Author's response to reviews

Title: High levels of mannose-binding lectin are associated with lower pulse wave velocity in uraemic patients

Authors:

Mads Hornum (mads.hornum@gmail.com)
Jakob T Bay (jakob.bay@regionh.dk)
Peter Clausen (pclaus@mail.tele.dk)
Jesper Melchior-Hansen (jesper.melchior@dadlnet.dk)
Elisabeth R Mathiesen (Elisabeth.Mathiesen@regionh.dk)
Bo Feldt-Rasmussen (Bo.Feldt-Rasmussen@regionh.dk)
Peter Garred (garred@post5.tele.dk)

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Author's response to reviews: see over
Dear Editor-in-Chief

Thank you for the review of our manuscript and for the opportunity to resubmit to BMC Nephrology.

We are grateful for your consideration of publication of our manuscript as an original article and the manuscript has been revised according to the comments of the referees and we find the manuscript much improved by these revisions.

We have added new references with data about normal values of MBL and vascular parameters regarding PWV, AIX, FMS and NID as requested by reviewer 1, and we have added the distribution of haemodialysis or peritoneal dialysis, and the use of an arterio-venous fistulae or a permanently haemodialysis catheter in the cohort, and there were no statistical difference in PWV, ISI, FMD, NID or augmentation index between the groups divided by MBL levels, and we have added new data about calcium, phosphate and PTH and patient characteristics, as requested by reviewer 2 and this did not change our conclusions.

However, data on the use of antihypertensive drugs between groups showed a significant difference, and this have been stressed in the text and in the conclusion.

The revised manuscript is in accordance with manuscript preparation guidelines of your Journal. We will, however, be pleased to assist if you or the referees need supplementary information or changes. All authors have read and approved the final version of the revised manuscript.
We hope that you will consider this revised version of our paper for publication in Nephron Clinical Practice

Yours sincerely,

Mads Hornum MD PhD

On behalf of the co-authors:
Jakob T. Bay MD
Peter Clausen MD PhD
Jesper Melchior Hansen MD, DMSc
Elisabeth R Mathiesen MD, DMSc
Bo Feldt-Rasmussen MD, DMSc
Peter Garred MD, DMSc
Authors’ response to the reviewers’ comments

Below we have responded to the comments from reviewer 1 and 2 giving a detailed point-by-point answer:

Reviewer 1
Manuscript Number: MS: 1400141810124294
This is perspective cohort study to evaluate the relationship between MBL levels and some instrumental and biochemical parameters of arterial stiffness in uremic subjects. Despite PWV is the only parameter correlated with the MBL levels, with statistical relevance, the issues of the paper are of potentially important clinical relevance, for further investigations.

However, a few minor revisions may be better pointed out.

1) I suggest to present the data with the distinction and comparison between hemodialysis and peritoneal dialysis subjects.

Author reply:
We have described the data according to haemodialysis and peritoneal dialysis distribution between the groups divided by MBL levels, and this did not change the results, as pointed out at page 8, line 201-3

2) Which is the normal level of MBL in healthy age-matched people? Please provide a control group regarding the vascular parameters (PWV, AIX, FMD, NID), to better explain the physiology and the importance of the process you want investigate

Author reply:
The normal levels for both MBL and vascular parameters PWV, AIX, FMD and NID was previously examined in age and BMI matched cohorts by our groups and the data has now been incorporated in the discussion on page 9 line 192 and line 197-200.

Reviewer 2:
Reviewer's report:
The manuscript by evaluate a potential impact of MBL on vascular parameters in uraemic patients.

Major limitations
It is well known that factors such as calcium and phosphorus homeostasis, hypertension and inflammation might be responsible for alterations of vascular tissue in patients affected by ESRD yet in the study no mention is given to some very relevant data:
There is no mention to calcium phosphate and PTH values that could be of help and could have a significant influence on vascular elasticity.

Author reply:
We agree with this important observation and have added calcium, phosphate and PTH values to table 1, no significant difference between groups and vascular parameters was seen.

No hint is given on the characteristics of the population in terms of comorbidities amount of antihypertensive drugs taken or in terms of the percentage of patients using peritoneal versus hemodialysis or the percentage of patients on hemodialysis having a fistula rather than a central venous catheter as a vascular access.

Author reply:
We agree with this important point and have added data on patient characteristics in table 1 and in the text on page 8 line 178-180. No difference was seen between groups regarding previous myocardial infarction, stroke and diabetes characteristics. However,
there was a significant difference in the amounts of antihypertensive drugs used, and this has been commented on in the text and the conclusion is modified.

Also no data is given regarding efficiency of depuration which may have a great influence on vasculature and inflammation, furthermore authors don’t even mention the number of patients on hemodialysis rather than on peritoneal dialysis and give no information on the type of hemodialysis rather than peritoneal dialysis used..

Author reply:
The patients were treated with standard dialysis treatment, no difference in efficacy between groups, and inflammation measured by CRP and homocystein was low and not different in/between both groups.
We added information on the number of patients on haemodialysis and peritoneal dialysis and pre-dialysis in table 1, no differences were seen.

Authors conclude that high levels of MBL are associated with lower PWV in a cohort of patients with ESRD awaiting kidney transplantation suggesting a beneficial role of high levels of MBL on arterial stiffness in uraemia, however unless we have better information regarding other possible confounding factors this conclusion lacks part of its strength.

Author reply:
We thank the reviewer for the most valuable comments, and have added new data to our manuscript and have in accordance with these data modified the conclusion.

Minor observations
Check for spelling errors ie antico-agulation

Author reply:
We thank for this comment and have carefully checked and corrected the manuscript for spelling errors
Editorial Comments:
Hornum and colleagues in the present paper evaluated the association of mannose-binding lectine (MBL) serum levels with vascular parameters in uraemic patients. Uraemia is in fact associated with a highly increased risk of cardiovascular disease and MBL has been shown to be involved in cardiovascular pathophysiology and a protective effect of MBL has been suggested. They studied a cohort of 98 ESRD patients and measured pulse wave velocity (PWV) and augmentation index (AIX) by tonometry and endothelial dependent flow-mediated (FMD) and endothelial independent nitroglycerin-induced (NID) dilatory capacities of the brachial artery by ultrasound. Serum levels of MBL were measured using Luminex x MAP bead array technology. Patients were divided into two groups according to their BML concentration (below or above the median concentration). They found that PWV was significantly lower in the group with high MBL levels which also had a slightly better AIX and higher insulin sensitivity (ISI) compared to the group with low MBL levels. No differences were found in FMD and NID.

Although the study by Hornum is well designed there are some major comments: The role of MBL in the pathophysiology of vascular disease is far to be clear. There are papers reporting a role of MBL in worsening vascular diseases as well as papers reporting the opposite. MBL is a well known activator of the complement leading to inflammatory processes. Furthermore the MBL serum level is quite variable in normal subjects and apparently it is not strongly associated with vascular diseases. The median value the authors have used to obtain two groups of uraemic subjects is meaningless since in the normal subjects the range of values is very large.

Author reply:
We divided the cohort by median MBL values thereby giving a balanced view of the data. We find that this is in accordance with normal scientific practice in especially MBL studies.

As the authors said the number of subjects of the study is quite limited, furthermore they studied only uraemic patients in waiting list for transplantation who surely had very small risk of cardiovascular diseases. Factors such as calcium and phosphorus homeostasis, hypertension and inflammation
might be responsible for alterations of vascular tissue in patients affected by ESRD yet in the study no mention is given to some very relevant data: There is no mention to calcium phosphate and PTH values that could be of help and could have a significant influence on vascular elasticity.

The paper lacks of tables with hemodialysis or peritoneal dialysis modalities and efficiency that may increase/decrease the risk factors

**Author reply:**
*Please see author reply to reviewer 1 and 2.*

Authors conclude that high levels of MBL are associated with lower PWV in a cohort of patients with ESRD awaiting kidney transplantation suggesting a beneficial role of high levels of MBL on arterial stiffness in uraemia, however apart for PWV the other parameters were not significantly different between the two groups, thus their conclusions lacks part of its strength.

**Author reply:**
*Please see author reply to reviewer 1 and 2.*