Author's response to reviews

Title: Physicochemical analysis of blood and urine in the course of acute kidney injury in critically ill patients: a prospective, observational study

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Answers to reviewers- Critical Care Forum

Reviewer 1

- Thanks for your review of our manuscript. In fact, this is a complex study in which many variables were analyzed. However, the subject is interesting and, although not new, it offers a new vision and way to interpret urinary biochemistry in the modern era of AKI. Since there is no previous similar study, we aimed to elucidate the subject in the most complete way. However, we recognize that there was too much information for a single paper. We decided to exclude our data regarding fractional excretion of K so that, instead of 8 figures, we have now only 5 figures. Although it remains complex, we hope you find it easier to follow. We believe that if we excluded more part of the results, it would generate many questions that would preclude its publication in an important journal. Of the 5 figures, two would only be present in the supplementary material.

Reviewer 2

- Thanks for your valuable review of our manuscript.
- As suggested, “critically ill patients” were included in the key words.
- In fact, there were 3 patients with COPD- 1 with no AKI, 1 with transient AKI, 1 with persistent AKI. The COPD patient with persistent AKI had a septic shock and low FECI and SIDA. The other two patients had higher SIDA (45-50 mEq/L) and consistent FECI > FENa, as expected in COPD.
- Page 5 line 11- “urinary strong ion difference” was included before its abbreviation SIdu.
- We included the term “loop” before the first mention of “diuretic” so that the reader can understand that every time we cite “diuretic” we mean “loop diuretic”. We also included “loop” in Table 1.
- It would be very hard to precise for each individual patient if he had used diuretic at the day of AKI diagnosis, at the two days before or after diagnosis as well as if he has used only a single day or many days or the dose of furosemide used. To answer this question it would involve a very complex statistical analysis and we think that this is beyond the scope of the manuscript. However, previous text in the literature (ref 8) has demonstrated that NaU (as well as FENa) are expected to increase with loop diuretic. It means that low values of FENa and NaU could not be attributed to diuretic use. We cannot exclude that decreases in these variables would be even greater in the absence of diuretics but the fact is that we found significant decreases in NaU and CIU.
preceding AKI diagnosis, having them received diuretics or not. Theoretically diuretic use is not taken into account for AKI diagnosis based in urine output; hence, we think that we should use this same rational for NaU: low values are a bad sign in the presence or absence of diuretic as well as a low urine output.

- We have creatinine values for many patients in the 3 months preceding ICU admission because many patients have ambulatory assistance in this same hospital. This was called baseSCr. For those patients without baseSCr, we used the lowest adjusted creatinine in the first 7 days of ICU admission to determine AKI at admission.

- We excluded the part of AUC for renal replacement therapy using FEK as suggested.

- The text and the amount of information in our manuscript were considered excessive by some reviewers. This limits our capacity to include additional comments (albeit interesting) in Discussion section.

- Since we excluded our results regarding FEK from this manuscript as suggested by reviewer 1 who believe that there was too much information from a single paper, we have not put this information in the key messages.

Reviewer 3

- In order to decrease the length of our manuscript and in agreement with reviewer 1, we excluded our data about fractional excretion of potassium since we believe that a separate manuscript can be written to specifically address this issue.

- As requested, we have changed the place in which we inserted “(AKI)” in order to not mislead the lectors. In the new place we think that the lector would be able to understand that AKI could be transient or persistent.

- Few previous studies have addressed this issue in the modern era of AKI and most of them have in fact looked at FEUr. Reference 8 (Pepin et al) also looked at FENa. Reference 10 (Darmon et al) also looked at FENa, U/P urea, U/P creatinine, NaU/KU.

- The reason why we have only chosen patients with an indwelling urinary catheter is practical. It would be hard to collect a daily urine sample simultaneously with blood in patients with no urinary catheter. Besides that, we would like to measure urine output. In addition, patients with urinary catheter are, theoretically, the patients with greater risk of AKI so that urine output monitoring was requested by the assistant physician. We included an additional commentary about this subject in the Discussion.

- The variables analyzed in our study were basically those that are part of a complete physicochemical (stewart) analysis, both in blood and urine. NGAL, for example, is not available in our Hospital. We used only variables that could be calculated using our routine lab exams.

- We were interested in the period near AKI diagnosis so that we have chosen to look at the 2 days before and after AKI. We believe that many days after AKI diagnosis other variables could interfere in the analysis. For example, a second insult to the kidney in a
patient recovered from a transient AKI could happen. Besides that, most reviewers considered the manuscript too long already.

- We used 2h creatinine clearance because it was very easy to measure since diuresis was discarded every 2 hours and we have urine volume, blood and urine creatinine values at the time that they collect the routine exams. We agree that it is not a gold standard but we think that it brings additional and valuable information in AKI development, perhaps better than serum creatinine alone. It is important to emphasize that in depth evaluation of 2h creatinine clearance was not a primary objective of this study.

- All diagnosis described refer to ICU admission. As suggested, we removed specific commentaries about sepsis. We intend to make a study specifically in septic patients in the near future.

- The study period, as mentioned in Methods section, ends when urinary catheter was removed or RRT was initiated, which of the two that occurred first. We agree that this is not clear in the present text. In this particular patient, both the transient AKI and the persistent AKI (that occurred long after recovery from transient AKI) were evaluated because the patient remained with the urinary catheter during all this time and the observation ended when dialysis was indicated. However, dialysis was not related to the first insult and we included only the first AKI diagnosis per patient, which was transient in this case. Mortality was evaluated both in the ICU and in the hospital as described in table 1. In order to make this more clear, we have made some alterations in the Methods. (see pages 5-6).

- We have excluded data of weak acids in table 3 and maintained just albumin and phosphate separately, as suggested.

- We agree that diuretic use influences FENa and FECl. It is known that loop diuretics increase FENa. The fact that median FENa was low (<1%) (except in AKIN stage 3) could not be attributed to diuretics. Perhaps FENa and NaU increase in AKIN 3 due to increased diuretic use or severe tubular impairment or both. Anyway, we believe that we don’t need urine biochemistry to diagnose AKI when it reaches stage 3. This Figure is mainly to demonstrate that, until advanced stages of AKI (stage 2), tubular capacity to retain sodium is well preserved (even in the presence of diuretics).

- We have modified the title of Table 1 so that the reader can understand that AKI status refers exclusively to the study period, which depends on time with a urinary catheter or dialysis need.

- As suggested, we have changed the symbols in Tables 2, 3 and 4. We have decided to express albumin in mEq/L because it refers only to the ionized part of albumin as the other variables in the same table (only ions).

- We have excluded 3 figures since we have excluded information regarding FEK in order to decrease the manuscript length. FEK would be a topic for another manuscript.
- Figure 2 was kept since tables are complex and this figure helps the reader to visualize better our results and the dynamism of the variables according to AKI duration.

- We have included references for each of the factors.

- It was not clear to us, what kind of reformulation the reviewer want us to make in the sentence.

- We removed the part of the sentence “probably not before nor on the day of AKI diagnosis”, as suggested since we don’t have this precise information.

- We have modified the sentence of “other concomitant sources of metabolic acidosis”, including an observation about persistent AKI group and its high vasopressor use, which may suggest frequent tissue hypoperfusion in this group as a source of additional metabolic acidosis.

- Our criticism regards categorization of NaU values in which values below 20 mean “pre-renal state” and above 40 mean “ATN”. We want to show that early AKI development includes progressive decreases in NaU values probably as a surrogate of decreased glomerular filtration and preserved/activated tubular sodium reabsorption. In AKI stage 3 there seems to have an impairment in the capacity to retain sodium and this may be due to ATN. However, stage 3 is a late stage of AKI. Our focus is in AKIN stages 1 and 2, in which a “pre-renal state” is progressively more intense. We refute this term “pre-renal” because it implies in renal hypoperfusion. Some articles have demonstrated that this so called “pre-renal state” may actually reflect microcirculatory impairment inside the kidney in the presence of a high renal artery blood flow (Langenberg, NDT 2006). The small differences in fluid balance among AKIN stages do not suggest hypovolemia or sub-therapeutic optimization as a main cause of our findings but this is hard to prove in this study and additional studies are required. We believe that persistent AKI do not usually represent ATN, since it frequently represents a “persistent pre-renal state”, with persistent low NaU values. This could be a result of intra-renal microcirculatory impairment. Note that in Langenberg et al NDT 2006, a urine biochemistry compatible with “pre-renal state” remained even with a high renal blood flow.

- Urine output (ml/kg/h) was similar at the day that AKIN max was reached among the different AKIN stages (1.13, 1.12, 0.97, 0.99), i.e., the majority of the patients that have reached AKIN 3 (creatinine-based) were not oliguric at that day. It is interesting to note that the median diuresis of the patients with advanced AKI differs from that of patients with less severe stages of AKI not in volume but in sodium and chloride content. In our sample, median creatinine at the day that AKIN max 3 was reached was 2.1 mg/dL (not too high) and it is possible that diuretics contributed to this similar volume among different AKINmax stages. This finding highlights the need to access not only urine volume but sodium and chloride content in the urine. We have included this result at the end of the Results section and a comment about this in the Discussion.
- As suggested, we have modified the sentence regarding low NaU values and kidney stress.

- Results regarding FEK were removed from this manuscript as suggested by other reviewers who think that the article is long enough and a paper addressing this particular issue of FEK might be an option.

- Although it was not statistically significant, we believe that increases in SIDu are partially explained by increases in KU. The graph represent only median values, and very similar values for median NaU and CIU values do not exclude that a greater fall in CIU in comparison to NaU could contribute to an increased SIDu in an individual basis. Our study is not able to answer that. Regarding NH4+ excretion, recent experiments in our lab not yet published have shown decreases in serum chloride in a few hours after CO2 retention in a pig model. We believe that this could be due at least in part to an increased chloriuresis (urine analysis was not yet performed). Hence, this previous idea that renal responses to acid-base disturbances take days might not be true. In addition, alterations in urine normally occur in a larger scale in order to prevent large oscillations in blood values. Small changes in blood acid-base status may occur secondary to more evident alterations in urine. A healthy person may have only small decreases in his serum base excess and SID secondary to saline infusion due to significant decreases in his SIDu. We agree that SIDu is not a variable very specific for ammonium excretion but, as the reviewer highlights, acid excretion is only possible if patient excretes enough sodium. We aimed to demonstrate that impaired natriuresis is a major characteristic of the early phase of AKI and thus this could also secondarily impairs acid excretion. We would like to demonstrate that an avid-sodium retaining state occurs in the early phase of AKI and this may occur even in the absence of volume contraction as demonstrated in experimental studies of septic AKI (see Langenberg et al. mentioned above). Thus, acid excretion is impaired (high SIDu) even in the presence of preserved tubular function, which is also compatible with our results (increases in SIDu would represent decreases in NH4+ excretion).

- In our conclusion, we only suggest that some of these alterations may precede creatinine elevation. In our opinion, this is a hypothesis-generating study. We mention that future studies are necessary to better explore this issue in larger scales. We believe that the relevance of our study is the need of a new look at urine biochemistry and the possible useful informations that it may provide.

**Reviewer 4**

- Tubular injury certainly occurs in AKI even in early stages. One of the interesting findings of this study is that tubular injury could be dissociated from urine biochemistry, i.e., patients may have tubular injury but still have a urine biochemistry compatible with “pre-renal”. This is probably secondary to the fact that tubular injuries are focal and, in the early stages, they do not seem to impair the global tubular capacity to retain sodium.