Author's response to reviews

Title: Copeptin Measurement as a Marker of Short-, Mid-, and Long-Term Mortality in All-Comers

Authors:

Kasper Iversen (kasper.iversen@dadlnet.dk)
Jens P Gøtze (jpg@dadlnet.dk)
Morten Dalsgaard (md@dadlnet.dk)
Henrik Nielsen (henrik.nielsen.06@regionh.dk)
Søren Boesgaard (soeren.boesgaard@regionh.dk)
Morten Bay (morten.bay@frh.regionh.dk)
Vibeke Kirk (v.kirk@dadlnet.dk)
Olav W Nielsen (own@dadlnet.dk)
Lars V Køber (lk@heart.dk)

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your letter and for offering us the possibility of submitting a revised manuscript. We have carefully made changes in the current version of the manuscript in order to comply with the editorial comments and the reviewer’s comments and suggestions. We have responded to each of these as detailed in the following pages. The reviewers’ comments are written with normal font, our responses are written in *italics*, deleted text/old text is underlined and added text is in **bold**.

We think that this version of the manuscript has been strengthened by the revision and we hope that you will now consider it suitable for publication in BMC medicine.

**Editorial comments**

Comment 1

Provide email addresses for all authors as follows:

http://www.biomedcentral.com/bmcmed/authors/instructions/researcharticle#formatting-title

*Our reply*

*We have made the changes as requested.*

**Old text**

Kasper Iversen,* Jens P. Gøtze, Morten Dalsgaard, Henrik Nielsen, Søren Boesgaard,  
Morten Bay, Vibeke Kirk, Olav W. Nielsen and Lars Køber

**New text**

Kasper Iversen,* – email: kasper.iversen@dadlnet.dk, Jens P. Gøtze, – email: jpg@dadlnet.dk,  
Morten Dalsgaard, – email: md@dadlnet.dk, Henrik Nielsen, – email: henrik.nielsen.06@regionh.dk,  
Søren Boesgaard, – email: soeren.boesgaard@regionh.dk,  
Morten Bay, – email: morten.bay@regionh.dk, Vibeke Kirk, – email: v.kirk@dadlnet.dk, Olav  
W. Nielsen, – email: own@dadlnet.dk and Lars Køber, – email: lk@heart.dk
Comment 2
Please include an Authors' contributions section before the Acknowledgements and Reference list and used the instructions at the link:
http://www.biomedcentral.com/bmcmd/authors/instructions/researcharticle#formatting-contributions

Our reply
We have already included the following prior to the list of references.
Please let us know if this is insufficient
Authors' contributions
MB and VK collected data and critically revised the manuscript. JPG, SB, MD, OWN and HN participated in drafting of the manuscript and interpretation of the data and critically revised the manuscript. LK participated in drafting of the manuscript, data analyses and interpretation of the data and critically revised the manuscript. KI wrote the first draft of the manuscript, performed the analyses and interpretation of the data.
All authors read and approved the final manuscript.

Reviewer 1
Comment 1
The choice of cut-off for "elevated" is based on the original assay paper written by me. Whilst I feel honored, I suggest you discuss the topic of choosing a correct Copeptin cut-off for outcome prediction. Particularly, since the Keller et al. JACC paper used a population of 5000 people to define 99th and also 97th percentile. The 97th percentile was at 13 pmol/L. One might point out, that this population contains already confounding patients, which are simply not yet identified, and who push the 97th percentile higher. Looking at your data, it would not really matter much, if you do this analysis with 11.6 or 13 pmol/L as the cutoff. But I think it would be an important point for everybody in the future trying
to apply your findings. Simply put: Which Copeptin in the ER do we call "elevated" and on what basis do we do that?

Our reply
Excellent comment. We do agree that a discussion of reference values in different population is essential. Secondly, as you hav noticed, it does not really matter whether 11.6 or 13 pmol/L is used.
We have made the following changes in the manuscript

New text
Discussion, previous studies , page 10
The cut-off level for elevated copeptin is in the present analysis based on the 97.5 percentile from the original assay article. Recent Keller et al has published slightly higher 97.5 percentile (i.e., 13 pmol/L) based on data from the Gutenberg health study. This population is however not a healthy population but a random population sample also including patients with a broad spectrum of different conditions and diseases. This might explain the slightly higher cut-off value in this population and is the reason why we chose to use the original published cut-off values for elevated copeptin.

Comment 2
What was the 97th percentile of Copeptin in your population? The 95th percentile is already very high.

Our reply
The 97.5 percentile in our population is 125.4 pmol/L
We have added this information to the manuscript

New text
Results, page 8
The 95 percentile, 97.5 percentile and 99 percentile in this population was 89 pmol/L, 125 pmol/L and 211 pmol/L.
Comment 3
Is it possible to express the increase in mortality risk per increase in copeptin concentration or log (ten fold) increase?

Our reply
We agree with the reviewer that this would be a good supplementary way of presenting the results.
We have added a line with these figures in table 3.

New text

| Per 1 increase in log copeptin | 6.66 (4.63-9.57) | 4.24 (3.29-5.47) | 2.76 (2.41-3.15) | 3.24 (2.07-5.08) | 1.96 (1.43-2.68) | 1.52 (1.29-1.79) |

Comment 4
It would be more reader friendly to replace the letters in Figure 2 by the clinical subgroup instead of having to refere to the legend.

Our reply
Very fine suggestion. We have changed the figure as suggested.

Reviewer 2
Comment 1
As the data on BNP is available (Heart 2003), it would add to the quality to show if copeptin adds to the risk-stratification achieved by BNP. Suggestion:
ROC-curves containing curve overlap copeptin/BNP

Comment 2
Multivariate analyses should be presented in more detail, showing that copeptin is an additional, independent predictor of mortality. Readers could more easily understand the message if a baseline model of age, sex, and comorbidity would be used for risk-stratification - and the additional value of copeptin should be shown in such a model.
Our reply
We agree with the reviewer that such an analysis is important. We have already included BNP in both our cox analyses and ROC analyses but agree that this might be a little unclear in the manuscript. We have changed this section accordingly. Furthermore we have included a model including only age, gender and comorbidities as suggested in comment 2.

Old text
Results, page 9-10
Receiver operating characteristic (ROC) analyses show that the area under the curve (AUC) for copeptin to predict one-week, short-, mid-, and long-term mortality was 0.84 (95% CI 0.77-0.92), 0.75 (95% CI 0.70-0.80), 0.71 (95% CI 0.67-0.74), and 0.70 (0.67-0.73), respectively. When a model containing all variables included in the final cox model +/- copeptin was used, we found a significant increase in AUC’s for one-week mortality (0.80 (95% CI 0.73-0.88) vs. 0.85 (95% CI 0.77-0.93), p = 0.04) and for three-month mortality (0.77 (95% CI 0.73-0.81) vs. 0.79 (95% CI 0.75-0.83), p = 0.01). There was no significant effect on AUC’s of adding copeptin to the model for one-year mortality or for the entire observation period.

New text
Results, page 9-10
Receiver operating characteristic (ROC) analyses show that the area under the curve (AUC) for copeptin to predict one-week, short-, mid-, and long-term mortality was 0.84 (95% CI 0.77-0.92), 0.75 (95% CI 0.70-0.80), 0.71 (95% CI 0.67-0.74), and 0.70 (0.67-0.73), respectively. Comparing a model with age, gender and comorbidity (model 1) to a model containing all variables included in the final cox model (i.e., N-terminal pro-brain natriuretic peptide (log-transformed), gender, age, liver disease, potassium, and hemoglobin) (model 2) and to the fully adjusted model with copeptin added (model 3), we found a significant increase in AUC’s for one-week mortality (0.70 (95% CI 0.65-0.76) vs. 0.80 (95% CI 0.73-0.88) vs. 0.85 (95% CI 0.77-0.93), p =
o.04) and for three-month mortality (0.72 (95% C.I. 0.70-0.75) vs. 0.77 (95% CI 0.73-0.81) vs. 0.79 (95% CI 0.75-0.83), p = 0.01), There was no significant effect on AUC’s of adding copeptin to the fully adjusted model for one-year mortality or for the entire observation period.

Comment 3
The concept of all-comers may be somewhat overstretched, as only hospitalized patients were enrolled (on average one third of all emergency patients), only patients over 40, and among those 3644 patients in the original cohort, 1320 copeptin levels were analyzed. Please comment and make changes accordingly. Please mention in limitations.

Our reply
We agree that the term all-comers might be overstretched.

We have therefore omitted the term in the manuscript. Instead we used the term unselected admitted patients ≥ 40 years of age.

In the limitation section of the discussion the risk of selection bias is mentioned and discussed (Some selection bias in the original cohort was inevitable due to missing informed consent from confused, mentally disabled, and frail patients. The mean age of the population in the study is high (74 years) and there was a high prevalence of comorbidity. Therefore, the results of this study cannot without caution be extrapolated to younger or non-hospitalized populations.)

Comment 4
Title: as the topic is risk-stratification, why not use it in the title, such as risk-stratification in emergency patients by copeptin?
Our reply

We agree with the reviewer and have changed the title according to the reviewers suggestions.

New text

Risk stratification in emergency patients by copeptin

Comment 5
Abstract:
please give adjusted hazard ratios

Our reply
We have changed the abstract as suggested and given the adjusted ratios (2.4; 1.9; 1.4) instead of the unadjusted ratios in the abstract

Comment 6
Abstract: "district hospital" is not an international term. inner-city hospital? regional center?

Our reply
We have changed the term district hospital to inner-city hospital throughout the manuscript

Comment 7
Abstract: triage is misused in this context (explained below), it should be "disposition", if taking the example of emergency medicine.

Our reply
We thanks the reviewer for this point and has changed the manuscript accordingly

Comment 8
Materials & methods: page 5, top: of 2506 patients admitted... this does not correspond to the original article this study refers to (3644 patients admitted in this period)
Our reply

Blood samples to the biobank were only collected in the last period of the study. The 2506 patients refer to patients admitted in that timespan. We do however agree with the reviewer that it would be more correct to refer admissions in the entire study period. Also, see our response on comment 9.

New text

Material and methods, page 5

In this period 3644 patients > 40 years was admitted to the hospital. During the last 10 months of the study blood samples were drawn from patients who gave written informed consent to participate in the study (n = 2230),

Comment 9

Results: page 7: ...from 1320 patients (58%)... percentage should be referred to the original population of 3644. That excluded patients were randomly spread is believable, but should be shown (comparison of age, sex, co-morbidity, mortality). Also, a distribution of main diagnoses should be shown

Our reply

As written above we do agree with the reviewer. We have furthermore added the following text and included an E-table 1 were comparison between included and not included patients appear.

New text

Results, page 7

There was no difference between included vs. excluded regarding baseline variables, comorbidity or discharge diagnoses (E-table 1), however mortality was slightly lower in the included patients (HR 0.89, 95% C.I. 0.82-0.97).
<table>
<thead>
<tr>
<th></th>
<th>Included patients (n = 1320)</th>
<th>Not included patients (n = 2309*)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>70.5</td>
<td>70.6</td>
<td>0.76</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>(69.7-71.3)</td>
<td>(70.0-71.2)</td>
<td></td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>536 (41)</td>
<td>956 (41)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>160 (12)</td>
<td>244 (13)</td>
<td>0.62</td>
</tr>
<tr>
<td>Ischemic heart disease, n (%)</td>
<td>281 (21)</td>
<td>369 (19)</td>
<td>0.15</td>
</tr>
<tr>
<td>Previous myocardial infarction, n (%)</td>
<td>132 (10)</td>
<td>224 (12)</td>
<td>0.13</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>349 (27)</td>
<td>455 (24)</td>
<td>0.08</td>
</tr>
<tr>
<td>Lung disease, n (%)</td>
<td>255 (19)</td>
<td>365 (19)</td>
<td>0.83</td>
</tr>
<tr>
<td>Liver disease, n (%)</td>
<td>39 (3)</td>
<td>58 (3)</td>
<td>0.91</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>141 (11)</td>
<td>220 (11)</td>
<td>0.47</td>
</tr>
<tr>
<td><strong>Discharge diagnoses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease, n (%)</td>
<td>260 (20)</td>
<td>384 (17)</td>
<td></td>
</tr>
<tr>
<td>Orthopedic disease, n (%)</td>
<td>211 (16)</td>
<td>349 (15)</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disease, n (%)</td>
<td>168 (13)</td>
<td>287 (12)</td>
<td></td>
</tr>
<tr>
<td>Hematological/oncological, n (%)</td>
<td>85 (6)</td>
<td>154 (7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Pulmonary disease, n (%)</td>
<td>85 (6)</td>
<td>150 (7)</td>
<td></td>
</tr>
<tr>
<td>Neurological disease, n (%)</td>
<td>133 (10)</td>
<td>258 (11)</td>
<td></td>
</tr>
<tr>
<td>Infectious disease, n (%)</td>
<td>187 (191)</td>
<td>329 (14)</td>
<td></td>
</tr>
<tr>
<td>Other diseases, n (%)</td>
<td>191 (15)</td>
<td>398 (17)</td>
<td></td>
</tr>
</tbody>
</table>

* Baseline data only available in 2309 of the 2324 not included patients (99%)
Comment 10
Results: page 8: ... reason for missing information was immigration (?) - EMIGRATION?

Our reply
Thank you for spotting this error. Immigration has been changed to emigration

Comment 11
Results:...at highest modest... please use weak correlation

Our reply
Has been changed as requested by the reviewer.

Old text
Copeptin showed a significant but at highest moderate correlation with other known markers of mortality

New text
Copeptin showed a significant but weak correlation with other known markers of mortality

Comment 12
Results: page 9:... value of copeptin in relation to elevated values of copeptin... did not understand, please explain

Our reply
We agree that this sentence is unclear and has changed it accordingly.

Old text
Results, page 9
Figure 1 shows Kaplan-Meier curves for survival in relation elevated levels of copeptin (Panel a), for quartiles of copeptin (Panel b).......
Results, page 9

Figure 1 shows Kaplan-Meier curves for survival for patients with normal and elevated levels of copeptin (Panel a), for quartiles of copeptin (Panel b)......

Comment 13

Results: multivariate model should be explained in more detail: has a standardized co-morbidity index, such as Charlson been used as covariate, or only the 7 clinical variables, of which 4 are related to the heart?

Our reply

We have not used a standard comorbidity index but instead used all available variables that might influence mortality as covariates.
For the multivariable analyses all the 20 variables included in table 1 was included. Variables were removed with backward elimination, and only 7 variables remained in the final model. We have clarified this.

Old text

Results, page 9

All variables from Table 1 were tested as covariates, and N-terminal pro-brain natriuretic peptide (log-transformed), gender, age, liver disease, potassium, and hemoglobin remained in the fully adjusted model as being significantly associated with mortality.

New text

Results, page 9

All the 20 variables from Table 1 were tested as covariates. After backward elimination N-terminal pro-brain natriuretic peptide (log-transformed), gender, age, liver disease, potassium, and hemoglobin remained in the fully adjusted model as being significantly associated with mortality.

Comment 14
Discussion: page 10: please put "unselected" into perspective (see top)

Our reply

As discussed in our reply to comment 3 we have modified this phrase.

Comment 15
Discussion: "previous studies": please omit biology from discussion

Our reply

We agree with the reviewer that this section not is within the primary scope of the present paper and has omitted it in the discussion section.

Deleted text

Discussion, previous studies, page 10.
Arginine vasopressin (AVP) is a central hormone in the hypothalamic-pituitary-adrenal axis. However, AVP is very unstable and therefore difficult to measure (34). Copeptin is the C-terminal part of pro-AVP and is released together with AVP during processing of the precursor. Copeptin is stable in serum and plasma at room temperature and can easily be measured ex vivo as a 'shadow' fragment of AVP in the circulation (4). Copeptin and AVP are secreted from the neurohypophysis upon hemodynamic or osmotic stimuli but also play a central role in the endocrine stress response (35, 36). The release of AVP (and copeptin) results in cortisol release (37). Cortisol is, however, difficult to measure in plasma and has a strong circadian rhythm; it therefore has only limited value as a marker for the endocrine stress level. Copeptin therefore seems to be a good marker for the stress response and has potential value in assessment of disease severity and prognosis in all-comers.

Comment 16
Discussion: strengths&limitations: "unselected nature", a weake expression may be preferrable

Our reply

We have changed the sentence as suggested.
Old text
The strengths of this study are the size of the cohort, its prospective and unselected nature, and the long follow-up period.

New text
The strengths of this study are the size of the cohort, its prospective and relative unselected nature, and the long follow-up period.

Comment 17
Discussion: if diagnostic groups are available, mortality on their background should be discussed. If not available, please state accordingly.

Our reply
We have discussed this comment, and we are not sure that we fully understand this comment. We would therefore kindly request the reviewer to explain this comment further.

Comment 18
Discussion: one major limitation should be mentioned: single center study. Population typical for Denmark? Europe? City - outskirts?

Our reply
We agree with the reviewer that this is an important concern and have added the following discussed.

New text
Discussion, strengths and limitations, page 11
The study was a single center study and this could potentially cause problems with generalizability. This concern is however partly countered by the fact that the hospital is placed in a part of Copenhagen including both high income and low income areas as well as inner city and suburb/country areas.

Comment 19
Discussion: clinical implications: copeptin could indeed be used in the ED, but triage, as internationally defined, is concerned with "who should not wait", and is therefore an instant clinical tool. Suggestion: as emergency medicine is concerned with triage, work-up, and disposition (observation, admission to ward or ICU, discharge), the clinical implication could focus on the latter. To give an example could help the reader to understand the concept (what if you knew, your patient has a 7-day mortality of 10.6%, would you discharge him?)

Our reply

Thank you for the clarification. We agree with the reviewer that an example would make the message of this paper clearer. We have added the following.

New text

Discussion, clinical implications, page 12

Knowing that your patients had a 7-day mortality of more than 10% or a one-year mortality of less than 3% might influence the clinician’s decisions regarding observation and treatment in the emergency department.