Author's response to reviews

Title: Urine IL-18, NGAL, IL-8 and serum IL-8 are biomarkers of acute kidney injury following liver transplantation

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Author's response to reviews: see over
Major Compulsory Revisions

1. The author didn't tell us the elevated time point the serum creatinine reported post operation and didn't tell us the average time point for serum creatinine increased more than 50% in AKI group. If the serum creatinine increased around or less than 24hrs post surgery. What's the benefit of these new biomarkers than serum creatinine?.

   The average time point the serum creatinine was increased was the same as the time measured from time of surgical incision to time of first blood samples= 21 hrs and 41 minutes as stated on Page 5 (Lines 111-112). Average time point between surgical incision and time to first blood sample was 21 hours and 21 minutes in the AKI group. This information has been added to paper on Page 5 (Lines 112-114).

   In the present study, biomarkers were only measured at the same time as serum creatinine. Our previous studies in AKI patients post cardiac surgery demonstrate that urine IL-18 and NGAL peak at 6 hrs post op. Thus it is possible that urine IL-18 and NGAL may peak earlier than serum creatinine. Future studies will determine whether these biomarkers increase before serum creatinine. A paragraph to this effect has been added to the discussion on Page 15-16 (Lines 352-358)

2. Please add the trends of serum creatinine change post operation, whereas not only the a value.

   The absolute change in serum creatinine (mg/dl) from pre-operative to 24, 48, 72, 96 and 120 hrs post-operative in AKI patients is now shown the Results (Lines 244-248) in new Table 4 (Lines 392-394).

   The author should tell us the post-operative serum creatinine in Table 2 is the value of which time point.

   Time point of serum creatinine and cystatin c was 21 hours and 41 minutes (See page 5) (Lines 112-114)

3. What's the reason of higher creatinine in non-AKI group than AKI pre-operative? Is there any possibility the is some other factors exist in AKI group, such as malnutrition (low albumin) and inflammation could affect the biomarkers level after surgery?

   Pre-operative (within 24 hours pre-op) serum creatinine was 0.81 in AKI group and and 1.13 in non-AKI group (P=0.0131). There was no difference in serum albumin or serum IL-6 pre-op between the AKI and no AKI groups suggesting that malnutrition or systemic inflammation is not the cause of the lower serum creatinine in the AKI group: Serum albumin (g/dL) pre-op was 3.15 in the AKI group and 3.08 in the non-AKI group (P=NS). Serum IL-6 (pg/mL) pre-op was 20.2 in the AKI group and 15.0 in the non-AKI group (P=NS). These new data is added to Pages 9 and 10 (Lines 210-217) of the revised manuscript.

4. The ROC-AUC were 0.833 for urine NGAL, 0.682 for serum IL-6, 0.773 for urine IL-8, and 0.742 for serum IL-8. However in the figure legend on the last page, the author mentioned P values were 0.3671 for urine NGAL,0.6682
for serum IL-6, 0.0949 for urine IL-8, 0.0803 for serum IL-8. It seems unreasonable. Why the large AUC for urine NGAL got P>0.05? Although the model was able to estimate Area Under the Curve, there were only 7 AKI events in the sample, thus the sample size was too small to find statistical significance using Logistic Regression.

5. As this is a study in the transplantation and some markers investigated are pro-inflammatory cytokines, we should consider if there are some rejection factors involved in the results. Please add the information of immunosuppression agents after surgery.

All the patients received either tacrolimus or cyclosporine during hospitalization. This information has been added on Page 5 (Lines 100-101). The exact timing of starting immunosuppression in each patient is not known. Regarding liver transplant rejection, the liver enzymes AST, ALT and alkaline phosphatase at 120 hrs post-op were not different between the AKI and no AKI. AST (U/L) was 160 in the AKI group and 161 in the non-AKI group (P=NS). ALT (U/L) was 417 in the AKI group and 328 in the non-AKI group (P=NS). Alkaline phosphatase (U/L) was 133 in the AKI group and 108 in the non-AKI group (P=NS).

Minor Essential Revisions
1. In authors’ part, there is no author marked unit 3. This has been corrected (Lines 5 and 10).
2. In the background, the author mentioned "To date, there have been no reports of using other established AKI biomarkers in OLT patients" except serum or urine NGAL. However it's not updated. Urine L-FABP has been reported as a diagnostic marker of AKI after OLT. Please refer to "Li Y, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) and L-type fatty acid-binding protein (L-FABP) as diagnostic markers of early acute kidney injury after liver transplantation. Biomarker. 2012; 17(4): 336–342"
This reference has been added on Page 4 (Lines 84-85) and new reference 19 (Lines 480-483).
3. In methods, please add the surgical approach and the time of an hepatic phase data in both groups # as these are considered related with AKI after OLT. We do not have surgical approach or an hepatic time. However we do have duration of surgery that was longer in the AKI group. Duration of surgery (hours) was 4.58 in non AKI vs. 6.0 in AKI (P<0.01). These data have been added to Table 1 (Line 378) and Results section on Page 8 (Lines 188-189).

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: 'I declare that I have no competing interests'
Reviewer 2 report
Reviewer: LIOTIER

Reviewer's report:
Please find the following report for the review of a manuscript that has been submitted to BMC Nephrology by Jeffrey C Sirota and colleagues. I accept with Minor Essential Revisions:
1. Is the question posed by the authors well defined? Yes
2. Are the methods appropriate and well described? Yes but can be improved:

Firstly, why you have chosen the urine NGAL rather than the serum NGAL for AKI?
The reviewer makes a good point. In future studies, plasma NGAL will be measured.

Secondly, renal insufficiency (acute or chronic) is not a exclusion criteria: there is only dialysis or kidney transplant. The results show however a mean pre operative creatinine of 0.81 mg/dL in the AKI group which excludes a priori renal failure but it is 1.07 mg/dL in the non AKI group (p=0.21).
Pre-operative (within 24 hours pre-op) serum creatinine was 0.81 in AKI group and and 1.13 in non-AKI group (P=0.0131). To determine whether low albumin or systemic inflammation contributed to the lower creatinine in the AKI group, serum albumin and serum IL-6 was compared between the Aki and no AKI groups. There was no difference in serum albumin or serum IL-6 pre-op between the AKI and no AKI groups suggesting that malnutrition or systemic inflammation is not the cause of the lower serum creatinine in the AKI group: Serum albumin (g/dL) pre-op was 3.15 in the AKI group and 3.08 in the non-AKI group (P=NS). Serum IL-6 (pg/mL) pre-op was 20.2 in the AKI group and 15.0 in the non-AKI group (P=NS). These new data is added to Pages 9 and 10 (Lines 210-217) of the revised manuscript.

Thirdly, why have you taken in the definition of AKI an increased of cystatin c while it was not written in the chapter material and methods and it is a poor marker of acute renal failure except for chronic renal failure? (Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. Am J Kidney Dis. 2002;40:221-6.)
Cystatin C has been added to the Methods section on Page 7 (Lines 155-157).
Reference has been added as suggested (Page 12, Lines 271-273) and Reference 25, (Lines 505-506)

3. Are the data sound? Yes but could you explain the low incidence of AKI in your population while it was expected highest (78% according your reference (1). The incidence of AKI after liver transplant is 17-95% depending on definition (Reference 1). This reference is now discussed in more detail on Page 3 (Lines 51-54) reflecting the variation in the incidence of AKI. The patients in the present study were not consecutive liver transplants and were from 2 different hospitals and may not reflect the true incidence of AKI.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data? Yes but why do not you apply a treatment to increase biomarkers as
Therapeutic studies based on the diagnosis of AKI using a panel of biomarkers, will be complex to perform, but will be possible in the future as new therapies for AKI emerge. A therapeutic study is beyond the scope of the resent study. The possibility of therapeutic studies is now mentioned on Page 16 (Lines 363-366).

6. Are limitations of the work clearly stated? Yes but some are missing:
First, there is lack of power in this study (only 40 patients)
We agree with the reviewer. The lack of power is discussed as a limitation of the study on Page 13 (Lines 342-346).

Second, creatinine is not the gold standard for the diagnosis of AKI. Unfortunately until biomarkers become FDA-approved for the diagnosis of AKI, serum creatinine, although far from ideal, remains the standard for the diagnosis of AKI (based of RIFLE, AKIN and KDIGO criteria).

Third, the choice of IL-18 and IL-8 is questionable because there are not the best AKI biomarkers (Liang XL, Liu SX, Chen YH, Yan LJ, Li H, Xuan HJ, Liang YZ, Shi W. Combination of urinary kidney injury molecule-1 and interleukin-18 as early biomarker for the diagnosis and progressive assessment of acute kidney injury following cardiopulmonary bypass surgery: a prospective nested case-control study. Biomarkers 2010;15:332-9.). We agree with the reviewer that a combination of biomarkers including KIM-1 may provide better results. This possibility is now mentioned on Page 16. (Lines 363-366)

Fourth, the choice of a measure 24 hours after liver transplantation is questionable because many biomarkers increased in the first hours postoperatively and you need respect kinetics of the biomarkers. We agree that biomarkers may be increased earlier than 24 hours. This possibility and the implications are discussed on Page 15 (Lines 352-358) of the Discussion.

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes
8. Do the title and abstract accurately convey what has been found? Yes
9. Is the writing acceptable? Yes

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
'I declare that I have no competing interests' below
Reviewer's report:
Authors describe the assessment of serum and urine biomarkers for detection of acute kidney injury (AKI) in patients undergoing liver transplantation (OLT), with the hypothesis that these would be higher in patients who develop AKI compared to patients who do not.
The question posed by the authors is well defined and the discussion and conclusions well balanced and supported by the data. Limitations are clearly stated. The writing is acceptable.

Major Compulsory Revisions
1. Authors should better describe methods for measurements of urine and serum markers (i.e. dosage was done on single or multiple samples, a brief description of process would be advisable for each interleukin).
Biomarkers were measured on single not pooled samples (Line 117). Methods for the measurements of the biomarkers are now detailed on Pages 5-7 (Lines 117-144) of the Methods section.

Our classification of AKI based on KDIGO is now described and referenced on Page 7 (Lines 152-157) in the Methods section and new reference 21 (Lines 489).

3. Authors excluded from the analysis patients who had an increase in serum creatinine that was not sustained for 24 hours. They justified this decision defining these patients as having pre-renal kidney injury. They do not specify the pathogenesis of AKI as inclusion or exclusion criteria, therefore they should comment this resolution.
Pre-renal AKI has been added to Exclusion Criteria on Page 5 (Lines 99-100).

4. Authors comment about the result of lower pre-operative serum creatinine levels in patients with AKI compared to patients without. They should consider that pre-operative serum creatinine in cirrhotic patients should be reduced for different reasons, such as reduced synthesis of creatine, malnutrition, reduced muscle mass.
Pre-operative (within 24 hours pre-op) serum creatinine was 0.81 in AKI group and and 1.13 in non-AKI group (P=0.0131). To determine whether low albumin or systemic inflammation contributed to the lower creatinine in the AKI group, serum albumin and serum IL-6 was compared between the AKI and no AKI groups. There was no difference in serum albumin or serum IL-6 pre-op between the AKI and no AKI groups suggesting that malnutrition or systemic inflammation is not the cause of the lower serum creatinine in the AKI group: Serum albumin (g/dL) pre-op was 3.15 in the AKI group and 3.08 in the non-AKI group (P=NS). Serum IL-6 (pg/mL) pre-op was 20.2 in the AKI group and 15.0 in the non-AKI group (P=NS). These new data is added to Pages 9 and 10 (Lines 210-217) of the revised manuscript.

There was no difference in MELD score between AKI and non AKI patients (Table 1). In a further analysis there was no difference in AST, ALT, alkaline phosphatase, albumin or bilirubin between AKI and non AKI patients. AST (U/L) was 84 in the AKI group and 95 in the non-AKI group (P=NS). ALT (U/L) was 43 in the AKI group and 68 in the non-AKI group (P=NS). Alk Phos (U/L) was 130 in the AKI group and 135 in the non-AKI group (P=NS). Serum albumin (g/dL) pre-op was 3.15 in the AKI group and 3.08 in the non-AKI group (P=NS). Bilirubin (mg/dL) was 2.4 in the AKI group and 3.6 in the non-AKI group (P=NS). This new data is provided in the Results section on Pages 8 and 9 (Lines 188-196) and discussed on Page 15 (Lines 337-341). References suggested above have been added (References 42 and 43) (Lines 571-576).

Minor Essential Revision
1. Glomerular filtration rate (GFR) is defined using a standard calibration formula on the basis of cystatin C levels. Authors should address this choose and explain why they did not use formulas based on serum creatinine.
   GFR formulas are mainly used for chronic kidney disease patients. As these patients had AKI and may not have been in a steady state of serum creatinine, we chose not to use GFR formulas.

2. Table 3 shows median pre-operative and post-operative biomarkers levels in patients who developed AKI and those who did not. Urine IL-8 results are reported in table but not in text.
   Urine IL-8 levels are in the text on Page 11 Line 239.

   This has been changed on Page 14 Line 330.

4. Number 3 of affiliations is not reported among authors. Please review it.
   No 3 of affiliations has been added Lines 5 and 10.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.