Author’s response to reviews

Title: Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation

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Author’s response to reviews: see over
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RE:
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Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation
Nils E Magnusson, Mads Hornum, Kaj A Jørgensen, Jesper M Hansen, Claus Bistrup, Bo F Rasmussen and Allan Flyvbjerg

Dear Christna Chap, PhD
Executive Editor
BioMed Central

Thank you for your kind Email regarding our delayed manuscript. Please find enclosed our revised manuscript entitled “Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation”

We would like to thank you for the thorough review of the paper and we recognize the importance of the points addressed by the reviewers and have therefore changed the manuscript accordingly. We have highlighted our responses (in bold) to the reviewer’s comments (in italic) below and we hope that you will find the corrections sufficient for publication.

Kind Regards

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Editorial request:
The name of the body which gave ethical approval is stated on page 4, line 19-20.

Reviewer Jirka Grosse:

In their manuscript entitled “Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation” by Magnuson NE et al. the authors showed that plasma NGAL is a novel marker of kidney function. They pointed out the correlation to serum creatinine, eGFR and duration of ESRD and to serum creatinine and eGFR after transplantation. This article is very important in the field. It should be accepted after a minor revision.

Few revisions appear necessary:
In the introduction the authors should give some epidemiological data concerning the incidence of nephropathy, dialysis and transplantation.
Page 3, line 17: Please write 'nuclear factor kappa B'.
Page 3, line 24: Please explain 'AKI'.
Page 5, line 22: Please write ‘...were stored at -80 °C’.

Abbreviations should be introduced only once. Please check the manuscript completely.
The layout of the figures is not suitable for publication. Please use a consistent layout (including consistent font) and a better resolution if possible.

We have performed the minor corrections indicated by the reviewer

Reviewer John Sayer:

Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation. This manuscript examines a cohort of CKD and ESRD patients. This patient group has previously been used for other published studies. The aim of the study is to “to investigate the possible relationship between plasma NGAL levels and clinical parameters in a prospective study of non-diabetic uremic patients.” I am not sure this aim has been fulfilled in the manuscript in its present form. The clinical parameters are not well described and there is too much emphasis within the paper of the methodology of NGAL.
measurement. I am unclear as to the significance of the association between homocysteine and NGAL and the data remains too preliminary for this association to be certain. In absence of this, the association of renal function and NGAL is not a novel finding. Perhaps the study should concentrate of the patients whom received transplants to show that NGAL correlates with rapidly improving renal function. Other interesting clinical questions need to be addressed. For example: Will the NGAL levels ever normalise in a transplant patient with excellent renal function and how long does this take? Will NGAL ever be a valid marker in renal transplantation given the confounders of renal mass, prednisolone therapy etc. A more clinically relevant discussion would be welcome.

Regarding the reviewers concern about the emphasis on methodology we feel that it is highly relevant to validate the method for measuring NGAL protein levels as these data are being used to interpret the relationship to clinical parameters. Wrongful measurements could skew the data and hence the interpretation. There is at present no golden standard for measuring absolute NGAL levels and we therefore think it is important to compare different measurement methods in terms of their relative performances.

In the paper we indicate that NGAL and kidney status does not seem to normalize in transplanted patients. Also, the data show that there are indeed significant difference between normal NGAL levels and eGFR and those found in transplanted patients after three and 12 months (see also table 2) suggesting that these parameters and hence kidney function will not be completely restored. We do not know the clinical importance of the association between NGAL and homocysteine which is indicated in the discussion with several references to previous findings.

Major comments:

Figure 1 is not well drawn, and needs to be redraw using different scales for the different time points, or an alternative method needs to be adopted to show the change in NGAL over time, perhaps as a time line for each individual?

Figure 1 has been redrawn and now includes an insert showing the time points three and 12 months with higher resolution.

Figures 2 and 3 relate to the NGAL assay and its methodology. This manuscript should focus on the clinical relevance of the results rather than be a methodological one.

Please see above
Figure 2 – lines on graph are not labeled and legend is insufficient to give meaning to the graph.

Figure 2 has been redrawn and graphs are labeled. The legend has been rewritten and a paragraph has been added to the result section, page 10, line 8-11.

Methodology includes no mention of homocystine measurements. Are patients who have been re-transplanted at risk of higher homocystine levels? Does homocystine relate to renal mass in any way?

Homocysteine was measured using standard laboratory methods. We have not been able to conclude that re-transplanted patients are at higher risk of higher homocystine levels or relate this to renal mass.

I am really not sure of the data in table 3; is this relevant to NGAL levels?

Table 3 related to eGFR and blood pressure. The table has been omitted and the significant finding added in the result section, page 9, and line 15-16.

The minor comments have been corrected according to the reviewers’ suggestions.

Concerning the phrase “public announcing”, “duration of renal end-stage disease” and the use of “Tx”. These phrases have been used before in our previous publications. Hence we feel that these phrases will be alright in the present manuscript:

Kidney transplantation improves arterial function measured by pulse wave analysis and endothelium-independent dilatation in uraemic patients despite deterioration of glucose metabolism. See also the reference in NDT below.


Minor comments

Page 2: The sentence “duration of end-stage renal failure” is misleading.

Page 4: “public announcing” needs to be rephrased.

Page 4: It is not clear to me how long before transplantation the NGAL assay was performed.

Page5: OGTT was performed –rather than “done”.

Page 5: -800C should read -80 degrees C

Page 6: “Data analyses using clinical data were done” – performed would be
better.

Page 6: Continuous data were done – performed would be better

Page 6: P value of <0.05 – the p value for each test should be stated as
significant p values will differ from test to test (esp. using non parametric tests).

Page 7- define CV.

Page 8 were hence significantly reduced – rephrase please.

Page 8: may be explained – possibly explained may be better

Page 9: Tx – non universal abbreviation

Page 11: define CVD

Page 11: List of 5 studies- needs to be rewritten. E.g. Firstly, and secondly etc.

Page 11: Typo : longitudinal

Reviewer Johann Bauer:

In the manuscript entitled “Plasma neutrophil gelatinase associated lipocalin (NGAL) is associated with kidney function in uraemic patients before and after kidney transplantation” Magnusson et al. describe a clinical study, which suggests that plasma NGAL is a reliable marker of kidney function. The study was thoroughly performed. The results are highly interesting. The journal’s criteria for accepting a paper appear to be met.

I have only this one concern: In 2011, a few papers appeared, in which a relationship between serum NGAL concentrations and kidney function is described. As far as I see, none of these papers is cited in this publication. Therefore I suggest that the authors should compare their study with similar recent studies and point out the novelty of their research results.

We have included a relevant reference from discussing the use of NGAL as a biomarker in kidney disease by Mike Smertka and Jerzy Chudek, 2011.