PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Sex-specific Mortality Prediction by Pro-C-type Natriuretic Peptide Measurement in a Prospective Cohort of Patients with ST-elevation Myocardial Infarction
AUTHORS	Mark, Peter; Frydland, Martin; Helgestad, Ole; Holmvang, Lene; Møller, Jacob; Johansson, Pär I; Ostrowski, Sisse; Prickett, Timothy; Hassager, Christian; Goetze, Jens Peter

VERSION 1 – REVIEW

REVIEWER	Zagidullin, Naufal Bashkir State Medical University, Internal Diseases
REVIEW RETURNED	11-Feb-2021

GENERAL COMMENTS	In this prospective cohort study 1760 patients the predictive efficasy of proCNP, take at admission was investigated according general mortality in 30 and 365 days after STEMI. The proCNP of higher quartile was shown to be risk factor in females. The study is accuratly planned, the impressive statistical analysis was performed including longitudinal studies, regression analysis, etc.. However I would recommend same issues to the study: 1) Please provide some information about NP family in the introduction. You may also discuss the obtained results compared to NTproBNP which is also proved to have predictive power (especilly with ST2/Pentraxin-3). 2) I would suggest to provide proCNP high concentration in female effecasy check (specifisity and sensitivity) concerning mortality. 3) Why have you analazed cardiovascular mortality? It is expected for proCNP to have bigger impact on mortality 4) I would suggest to draw the design of the study in the figure. 5) I would suggest to introduce also GFR in the tables and change Kidney diseases to CKD .... 6) In discussion you speculate about menopausal impact on mortality but do not prove the results for > 50 year female cohort. After answereing the question/corrections above the manuscript could be published.

REVIEWER	Araujo, Gustavo N. Imperial Hospital de Caridade
REVIEW RETURNED	17-Feb-2021

GENERAL COMMENTS	Major comments: Comparison between male and female patients overall baseline characteristics are not shown in this paper. Thus, conclusions about sex differences are unsubstantiated. In order to have valid conclusions, the authors should have performed, for example, a

	multivariate Cox model and propensity score methods (i.e. sex and age-matched controls of a female cohort). The sex differences found seem to have been an exploratory analysis, and it is shown as a main result in title and conclusions. Thus, it should me more exposed in the objectives and methods section (was it pre-specified?). While performing mortality analyses only on proCNP concentrations ≥ median, I am not sure if the authors can have such conclusions about overall proCNP prognostic capacity. The results and discussion are not displayed objectively. There is too much information, too many analyses, but the reading has no flow. The idea of the study is interesting and the number of patients is probably enough. However, I will recommend major revision with proper statistical analyses. Specific comments: Abstract, page 3, line 9: the authors should define the primary outcome: 30-day vs. one year mortality. Sample size should be calculated based on one of the outcomes. Introduction, page 6, line 5: Although CNP plays a protective role in cardiac pathophysiology, it was a predictor of mortality in your study. Please comment Introduction, page 6, line 18 (third paragraph): the authors include population details and outcome measurements, which should be detailed in the methods section. Methods, page 7, line 17. Reference intervals of CNP should be detailed further (section “biochemical analyses). Methods, page 8, line 18. Please specify more details about biochemical measurement (i.e. timing of blood withdrawal). Methods. Outcomes (primary, secondary) are not detailed on this section. Statistics, page 9, line 7. Reference population should be described above, in methods section. Statistics, page 10, line 12. Multivariable cox proportional hazard model inclusion criteria is not clear. Statistics. Sample size calculation is recommended. Results, page 11, line 6. The first paragraph of the results section show results of another study. Results, page 11, line 17. Decreased proCNP concentrations are probably variations within normal values, and should not be excluded from analysis. Results. A flowchart with included and excluded patients is recommended.

	Table 1: Too much information. Table 2: It is unclear if the ProCNP used in the model is quantitative (per 1 pmol/L increase) or qualitative (proCNP > median). Table 3: models 1 and 2 are not explained.

REVIEWER	Yang, Jian Department of Cardiology, the First Affiliated Hospital, College of Medicine, Zhejiang University
REVIEW RETURNED	18-Feb-2021

GENERAL COMMENTS	Introduction: 1. Studies have explored the proCNP prognostic value in general population and ACS patients. What is the significance of the author's further research in the STEMI population? Obviously, the population in this study has higher selectivity and fewer clinical end points observed. The author should introduce the innovation and clinical significance of this research in depth. Methods: 1. In the Ethics section, the author should give the complete ethics committee name. 2. Although the design of the cohort has been previously reported, is the study registered on the relevant website? If so, it should be stated. 3. The author analyzed longitudinal plasma sample data in a subgroup of 287 people. However, how was the 287 people obtained? Is it randomly selected from the total cohort, or is there only 287 people with longitudinal plasma samples? Related content should be stated in the methodology. Results: The results section should be subtitled. Discussion: 1. Can the conclusions obtained in the reference cohort based on the Nordic sample database and the Danish cohort be extrapolated to the general population? The author should summarize the relevant literature and discuss the differences of CNP among races to explore the generality of the conclusions of this article.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Naufal Zagidullin, Bashkir State Medical University Comments to the Author:
In this prospective cohort study 1760 patients the predictive efficasy of proCNP, take at admission was investigated according general mortality in 30 and 365 days after STEMI. The proCNP of higher quartile was shown to be risk factor in females. The study is accuratly planned, the impressive statistical analysis was performed including longitudinal studies, regression analysis, etc.. However I would recommend same issues to the study:

1) Please provide some information about NP family in the introduction. You may also discuss the obtained results compared to NTproBNP which is also proved to have predictive power (especilly with

ST2/Pentraxin-3).

Reply: We have added some information in the introduction on the natriuretic peptide family (page 6, line 2-4). In the present study, we measured proANP instead of NT-proBNP, and adjusted for proANP in the multivariate Cox regression models. MR-proANP has been shown to be non-inferior to NT-proBNP as biomarker of heart failure (Maisel et al. JACC 2010). However, as the ANP gene is also driven by hypoxia (a HIF motif in the promoter region) in the absence of heart failure, we believe our choice of biomarker makes the most sense in STEMI; both in terms of underlying heart failure and in terms of ongoing myocardial hypoxia (Goetze JP. Coronary artery disease, heart failure, and cardiac natriuretic peptides in the middle. Eur Heart J 2005;26:2603-4).

2) I would suggest to provide proCNP high concentration in female effecasy check (specifisity and sensitivity) concerning mortality.

Reply: We have now added information on sensitivity and specificity in the Supplemental Material (page 10, line 6-9).

3) Why have you analazed cardiovascular mortality? It is expected for proCNP to have bigger impact on mortality

Reply: We agree that cardiovascular mortality would be an interesting outcome. Unfortunately, we only have information on all-cause mortality within one year. However, we feel confident that the one-year motality following AMI mostly will reflect cardiovascular death.

4) I would suggest to draw the design of the study in the figure.

Reply: Thank you for the constructive suggestion. We have made a figure of Study Design and added it to the manuscript (Figure 1, page 34)

5) I would suggest to introduce also GFR in the tables and change Kidney diseases to CKD ....

Reply: We have now added data on estimated GFR to Table 1 and changed kidney disease to chronic kidney disease (CKD) throughout the manuscript.

6) In discussion you speculate about menopausal impact on mortality but do not prove the results for > 50 year female cohort.

Reply: As you note, we do speculate in the discussion section on that high concentrations of proCNP in elderly females may be partly related to a disadvantageous vascular transition after the menopause. Unfortunately, we do not have data on menopausal status in the present study and we, thus, cannot examine a potential association to proCNP concentrations. We hope that the reviewer will allow this speculation from our side, where the difficult matter for future clarification will be to collect samples from women with STEMI prior to menopause (it is not common).

After answereing the question/corrections above the manuscript could be published.

Reviewer: 2
Dr. Gustavo N. Araujo, Imperial Hospital de Caridade Comments to the Author:
Major comments:
Comparison between male and female patients overall baseline characteristics are not shown in this paper. Thus, conclusions about sex differences are unsubstantiated. In order to have valid conclusions, the authors should have performed, for example, a multivariate Cox model and

propensity score methods (i.e. sex and age-matched controls of a female cohort).

Reply: Our study demonstrates an interaction of sex and proCNP concentrations on mortality of STEMI patients and the primary aim of our manuscript is to show that proCNP measurement holds a distinct prognostic and descriptive value for females, that is not replicated in males. Thus, we have not focused on overall comparisons of female and male patients with STEMI as this has been the objective of several other studies and is therefore well-described.
We do indeed perform multivariate Cox models (Model 1 and 2 of Table 2) with adjustment for a number of other variables. The purpose of propensity score methods (as suggested) would, to our knowledge, be to compare proCNP measurement of female STEMI patients to matched females in a cohort without STEMI, to test whether a predictive value can be extrapolated to females more generally. This is certainly an interesting question but is beyond the scope of the present study. We believe that our conclusions reflect that the findings only concern patients with STEMI.

The sex differences found seem to have been an exploratory analysis, and it is shown as a main result in title and conclusions. Thus, it should me more exposed in the objectives and methods section (was it pre-specified?).

Reply: The difference in the predictive potential of proCNP between females and males was an exploratory finding. We have now changed the text of MAIN OUTCOMES in the methods section to clearly reflect that sex-specific findings was not a pre-specified hypothesis (page 9, line 13-16).

While performing mortality analyses only on proCNP concentrations ≥median, I am not sure if the authors can have such conclusions about overall proCNP prognostic capacity.

Reply: We appreciate your comment. In the revised manuscript, we have now corrected the text of the conclusion of both the abstract and “Conclusions” to reflect that the independent predictive potential of proCNP measurement solely concerns proCNP concentrations ≥median (page 4, line 6 and page 20, line 6).

The results and discussion are not displayed objectively. There is too much information, too many analyses, but the reading has no flow.

Reply: We have sub-titled the results section to improve the reading flow.

The idea of the study is interesting and the number of patients is probably enough. However, I will recommend major revision with proper statistical analyses.

Specific comments:
Abstract, page 3, line 9: the authors should define the primary outcome: 30-day vs. one year mortality. Sample size should be calculated based on one of the outcomes.

Reply: We have corrected the text of the abstract, where one-year mortality is defined as the primary outcome (page 3, line 9-10). Regarding sample size calculation: see later comment.

Introduction, page 6, line 5: Although CNP plays a protective role in cardiac pathophysiology, it was a predictor of mortality in your study. Please comment.

Reply: Thank you for the comment. In studies of the predictive potential of NT-proCNP in acute coronary syndrome and heart failure, high concentrations are also associated with adverse outcomes despite the beneficial cardiovascular effects of CNP. Thus, increases in proCNP may be a compensatory response to cardiovascular disease. We have added this aspect to the Introduction

section (page 6, line 17-18).

Introduction, page 6, line 18 (third paragraph): the authors include population details and outcome measurements, which should be detailed in the methods section.

Reply: We have removed inclusion details and numbers from the introduction (page 7, line 4-13).

Methods, page 7, line 17. Reference intervals of CNP should be detailed further (section “biochemical analyses).

Reply: Details on reference intervals of proCNP are in the Supplemental Material. We have added a reference to this in the main manuscript (page 10, line 3-4).

Methods, page 8, line 18. Please specify more details about biochemical measurement (i.e. timing of blood withdrawal).

Reply: Details on biochemical measurement are in the Supplemental Material. We have added more details in the main manuscript (page 8, line 13-15) and in the Supplemental Material (page 1, line 13).

Methods. Outcomes (primary, secondary) are not detailed on this section.

Reply: We have added a paragraph on main outcomes in the Methods section (page 9, line 13-16).

Statistics, page 9, line 7. Reference population should be described above, in methods section.

Reply: We have described the selection of reference individuals in the beginning of the Methods section. For consistency purposes, statistical information on calculation of reference intervals is kept in “Statistics” of the Methods Section.

Statistics, page 10, line 12. Multivariable cox proportional hazard model inclusion criteria is not clear.

Reply: We appreciate your comment. We have elaborated the text to clarify that the inclusion of only patients with a proCNP ≥median is based on assessment of smooth spline plots of the hazard rate based on proCNP as a continuous variable. Also, we have specified that median cut-off values are based on the sex- and age-specific concentrations of the reference population (page 11, line 2-7).

Statistics. Sample size calculation is recommended.

Reply: We agree that, generally, sample size calculation is preferable prior to conduction of a study. In our study, the STEMI cohort was established for the investigation of biomarkers for predicting late cardiogenic shock in a different substudy. Therefore, a sample size calculation with respect to proCNP mortality prediction was not done. Still, given the size of our cohort, we find that our study has sufficient power to thoroughly examine the sex-specific predictive potential of proCNP.

Results, page 11, line 6. The first paragraph of the results section show results of another study.

Reply: We have subtitled and changed the wording in the Results section to improve clarity (page 12, line 1-2).

Results, page 11, line 17. Decreased proCNP concentrations are probably variations within normal values, and should not be excluded from analysis.

Reply: Decreased proCNP concentrations may be variations within “normal” concentrations. However, we find it necessary to be consistent about cut-off values for both increased and decreased proCNP and not reclassify patients based on speculations. Since only five patients have a decreased proCNP concentration, either approach lead to comparable conclusions.

Results. A flowchart with included and excluded patients is recommended.

Reply: We agree and a flowchart has been added to the Supplemental Material (Figure 1, page 3-4).

Table 1: Too much information.

Reply: We have moved information on culprit vessel, number of vessels affected and TIMI flow to the Supplemental Material.

Table 2: It is unclear if the ProCNP used in the model is quantitative (per 1 pmol/L increase) or qualitative (proCNP > median).

Reply: Thank you for the comment. We use proCNP ≥median as an inclusion criterium in Cox regression model to test the predictive value of proCNP as a continuous variable (per 1 pmol/L increase). We have added a clarifying text to Table 2 (page 31).

Table 3: models 1 and 2 are not explained.

Reply: Models 1 and 2 are multivariate models testing other variables than time to explain changes in proCNP concentration. We have made some changes in the text of the table to clarify the models (page 33).

Reviewer: 3
Dr. Jian Yang, Department of Cardiology, the First Affiliated Hospital, College of Medicine, Zhejiang University Comments to the Author:
Introduction:
1. Studies have explored the proCNP prognostic value in general population and ACS patients. What is the significance of the author's further research in the STEMI population? Obviously, the population in this study has higher selectivity and fewer clinical end points observed. The author should introduce the innovation and clinical significance of this research in depth.

Reply: Our study is the first to investigate the prognostic potential of proCNP-derived peptides in plasma in an acute setting, where plasma is sampled just after a myocardial infarction and before coronary intervention. Previous papers have reported on the long-term prognosis, where proCNP is measured in a stable phase. Thus, our approach provides novel insight into the association of the plasma proCNP concentration immediately after the event and mortality (covering both the first 30 days, where mortality is highest, and the subsequent 11 months). Moreover, by including a large and well-characterized cohort of only patients with STEMI, we have sufficient power to demonstrate sex-specific differences within this disease, where males constitute the majority.
We have made some changes in the Introduction to highlight these aspects (page 6-7, line 18-8).

Methods:
1. In the Ethics section, the author should give the complete ethics committee name.

Reply: We have added the complete name of the ethics committee (page 9, line 2).

2. Although the design of the cohort has been previously reported, is the study registered on the

relevant website? If so, it should be stated.

Reply: No, the study is not registered on clinicaltrials.gov as we were not aware of this aspect, when the Cohort was established.

3. The author analyzed longitudinal plasma sample data in a subgroup of 287 people. However, how was the 287 people obtained? Is it randomly selected from the total cohort, or is there only 287 people with longitudinal plasma samples? Related content should be stated in the methodology.

Reply: The longitudinal plasma samples were obtained from a consecutive subgroup of the cohort from January to March, 2016, at Rigshospitalet. The 287 patients are all the patients from the subgroup with longitudinal plasma samples, so they are not randomly selected. This information is provided in Supplemental Material, where we have also added some text (page 1, line 14-19).

Results:
The results section should be subtitled.

Reply: We have added sub-titles in the results section.

Discussion:
1. Can the conclusions obtained in the reference cohort based on the Nordic sample database and the Danish cohort be extrapolated to the general population? The author should summarize the relevant literature and discuss the differences of CNP among races to explore the generality of the conclusions of this article.

Reply: Thank you for a highly relevant comment. A couple of aspects make it difficult to speculate whether our results can to extrapolated to other populations. Firstly, the vast majority of patients from the STEMI cohort and individuals from the reference population are Caucasian. Secondly, few studies have investigated circulating proCNP-derived peptides in population or cohort studies, partly due to the scarcity of reliable methods of measurement. We have added a text in the Discussion describing this aspect (page 18, line 3-6). As the reviewer correctly addresses, differences between races are, unfortunately, not studied enough.