Author's response to reviews

Title: Impact of mannose-binding lectin deficiency on radiocontrast-induced renal dysfunction: a post-hoc analysis of a multicenter randomized controlled trial.

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Author's response to reviews: see over
POINT BY POINT REPLY

We thank for the careful review of our manuscript and the opportunity to respond to the important concerns raised by the reviewers.

Comments from reviewer #1:

In their article “Impact of mannose-binding lectin deficiency on radiocontrast-induced renal dysfunction: a posthoc analysis of a multicenter randomized controlled trial” the authors investigated the association of mannose-binding lectin (MBL) levels and the incidence of contrast-induced nephropathy (CIN) in a prospective study. Renal function was measured by serum creatinine and serum cystatin C. The incidence of creatinine-based CIN was 6.5% and the incidence of cystatin C based CIN was 24%. The cohort included 246 patients. MBL levels were not associated with the occurrence of creatinine-based CIN, but was associated with cystatin C based CIN. MBL deficiency was an inverse predictor of a cystatin C increase ≥ 10%. The data is clearly presented. However, the present study has some limitations. Only surrogate marker (serum creatinine, cystatin C) were tested as primary endpoint and no association of MBL deficiency with superior clinical outcomes was demonstrated. To investigate these clinical outcomes the study was not powered. Analysis of MBL deficiency was solely relied on MBL phenotype and genetic material was not available. However, these limitations are clearly discussed in the present manuscript. The authors should include a sentence in the discussion if MBL levels are varying with degree of renal failure.

Several studies have compared MBL levels in healthy subjects vs. pre-dialysis and dialysis patients [1-8]. Of note, all these studies have been conducted in Asia, hence data for other population groups are lacking. These studies have yielded conflicting results which can at least partially be explained by the different types of ELISA used in these studies. In general, studies that used a functional MBL ELISA (where MBL binds to coated mannan) have shown lower MBL levels in pre-dialysis and dialysis patients vs. healthy subjects [4, 7] with the exception of Akbari R et al.[1]. Conversely, using a sandwich MBL ELISA (where MBL is captured by a coated antibody) MBL levels were found to be markedly increased in pre-dialysis and dialysis patients compared to healthy subjects [2, 3, 6, 7]. In addition, lectin pathway activity was also increased in dialysis patients vs. healthy subjects in one study [2]. Interestingly, MBL mutation rates were similar in dialysis and healthy controls [2, 3]. A recent study from Satomura A et al. [7] compared both ELISAs and confirmed the inverse relationship in pre-dialysis, dialysis and healthy subjects with the highest “functional” MBL levels found in healthy subjects and the highest “sandwich” MBL level found in hemodialysis patients. The reason for this discrepancy remains speculative at the moment. The functional MBL ELISA might measure mainly higher order oligomeric structures of MBL which are possibly inhibited by uremic
toxins in hemodialysis patients. A recent study by the same authors has found an increase of MBL levels measured by the functional ELISA after initiation of hemodialysis [8]. Higher serum MBL levels in pre-dialysis or dialysis patients consistently reported with the use of the MBL sandwich ELISA might be related to increased inflammation in those patients as compared to healthy controls. MBL is a weak acute phase reactant and its production in the liver has been shown to be up-regulated by IL-6. However, above mentioned studies could not demonstrate a correlation of CRP and MBL in patients on hemodialysis vs. healthy controls [7, 8]. Moreover, MBL levels did not correlate with the degree of glomerular filtration rate, and MBL levels were not measurable or increased in the urine of pre-dialysis and dialysis patients [6].

In summary, MBL levels as measured by sandwich ELISA (as we did) have been shown to be significantly higher mainly in patients on peritoneal or hemodialysis, but to a lesser degree in patients with pre-dialysis chronic renal failure. Importantly, patients with chronic renal failure not on dialysis included in these studies had a mean estimated glomerular filtration rate (eGFR) of 19.4 and 23.3 ml/min compared to a mean eGFR of 43.5 ml/min in our study (median 44, IQR 35-52 ml/min).

Hence, it is speculative if this degree of renal impairment is associated with an increase in MBL levels as measured by sandwich ELISA in our study population.

We have added the following sentence in our discussion as requested (page 14): MBL levels (as measured by sandwich ELISA) have been shown to be significantly increased in Asian pre-dialysis and dialysis patients compared to healthy controls. To the best of our knowledge comparable studies in a Caucasian population with moderate renal impairment (similar to our study patients) are lacking.

Comments from reviewer #2:

Thank you for this interesting report. It is well written. Just two minor comments.

1. How was normality tested?

Normality for MBL levels was assessed by calculating the Shapiro-Wilk, the D'Agostino & Pearson omnibus and the Kolmogorov-Smirnov normality tests, which all showed a p value of <0.0001 indicating that MBL levels significantly deviated from a Gaussian distribution in our study. In addition, normality was determined graphically by using a Q-Q plot (Figure 1). As the data points strayed from the line in an obvious non-linear fashion with a distinct subgroup of very low values, we concluded that MBL levels do not follow a Gaussian distribution in our study, and hence used non parametrical tests for statistical analysis. This finding is consistent with our previous experience [9] and with what has been reported by several research groups previously [10-12]. Interestingly, the distribution of L-ficolin, another initiator of the lectin pathway of complement was found to be normal in a previous study [10].

We have added the following sentence in the methods section (page 7): Due to the non-Gaussian distribution of human MBL levels (as determined by Q-Q plot and normality tests) two-group comparison of serum MBL levels were performed using a Mann-Whitney-U-test, whereas a Kruskal-
Wallis one-way analysis of variance or Friedman test was used for multigroup comparison where appropriate.

![Q-Q plot for MBL levels](image)

Figure 1: Normal Q-Q plot for serum mannose-binding lectin levels.

2. Could you speculate on the influence of (inflammatory) disease state on serum levels of MBL? Did you check serum levels on just one day?

Human MBL levels are mainly influenced by polymorphisms within the coding and promoter regions of the MBL2 gene. During lifetime individual MBL serum levels remain remarkably stable highlighting the dominating influence of genetics as compared to environmental factors. With respect to the latter thyroid dysfunction [13], severe renal (pre-dialysis or dialysis) or liver impairment [14] and inflammation have been shown to impact significantly on MBL levels [15]. However, even during acute inflammation, low MBL serum concentrations are still predictive for moderate to severe deficiency as the above mentioned factors only significantly influence MBL levels in individuals without preexisting MBL deficiency as shown in acute pneumonia [16], after allogeneic stem cell transplantation [17], after surgery [18] or in the setting of thrombolysis for acute ischemic stroke [9]. Therefore, measurement of MBL serum levels by ELISA allows reliable quantification of the functional activity of the MBL pathway in vivo in these setting.

In our study serum MBL levels were only determined on the day prior to the contrast procedure. Thyroid hormones were not measured, but acute inflammation was noted in a subgroup of patients.
(elevated C-reactive protein), indicating that median MBL levels in our study might have been higher than median levels measured after patients had fully recovered from their disease. Importantly, the actual and not the baseline MBL level should be of clinical relevance with respect to ischemia/reperfusion injury, and low MBL levels should still be predictive for MBL deficiency.

Editorial comments:

1. Please provide the full name of all ethical committees in the Methods section of the manuscript.

As requested by the editors we have added the full name of all ethical committees in the Methods section of the manuscript (page 5).

The study was conducted according to the principles of the revised Declaration of Helsinki, had been approved by the local ethical committees (Ethikkommission beider Basel, Basel/Liestal, Switzerland; Comitato Etico Centro Cardiologico Monzino, Milano, Italy), and all participants gave written informed consent for the study.

References:


