Author's response to reviews

Title: The role of urine neutrophil gelatinase - associated lipocalin (NGAL) in acute heart failure in patients with ST - elevation myocardial infarction

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Author's response to reviews: see over
Dear Editor

We thank you for considering the submitted paper “The role of urine neutrophil gelatinase-associated lipocalin (NGAL) in patients with ST-elevation myocardial infarction”. We have considered the reviewer’s well pointed comments and revised the manuscript accordingly. The responses to individual suggestions and concerns (written in italic letters) are detailed below.

Response to Mr/Mrs Tinne Tranberg review

“The sample size is very small (n=61). The information about the participating patients is limited and the information about the non-participating patients has not been reported. Why are only 61 STEMI patients included during April 2010 to July 2011? Selection bias? I would say this is quite few. What are the inclusion and exclusion criteria? Are there differences between those included in the study and those who were excluded or not participating (e.g. chronic heart failure chronic lung disease, thyroid dysfunction)? How many of the participating patients had chronic heart failure (HF) prior to STEMI or enrolment?”

The inclusion criteria were STEMI for which a PPCI was performed. Exclusion criteria were prior malignancy, infectious disease, sepsis and death or transfer to another hospital within 24 h of admission to the ICU. The latter is the main cause for the seemingly low number of patients, because many do not require ICU treatment and are transferred back to the referring hospital soon after PPCI.

We have no reliable data on prior heart failure or echocardiography in our participants. They were admitted in an emergency setting and many either had not past medical data on cardiac function or this information could not be obtained and we therefore decided not to include it in the analysis because of bias.
“The authors have reported their results as mean ± SD in the text and in the tables. However, it is doubtful whether the values of NGAL, Troponin I, NT-proBNP, creatinine etc. are normally distributed due to the small sample size and the subdivision into small groups; n=41 vs. n=20 and n=52 vs. n=9. In case of non-normally distributed data the Student’s t-test is not the correct choice of a statistical test.”

We thank the reviewer for this comment. We revised the statistical analysis and used the Mann Whitney test for comparisons of numeric variables between subgroups. The outcomes of the study have not changed.

“In general the legends of the tables should include a statement of how the results are presented (mean ±SD etc.).”

The tables have been corrected.

“Table 3. Killip >2 (%): I am not sure I understand the percentage 28 and 78 = 106%? And in the Result section the authors state that 36% of the STEMI patients were in Killip classes # II-IV.”

There was a typing error and the numbers have been corrected.

“A limitation section is lacking in the manuscript.”

A discussion of the study limitations has been added to the manuscript.

“The quality of the written English is below standard. The text would benefit from an English Editing revision, as there are several grammatical errors, which is an impediment to understanding.”

The text has been edited and we believe the standard of the language is improved.
“The title does not clearly express what is being investigated (markers of heart failure).” We suggest the title be changed to “The role of urine neutrophil gelatinase – associated lipocalin (NGAL) in acute heart failure in patients with ST – elevation myocardial infarction”.

The title has been changed.

“In the methods section, the authors have decided to use the cut-off urine NGAL level of 50 ng/ml. I miss a statement why the authors use this particular cut-off value?”

When we began analyzing data, we noted low urine NGAL levels in almost all participants, but higher levels in those with high NT-proBNP; it seemed that higher values in our sample, which were still well below the usual cut-off 137 ng/ml, were specifically associated with heart failure. We therefore constructed an ROC curve with NGAL as the test and NT-proBNP as the state variable, which confirmed high specificity of NGAL. The desired 90% specificity was reached at 46.95 ng/ml. The cut-off 50 ng/ml was therefore used in statistical analysis in addition to the 137 ng/ml limit.

“In the result section it would be appropriate to report the number of patients in each Killip class I-IV.”

This information has been added to the result section.
Response to Mr Josef Dankiewicz review

“Although not necessary to assess correlation I would suggest that the authors consider some type of multivariate analysis to predict acute heart failure. At a minimum this would require a logistic regression model with comorbidities, age and troponin levels. Does including NGAL improve the model?”

In a multivariate regression model NGAL was associated with NT-proBNP as a marker of heart failure and this has been included in the revised version.

“I would recommend reducing the number of variables in all tables. Is it necessary to show data on leukocytes and CRP and three different time points while there is only data on NGAL at admission and 12h? Additionally I recommend that they align the text to the left.”

We thank the reviewer for this comment, the tables have been redesigned.

“The authors should consider what their main question is. Is it the correlation between NGAL and inflammation, or NGAL and development of acute heart failure?”

The main question of the study is association of NGAL with development of acute heart failure after myocardial infarction.

The association of NGAL levels with inflammation and of inflammation with heart failure has been well established in previous studies and we have included CRP levels and leucocyte count in the statistical analysis as possible confounding factors. In our sample, NGAL levels are correlated with CRP level and leucocyte count, but this was not the hypothesis in our study. We have re-written the paper with a hopefully clearer focus on the main study question.
“In my opinion the results section is too cluttered with numbers. I recommend reducing the number of results displayed in this section, especially when they are available in a table.”

We thank you for this comment and have reduced the numbers of results in the result section.

“I would suggest changing the term correlation to association when discussing a statistical significant chi2-test.”

The term has been changed.

“The authors should make the main results in table three clearer by moving them to the top of the table. In my opinion EF, Killip >=II, and NT-proBNP are the most interesting results.”

Table 3 has been designed in accordance with this suggestion.

“In reading work on biomarkers it can be quite difficult to appreciate the distribution of the (urine) levels through numbers alone. The authors should include a boxplot or scatterplot of the urine levels of NGAL. This would ideally be dichotomized by acute heart failure. Further, regarding the distribution of NGAL I think it necessary to formally test for normality with a statistical test. If the assumption does not hold differences in continuous variables should with a non-parametric test (the authors already use a non-parametric test for correlation: spearman Rho). The distribution should be shown with IQR.”

The scatterplot of urine NGAL levels is now included in the paper (Figure 2) and the distribution is shown with the inter-quartile range as suggested by the reviewer. The statistical analysis has been re-done using non parametric tests.
“The authors state that NGAL levels correlated (were associated) with acute HF. Although they only state association the obvious clinical use of a biomarker in this case would be to predict acute HF. So even if they don’t state correlation I would like them to comment on the possible confounding due to acute kidney injury (which has been the main use of NGAL in prior studies). It seems to me that large infarctions, longer PCI-times and more lesions are associated both with acute HF and acute kidney injury due to cardiogenic shock. The authors should comment on this and provide data on the incidence of cardiogenic shock and mortality.”

We have now stated more clearly that the source of urine NGAL cannot be determined by our study design and discussed chronic heart failure and kidney injury as probable confounding factors. In our samples urine NGAL levels were specifically associated with acute heart failure and were independently associated with BT-proBNP concentration. More and more studies are interested in early elevation of urine and serum NGAL in patients with acute heart failure and, we agree, whether or not it is the combine effect of heart and kidney failure still has to be investigated.

We have some data of incidence of cardiogenic shock (6 of our patients were in Killip class IV) but no relevant data of mortality.

“I would like the authors to comment on how they arrived at the cut-off value of 50ng/ml. Do they have a reference for this? It not they should clearly state how they arrived at that level.”

When we began analyzing data, we noted low urine NGAL levels at admission and after 12h in patients who did not develop heart failure. In the next step, it seemed that higher values in our sample, which were still well below the usual cut-off 137 ng/ml, are specifically associated with heart failure. We therefore constructed an ROC curve with NGAL as the test and NT-proBNP as the state variable, which confirmed high specificity of NGAL. The desired 90% specificity was reached at 46.95 ng/ml. The cut-off 50 ng/ml was therefore used in statistical analysis in addition to the 137 ng/ml limit.
“The authors provide information on some co-morbidities but prior heart failure and ejection fraction, which is probably the most important factor in this case are not included. If pre-STEMI ejection fraction or NYHA-class is not available I would suggest a binary variable, CHF before STEMI or not.”

We agree with this and other reviewers that information about previous heart failure would be important, but we assessed that the available data were not reliable enough and decided not to include them in the analysis.

“The authors state that mean creatinine was normal. I would be interested in the incidence acute kidney failure and/or the use of renal replacement therapy (if any).”

None of the participants had acute kidney failure and none need renal replacement therapy.

“The authors should comment on what normal values of NGAL are in healthy individuals. Does a urine level of 50ng/ml constitute a meaningful elevation? What is the margin of error in the assay?”

Normal values in healthy individuals are 131.7 ng/ml and we had intra-assay coefficient variability of 4.6 %.
We found only two patients with urine NGAL levels higher than normal, therefore we decided to construct a ROC curve with NGAL as the test and NT-proBNP as the state variable, which confirmed high specificity of NGAL. The desired 90% specificity was reached at 46.95 ng/ml and so we used cut-off 50 ng/ml in statistical analysis.
Responses to Sebastian Wiberg review

_The authors should comment on the limitations of the study in the discussion section.”_

A section on study limitations has been added to the manuscript.

_“Questions for the authors: The authors write that they included 61 consecutive patients in a 15 months period. Does that mean that only 61 patients had PPCI performed on the indication “STEMI” in a 15 months period, or were the included patients in fact not consecutive?”_

We did include all consecutive patients who met the inclusion criteria. The main reason for the seemingly low number of patients is the criterion they had to stay in the ICU for at least 24 hours after PPCI and many patients are transferred to regional hospitals in less than a day.