg. mean (95 % CI)</td>
<td>−43.7 (−41.1 to −44.0)</td>
<td>−44.0 (−48.4 to −43.5)</td>
<td>−45.4 (−52.0 to −43.5)</td>
<td>−43.3 (−39.5 to −44.4)</td>
</tr>
<tr>
<td>II</td>
<td>0.48</td>
<td>0.50</td>
<td>0.46</td>
<td>0.40</td>
</tr>
<tr>
<td>Estimated Harris–Brown ratio</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Predicted Harris–Brown ratio</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A, not applicable; FF, presumed fertile females; *results from Bayesian approach is presented with 95 % credibility interval.
Research ethics: The study was conducted according to the Declaration of Helsinki Ethical Principles and Good Clinical Practices. The respective regional ethics committee approved the protocol at each center: The Regional Committee for Medical and Health Research Ethics in Bergen (Bergen and Oslo) (ID number 2018/92), South Central – Berkshire Research Ethics Committee (London). All volunteers gave an informed written consent before participating.

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: K.M.A. is an Associate Editor of Clinical Biochemistry and Chair of the IFCC Committee on Clinical Application of Cardiac Bio-Markers. She has served on advisory board for Roche Diagnostics and SpinChip, received consultant honoraria form CardiNor, lecturing honorarium from Siemens Healthineers and Snibe Diagnostics and research grants from Siemens Healthineers and Roche Diagnostics. T.O. is an Associate Editor of Circulation and has received speaker and/ or consultancy honoraria from Abbott Diagnostics, Bayer, CardiNor, Roche Diagnostics and Siemens Healthineers, and has received research support from Abbott Diagnostics, Novartis, Roche Diagnostics, via Akershus University Hospital. M.M, E.A.R, N.A.G, K.V, M.S.S, H.S, J.T, B.A, P.T report no disclosures.

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References