Author’s response to reviews

Title: Trough colistin plasma level is an independent risk factor for nephrotoxicity: a prospective observational cohort study

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Author’s response to reviews: see over
Dear Dr Harris,

Thank you very much for your consideration of our manuscript (MS: 5430024648283306) now titled “Trough colistin plasma level is an independent risk factor for nephrotoxicity: a prospective observational cohort study” and your request for a revised version.

We have attached all reviewers’ comments, and addressed each one individually. As you will see, we have made every attempt to incorporate these suggestions as thoroughly as possible. We hope you will agree that the revised manuscript is now suitable for publication in BMC Infectious Diseases. We look forward to hearing from you at your earliest convenience.

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Yours sincerely,
Luisa Sorlí, MD,
Point-by-point responses to the reviewer’s comments on the manuscript

Steady-state plasma level is an independent risk factor for nephrotoxicity: a prospective cohort study

The authors would like to thank the Reviewers for their careful review of our manuscript and for providing us with their comments and suggestions to improve the quality of the manuscript. The following responses have been prepared to address all of the reviewers’ comments in a point-by-point fashion.

Reviewer: Jason J Pogue

Reviewer's report: Sorli and colleagues present a very interesting paper associating day 3 colistin levels with toxicity at day 7 and day 14. This paper has the opportunity to be a significant add to the literature base surrounding colistin nephrotoxicity, however, the following need to be addressed first.

Authors: The authors would like to thank the reviewer for their positive evaluation of our manuscript and for accepting it for publication after revision. We have incorporated the following reviewer’s specific comments in preparation of a revised version of the manuscript.

Major:

1. While I would agree that the concentration time profile of colistin in patients appears to be relatively flat, I think it is inaccurate to call these levels C_{ss}, as the authors did not verify it in their patient population. Additionally, with no data given regarding the dosing schedule this would be impossible to ascertain. The authors should call these levels C_{min}, which is a more accurate measure of what they are. They are free to comment in the discussion that they feel they are an accurate marker for C_{ss}, but in the context of this trial, they are trough levels.

Authors: We agree with the reviewer that the levels that we reported in the paper were trough levels. In the revised version, we have reported the peak levels (C_{max}) and the trough levels (C_{min}). However, we have only included C_{min}
in the logistic regression model for the following reasons:
- There were limited fluctuations in colistin plasma concentration vs. profiles at steady state as you can see in the Results section ($C_{max}$ and $C_{min}$ had almost the same value).
- For the purposes of drug monitoring, it would be more advisable to sample immediately before CMS dosing not only because this is frequently more convenient from a practical viewpoint, but because CMS concentrations would then be minimal and the risk of colistin concentration overestimation resulting from post-sampling CMS hydrolysis would therefore be considerably reduced.

In view of this, the authors have considered changing the title of the paper to “Trough colistin plasma level is an independent risk factor for nephrotoxicity: a prospective cohort study.

2. Dosing. The authors should describe in their methods, as well as the results, the dosing regimens used (methods) and average daily doses (results). While I appreciate that a wide range of doses might have been used, they should do a better job of describing daily doses. This is of particular interest to those of us in the USA, who will likely use higher daily doses (assuming the majority of these doses are in the 6-9 M IU range.

Authors: We have included in the Results section a table with the dosing regimens and the main characteristics of patients receiving each dosage regimen (Page 26). However, only 16 patients were in the group of 9 million IUs daily and this fact could make comparison difficult with patients of other studies receiving higher CMS daily doses (particularly in the USA).

3. Along the same lines as question #2, I am very interested to know if there was a) an association between daily dose and toxicity and b) an association between daily dose and levels in patients. I understand that the authors might not have these data readily available (particularly the association between dose and levels) and if they cannot put them in the manuscript they need to comment on this as a limitation. In the results section they twice comment on patients having higher doses of CMS (one says "doses of CMS" at day 7, and the other says "CMS cumulative dose" at EOT)- are these the same variable? different? is this first one daily and the second cumulative? please clarify.

Authors: In the univariate analysis, we found an association between colistin
daily dose and toxicity but it disappeared in the logistic regression analysis (Table 4, page 27). On the other side, there was a correlation between colistin daily dose and plasma levels suggesting a linear pharmacokinetics of this antibiotic (Table 3, page 26). We have included these results in the Results section (Table 4, page 27 and Table 3, page 26) and have discussed them in the Discussion section (Page 13, line 7).

Regarding your question about the Results section, we are talking about two different “cumulative doses”. When we talk about AKI risk factors at day 7, we mean the CMS cumulative dose to day 7. When we are talking about AKI risk factors at the end of treatment, we mean the CMS cumulative dose for the entire treatment (Page 10, line 5 and line 8).

4. Is there a reason the authors only looked at toxicity on day 7 and EOT? Any info on what day toxicity occurred?

Authors: This is an observational study in which the investigators did not intervene in the daily clinical practice of the responsible physicians. We looked at the toxicity on day 7 and at the end of treatment because all inpatients had at least one blood sample test weekly and at the end of treatment. We have explained this in the “Methods section” (Page 5, line 22). A low percentage of patients had a blood test before day 7. This fact made any other type of analysis difficult. For the same reason, we cannot give accurate information about the day in which toxicity occurred. However, we agree with you that this could be useful information that should be explored in next works.

5. Results: "Globally 51.9% developed AKI during treatment," but then you list RIFLE rates as 25% and 49%. Which one is accurate?

Authors: If we consider the whole treatment period, 51.9% of patients developed AKI. The averages of 25% and 49% refer to the day 7 time point and the end of treatment. To avoid confusion, we have changed this in the text (Page 9, line 10).

6. Is it possible to get into more detail on those who had dose adjustments and recovered their baseline renal function? I think this would be a huge contribution to the literature if you could better explain. What were the numbers of patients that did versus didn’t? Was there a dose adjustment (when compared with others) that was associated with return of renal function?

Authors: We agree with you. We have added a paragraph in the Results section
giving more details about the dose adjustments (From page 9, line 11, to page 10, line 2). However, the small size of the sample (only 14 patients) doesn’t let us draw conclusions about the best strategy of dose adjustment.

7. Please show multivariate model in results- this one is just a suggestion.

Authors: We have added a table with these results (Table 5, page 29).

8. Is it possible to get into more detail regarding the levels and toxicity rates? Looking at the results table there is a huge difference between levels in those with and those without toxicity, and the cutoff levels associated with toxicity (particularly the 3.3 level at day 7) were much higher than I would have thought they would be. This is also more of a suggestion, but I think it would add great value if you added a table that showed rates of toxicity when the concentration was <= 1.0; between 1.0-1.9, 2.0-2.9, 3.0-3.9, etc. As clinicians this would help us assess the risk better of shooting for a higher level from an efficacy standpoint.

Authors: We have added a table that describes the relationship between colistin levels analyzed as a continuous variable and nephrotoxicity (Table 6, page 30).

9. Did any of the patients develop toxicity before the levels were drawn? Could lead to falsely elevated levels.

Authors: Effectively, some patients had developed acute renal failure at baseline, and as you say, patients with acute kidney failure at baseline had higher colistin plasma levels. We have added these data in the Results section (Page 10, line 11). This fact could be explained by the inverse relationship between creatinine clearance and colistin plasma levels previously demonstrated by other authors. This has been added in the Discussion section (Page 13, paragraph 2).

10. Suggestion- I think in the discussion readers would benefit from a bit clearer explanation with regard to the statement "these results confirm this is a drug with a narrow therapeutic range." What i mean is that the authors could tell us more about why this toxicity occurring at 2.5-3.3 is so concerning (as the needed levels to achieve AUC/MIC goals are right there as well)

Authors: We have made changes to clarify this concept (Page 15, first paragraph).
Point-by-point responses to the reviewer’s comments on the manuscript

Steady-state colistin plasma level is an independent risk factor for nephrotoxicity: a prospective cohort study

The authors would like to thank the Reviewers for their careful review of our manuscript and for providing us with their comments and suggestions to improve the quality of the manuscript. The following responses have been prepared to address all of the reviewers’ comments in a point–by-point fashion.

Reviewer: Nikolaos Markou

Reviewer's report:
This is a highly interesting study on colistin nephrotoxicity, which for the first time explores associations between colistin trough concentrations and AKI.

Authors: The authors would like to thank the reviewer for considering our manuscript interesting and accepting it for publication after revision. We have incorporated the following reviewer’s specific comments in preparation of a revised version of manuscript.

Reviewer's report:
Although presence of a control group would be desirable (a case-control study probably), the finding of a strong association between colistin trough levels and AKI is unequivocal proof of colistin toxicity and perhaps this should be stressed more emphatically in the paper.

Authors:
We agree with the reviewer that the lack of a control group is a limitation of this work and we have added this point in the limitations paragraph (Page 15, paragraph 2).

Reviewer's report:
Although I understand the difficulties of acquiring full pharmacokinetic curves for colistin, I would still wish for at least peak levels. Also, I believe that more precise information about dosing is needed. What was daily colistin dosage schedule?
Authors:
As you say, it is difficult in clinical practice to obtain full pharmacokinetic curves. To address this point, we have added colistin peak level values and introduced this level in the univariate analysis (Tables 3 and 4, pages 26-27).
Because there was limited fluctuation in colistin plasma concentration vs time profiles at steady-state (you can see that $C_{\text{min}}$ and $C_{\text{max}}$ are almost equal), we only included colistin trough levels in the multivariate analysis. We have explained this in the paper (Page 8, Statistical analysis section).
Regarding dosage schedule, we have included a new table describing the characteristics of patients with different colistin dosage regimens (Table 3, page 26).

Reviewer’s report:
Did dosage schedule affect outcome? What was the survival in patients who developed AKI vs those who did not? Was mortality associated with AKI or with levels of colistin?

Authors:
In this group of patients, there was not a relationship between colistin dosage regimen and clinical outcome. However, mortality was higher in patients who developed AKI and in those with higher colistin plasma levels. We have added this in the Results section and the Discussion section (Page 11, line 6 and page 14, line 19).

Minor comments:
1. Reviewer:
   Abstract
   In the paper, colistin concentration at steady state (Css) refers to colistin trough levels and this should be made clear in the abstract.
   Authors: We agree with the reviewer and have changed this item throughout the paper.
   In view of this, the authors have considered changing the title of the paper to “Trough colistin plasma level is an independent risk factor for nephrotoxicity: a prospective cohort study.”

2. Reviewer:
   Background
   Page 4
   Line 1: ‘Colistin is an old class … bacteria (MDR-GNB)’: rephrase (colistin is
an antibiotic and not a class of antibiotics). Increase in MDR-GNB is one reason but retained antibacterial activity of colistin against MDR-GNB is another for the resurgence of this drug.

Line 6: Provide detailed references for the individual studies in this paragraph as well as in the next.

Authors: We have written this paragraph according to the reviewer’s suggestion and have provided the references that the reviewer has requested (Page 4, lines 1, 3 and 8).

3. Reviewer:

Methods

3.1. As regards definitions: RIFLE criteria were clearly used to not only for stratification but for detection of AKI as well. Rephrase and substitute ‘AKI’ for ‘nephrotoxicity’, as AKI is the evaluated outcome and nephrotoxicity a possible explanation. It should also be added that RIFLE was estimated with exclusion of the urinary output criterion. In addition to chronic kidney disease at baseline, were there also any patients with already established AKI at baseline? What about patients with increased creatinine at baseline and no information as regards chronicity?

Authors: We have rephrased the paragraph according to the reviewer’s suggestions (Page 6, line 6, 7 and 8). Regarding patients with already established AKI at baseline, we have defined a group of patients with acute renal failure at baseline (creatinine > 1.4 mg/dL or GFR < 90 ML/MIN/1.73m² with no prior data regards chronicity) and have included this group in the univariate analysis (Page 6, line 1, 2 and 3 and page 27, table 4).

Acute renal failure at baseline was not related to the development of AKI at day 7 or at EOT (Table 4, page 27).

3.2. APACHE II is used on admission to the ICU and outside this context its use is problematic.

Authors: We completely agree with reviewer (Page 6, line 7, 18 and 19). We have calculated the SAPS II score for patients not in the ICU and included this in the analysis (Table 4, page 27).

3.3. Explain why that particular time limit (4 days from start of treatment) was selected for evaluation of nephrotoxicity
Authors: We selected 4 days not for evaluation of AKI but for the evaluation of colistin plasma levels. AKI was evaluated on day 7 and at the end of treatment because this is an observational study and some patients had no data before this time. However, all inpatients have a weekly blood test (Methods section, page 5, line 19).

3.4. Line 5: ‘all consecutive’ - all is superfluous.

3.5. Paragraph 4- line 5: I believe that “with or without kidney damage” is superfluous.

Authors: We agree with the reviewer. We have rephrased these sentences.

3.6. Paragraph 4: Follow up after termination of treatment; obviously follow up was also interrupted in case of death or earlier exit- note should also be made of this.

Authors: We have made a note of this in the paper (Page 6, line 11).

3.7. In the statistical analysis section, rephrase 1st and 2nd paragraph.

3.8. 2nd paragraph: replace ‘range’ with ‘interquartile range’.

3.9. 3rd paragraph: p value of 0.2

Authors: We have rephrased these paragraphs (Statistical Analysis, Page 8).

4. Reviewer:

Results

4.1. I would stress that none of the patients who developed AKI had need of RRT, while all survivors had full recovery of renal function. Before observing in the text that early recovery was associated with colistin dose reduction, some additional information about the time-course of renal recovery would be welcome.

Authors: We think that this could be useful information and we have attempted to describe the results of the dose modification in our patients. However, the small sample size (only 14 patients who developed AKI had a dose adjustment) made it difficult to draw conclusions. (From page 9, line 10, to page 10, line 2)

4.2. ROC analysis for nephrotoxic Css levels: please provide data on sensitivity and specificity of the selected breakpoints

Authors: We have provided this in the text (From page 10, line 23 to page 11, line 2).

5. Reviewer

5.1. Discussion
While colistin levels were the strongest predictor of AKI, no relationship was found between AKI and colistin daily or cumulative dose. This discrepancy merits probably some discussion: was it the result of a low association between colistin dose and colistin levels - eg great variability in colistin levels for the same colistin dose? Were lower troughs of colistin associated with less frequent drug administration?

**Authors:** We agree with the reviewer. As we explain in the new manuscript, we think that this fact is due to the great variability in colistin levels for the same colistin dose. This variability is possibly due to variations in creatinine clearance and other factors not well studied at present (Page 13, paragraph 2).

5.2. Conclusion

I would transfer discussion of limitations to the discussion section. I would site as additional limitation the lack of a control group.

**Authors:** We have transferred the limitations to the Discussion section and added as a limitation the lack of a control group (Page 15, second paragraph).

6. **Reviewer:**

Table 1-legend: RIFLE criteria are not used for assessment of nephrotoxity but for assessment of renal injury. Correct the definitions for RIFLE L and E in the table.

Table 2: correct alignment in the row referring to patients with nephrotoxicity at the end of treatment.

**Authors:** We have made these corrections (Tables 1 and 2, pages 24 and 25)