**Peer review file**

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**Reviewer A**

**Comment 1:** Small sample size and low number of events.

**Reply 1:** Small sample size of our single-center study is associated with the inclusion criteria – we evaluated patients with first presentation of acute myocardial infarction. We attempted to evaluate a novel hypothesis that copeptin concentration assessed at two different time points might have prognostic significance in patients with AMI. We are fully aware that our results are preliminary and should be treated with attention to the limitations of a small study group. Although small sample size and low number of events may limit the external validity of the study, we believe our results may indicate an interesting direction for further research. We have emphasized this limitation of our study and discussed it in context of future research in this topic.

**Changes in the text 1:** We have rephrased the paragraph "Study limitations" adding information presented above (see Page 16, Line 3-11 )

**Comment 2:** When assessing the impact of a new factor on outcomes it is important to show what does this factor adds to the currently recommended prognostic tools. In this sense which variables were included in the multivariate analysis. What about GRACE score? Coronary anatomy? Residual SYNTAX score?

**Reply 2:** We would like to thank the Reviewer for this valuable comment. The following laboratory and clinical factors were included into the multivariate analysis: copeptin – 1,

copeptin – 2, NT-proBNP – 2, CRP – 1, CRP – 2, creatinine, ejection fraction (EF) and age. Residual SYNTAX score was not incorporated in the analysis but we have included the presence of multi-vessel disease. We have also calculated and included the GRACE score. GRACE score emerged as a prognostic factor for MACE in univariate analysis but did not change the results of multivariate analysis. These data required clarification in the manuscript so we have supplemented the missing information as decribed below. We have also presented the results of the multivariate analysis in Table 4.

**Changes in the text:** We have explained abbreviations CRP – 2 and NT-proBNP –2 (see: Page 7 line 8-14), we have added data about the GRACE score in univariate analysis (see: Page 10, line 18-19) and factors included in the multivariate analysis (see: Page 10, Line 23-25). Multivariate analysis results are presented in Table 4.

**Comment 3**: In the abstract, the authors describe an association between copeptin and outcomes however no objective data are provided.

**Reply 3:** We are grateful for pointing this out. We have included the results of the analysis in the abstract.

**Changes in the text:** We have added the results of the statistical analysis (see Page 2, Line: 12, 18, 20-21, 24)

**Comment 4**: Which are the clinical implications of these findings?

**Reply 4:** We appreciate this question as it forced us to present our results in a broader contex. In this study we attempted to clarify whether copeptin, used so far as a diagnostic biomarker supporting cardiac troponin in patients with chest pain, might have prognostic value in patients without preexisting heart failure – in our research represented by a cohort of patients

with first presentation of acute myocardial infarction. We hypothesized that copeptin release in response to myocardial injury could provide information about the course of hospitalisation and outcomes after PCI. What is more we attempted to check if second assessment of copeptin, 4/5 days after PCI, could indicate the effectiveness of implemented treatment and predict the incidence of MACE. Although high-sensitivity cardiac troponins marginalized the diagnostic usage of copeptin, its potential in prognosing adverse cardiovascular events could justify additional laboratory test in patients presenting with AMI. Our results suggested that copeptin concentration assessed 4/5 days after successful PCI in first myocardial infarction may have prognostic significance in one-year observation. As a non-specific marker of endogenous stress it may reflect complex processes that may influence the prognosis after AMI. If these findings are confirmed by studies on larger groups, copeptin assessment could indicate the need for more intensive treatment and more frequent post-hospital monitoring of patients with first AMI in their lives.

**Changes in the text:** We have modified our text in the Discussion section (see: Page 15, Line 8-11, 13-15, 17-23).

**Reviewer B**

**Comment 1**: Good points: First presentation with AMI, Interventions preformed within recommended time frame for NSTEMI and STEMI, no loss to follow-up.

**Reply 1:** Thank you very much for emphasizing this. What is more we sincerely appreciate your time and effort to write all the following thoughtful comments that were extremely helpful in improving our work and enabled us present our results in more understandable way.

**Changes in the text:** No needed for this comment.

**Comment 2**: A mixed cohort though mostly STEMI (84%) though. Would results change if analysis was performed in the STEMI group on its own?

**Reply 2:** We appreciate this comment because it pointed out that more detailed information is needed. As stated in the manuscript, we evaluated consecutive patients admitted to our hospital who met the criterion of first presentation with acute myocardial infarction and required PCI as a primary strategy. This group included STEMI patients and patients with symptoms of ongoing ischemia who finally did not meet STEMI criteria. Because both groups were treated accordingly to current recommendations with primary PCI, we analyzed them together. During analyses we have noted that NSTEMI group had higher concentration of copeptin and being aware of inconclusive data in this subject in the literature, we presented our observation. The aim of this study was to evaluate copeptin as a prognostic factor in patients with first AMI treated with current standards. Separate analysis depending on the type of myocardial infarction require the inclusion of larger group of patients and further research. Because the presentation of this issue in the manuscript was too succinct and could therefore be unclear for the readers, we have clarified this information.

**Changes in the text:** We have added modified the text as stated above (see: Page 5, Line 21-22; Page 8, Line 8-11).

**Comment 3**: Why did patients have a long hospital stay? Does not appear to be clear from the results of the discussion – I note however that 30% had a Killip class II-IV.

**Reply 3:** In our hospital AMI patients treated with successful PCI are discharged after 4/5 days if no complications occur. Average time of hospitalisation was 6,85 ±3,31 days. Hospital stay of 10 days and more concerned 13 patients and was due to cardiogenic shock in 5

patients, 3rd – degree atrioventricular block in 3 patients, acute kidney injury in 3 patients, mechanical complications of myocardial infarction (free wall rupture and cardiac tamponade) in 1 patients and pericarditis in 1 patient. This information has been included into the manuscript. We would like to emphasize that copeptin – 2 concentration was assessed at a fixed time point, independently of the time of hospital stay.

**Changes in the text:** We modified Table 1, adding the information about the length of hospitalisation (see: Table 1) and added the rest of information in the manuscript (see: Page 10, Line 3-6).

**Comment 4**: Copeptin measured on day 4/5 of admission might not have been the best time for rechecking copeptin – as stated by the authors, copeptin peaks within 4 hours of onset of symptoms so maybe copeptin should have been checked 6 hours and 24 hours from intervention to be more meaningful.

**Reply 4:** Thank you for pointing this out. In fact, in the presence of AMI, copeptin highest concentration is observed early after the onset of symptoms and then it decreases rapidly within first 24 hours. This temporal release pattern of copeptin in AMI was noted in several studies independently of the type of implemented therapy (coronary intervention, thrombolysis, conservative treatment). Moreover, the decrease in copeptin concentration within 24 hours from ischemic stimulus was observed also by Liebetrau et al. in patients with hypertrophic obstructive cardiomyopathy undergoing transcoronary alcohol ablation of septal hypertrophy as a model of AMI. In that case myocardial damage was permanent and still the decrease in copeptin concentration was observed and its release kinetics was similar (1). The explanation for this may be organism adaptive reaction for endogenous stress stimuli or pain relief, although the exact mechanisms remain unknown. Selecting time points at which second measurements were done, we based on the assumption that maintained activation of the

vasopressin system after AMI (until standard discharge time after 4/5 days of hospital stay) may indicate future unfavorable events, as it was observed in patients with heart failure. Although all the subjects in our study underwent coronary intervention and received recommended pharmacological treatment, copeptin levels on day 4/5 were higher than on the admission to the hospital and ultimately seemed to have prognostic implications in patients with first AMI.

(1) Liebetrau C, Nef H, Szardien S, et al. *Release kinetics of copeptin in patients undergoing transcoronary ablation of septal hypertrophy.* Clin Chem. 2013 Mar;59(3):566-9.

**Changes in the text 4:** No needed for this comment.

**Comment 5**: There was no clear relation of copeptin level to time of reperfusion as well which is a limitation.

**Reply 5:** In order to properly address this comment, we clarified the way these data were presented. As stated in the manuscript (page 8, line 11-12) the time since onset of symptoms to coronarography (defined by the moment of arterial sheath insertion) was taken into account in the analysis. As the data in Table 1. may not be presented clearly enough, we have rephrased it. In our study no correlation between time of symptoms onset and copeptin concentration determined with the Spearman's rank correlation test was observed. However, if patients were divided into four groups according to the time of symptoms onset, highest median copeptin value was observed in patients presenting between 3 and 6 hours after the symptoms started (however statistically not significant), supporting findings from other studies. We have presented these data in Table 3.

**Changes in the text 5:** We have added the above data in the text (see: Page 9, Line 1-4) and in Table 3.

**Comment 6**: A significant number of patients had moderately and severely impaired LV function (72%, and 22% respectively) which raise the question: is this a higher risk group already? (NB: at least 50% of the patients had 3 risk factors for CAD), and note long hospital stay again could be related to higher incidence of complications in the recruited cohort. Ejection fraction is related to MACE according to this study in its univariate analysis with a p value of 0.0087 which was more significant than the association with copeptin with a p value of 0.043.

**Reply 6:** The study cohort included patients with first presentation of AMI in their lives and without known previous LV function impairment. LV function was assessed by transthoracic echocardiography performed before discharge. Patients' profile in terms of cardiovascular risk factors in the study cohort was consistent with that in general population of AMI patients in our hospital. Long hospital stay was associated with complications described in reply to comment 3 (above). Copeptin as a nonspecific marker of endogenous stress may reflect the combined influence of various factors on the occurrence of complications, although it did not directly correlate with any of the above. We have presented data about the correlation of copeptin and LV systolic function in Figure 3. In our study, in both univariate and multivariate analysis, copeptin and EF were statistically significant predicting factors for MACE with p values < 0,05, as stated in the manuscript.

**Changes in the text 6:** We have added Figure 3 and Table 4 to the manuscript.

**Comment 7:** Was there any relation between EF and copeptin? Needs to be clarified.

**Reply 7:** We have followed reviewer's instructions and added information about the correlation between EF and copeptin. In the manuscript (page 9, line 11-13) we have stated

there was no relation between copeptin and LV systolic function (considered as preserved, moderately or severly impaired) but we have completed the data presenting results of Spearman's rank correlation test between EF and copeptin – 1 (p=0,428) and copeptin – 2 (p=0,306).

**Changes in the text 7:** We have modified the manuscript text (see Page 9, Line 11-15) and added Figure 3.

**Comment 8:** 136 consecutive patients in a 12 months period? Is this a low-volume center? Complications related to this which may explain the paper findings.

**Reply 8:** We understand reviewer's concern. In the study, period consecutive patients (excluding weekend admissions) were evaluated in terms of first presentation of AMI and requirement of primary percutaneous coronary intervention strategy. The total number of AMI patients treated with PCI in our hospital is a result of a high concentration of interventional cardiology centres in our region. According to our database, there are approximately 400-450 PCIs performed in patients with acute myocardial infarction (STEMI and NSTEMI) every year in our hospital which stay in line with data presented in a summary report of the Association of Cardiovascular Interventions of the Polish Cardiac Society and Jagiellonian University Medical College (1). Our research is a single-centre study based on a relatively low number of patients and our observations are preliminary and definitely require validation in larger, multicentred studies. The small number of observations and several factors taken into account in a multivariate analysis may have had impact on the results. However, we believe our work could bring interesting and valuable information in the area of research on the importance of copeptin in patients with myocardial infarction. We focused on the prognostic value of copeptin in patients with AMI treated with current standards, and there is very little literature available on this subject so our results may indicate an interesting

direction for researchers.

**Changes in the text 8:** No needed for this comment.

**Comment 9:** NSTEMI having significantly higher levels was strange – why? Not clear.

**Reply 9:** The data available in the literature do not clearly indicate which type of myocardial infarction (STEMI or NSTEMI) is associated with greater copeptin secretion (see References 102, 110, 113, 134, 143 in the Manuscript). Although myocardial injury related to coronary artery total occlusion probably leads to higher copeptin release, NSTEMI patients in our cohort were high-risk patients with ongoing chest pain and dynamic ECG changes. Our findings are in line with the results presented by Afzali et al. The above explanation were included in the discussion.

**Changes in the text 9:** We have added the above text into the manuscript (see: Page 13, Lines 3-9).

**Comment 10:** Was there any correlation of copeptin with blood pressure? This was not mentioned. Also, were there any cases with cardiogenic shock in this cohort?

**Reply 10:** Thank you very much for your careful analysis that helped us present better our results. Copeptin did not correlated with systolic blood pressure on admission (r=-0,585, p=0,563). In our cohort there were 13 cases of cardiogenic shock. Patients with cardiogenic shock were not different in terms of copeptin –1 and copeptin –2 concentration from subjects without this complication (p=0,098 and p=0,264, respectively) – we have added this information in the text.

**Changes in the text 10:** We have added above information (See: Page 9, Line 15-18)

**Comment 11**: Table 1 is listing the number of vessels involved: what is the difference between 3VD and multi-vessel disease? This needs to be clarified.

**Reply 11:** Thank you very much for pointing this out because in fact for readers this could be unclear. According to the definition of multi-vessel disease (MVD) we have included into this group patients with significant luminal stenosis (>70%) in at least two major coronary arteries. Although the definition of MVD comprises also significant stenosis (>70%) in one major coronary artery in addition to 50% or greater stenosis of left main trunk, there were no such patients in our cohort. To avoid confusion, we have combined these two groups into one in Table 1.

**Changes in the text 11:** We have modified Table 1.

**Reviewer C**

**Comment 1:** The main problem with the manuscript is the description of the results and discussion of their significance. In this study, I considered that elevated copeptin concentration was identified as a predictor of major adverse cardiovascular events. However, you did not show the main results in tables or figures. You need to review the tables and figures for the results of this study.

**Reply 1:** Thank you very much for your suggestion. We have made the following changes in the manuscript:

**Changes in the text 1:**

- We have added Table 2 presenting correlation of copeptin with clinical and demographical variables

- We have added Table 3 presenting data on copeptin concentration in relation to time since the onset of symptoms
- We have added Table 4 with the results of multivariate analysis
- We have added Figure 2 presenting changes in copeptin concentration in individual patients.
- We have presented the correlation between copeptin – 1 or copeptin – 2 concentration with left ventricular systolic function in Figure 3.

**Comment 2:** Did you need to analyze the ROC curve for the relationship between the incidence of AF and the copeptin concentration shown in Figure 2? Because the result was not the main purpose of this time, I think the emphasized result would confuse the conclusion.

**Reply 2**: We appreciate your comment. In our study, we were analyzing the course of the entire hospitalisation in relation to copeptin concentration to determine its in-hospital prognostic value although in fact it was not the main purpose of the study. We rephrased the Results and Discussion paragraphs so as not to confuse the conclusion, as advised.

**Changes in the text 2:** We have removed the analysis of the relationship between copeptin and AF from the Discusion (see Page 13, Line 4-24), removed part of the data from the Results (see Page 9, Line 21-23) and removed Figure 3.