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
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:**

3.1. **Methods**

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, line10, 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 page10, 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 nephrotoxicity 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)