Author's response to reviews

Title: Procalcitonin levels and bacterial aetiology among COPD patients admitted to the ICU with severe pneumonia: a prospective cohort study

Authors:

cédric daubin (daubin-c@chu-caen.fr)
jean-jacques parienti (parienti-jj@chu-caen.fr)
sabine fradin (fradin-s@chu-caen.fr)
astrid vabret (vabret-a@chu-caen.fr)
michel ramakers (ramakers-m@chu-caen.fr)
nicolas terzi (terzi-n@chu-caen.fr)
françois freymuth (freymuth-f@chu-caen.fr)
pierre charbonneau (charbonneau-p@chu-caen.fr)
damien du cheyron (ducheyron-d@chu-caen.fr)

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Author's response to reviews: see over
Dear Editor,

Thank you for your interest. Please find below my responses to the reviewers comments for the above referenced paper.

We would like to thank the four reviewers for their comments, as we believe that our revised manuscript clearly improved as compared to the first submission.

Looking forward to hearing from you,

Sincerely,

Cedric Daubin
Corresponding author

Response to reviewers

Reviewer 1: Saad Nseir

Major Compulsory Revisions:

1) Pneumonia was diagnosed based on chest radiograph. Unfortunately, portable chest is inaccurate in diagnosing chest infiltrate in ICU patients, especially in COPD patients. CT scan should have been performed in order to diagnose pneumonia in this population.

We agree with this comment. Pneumonia is a difficult clinical diagnosis specially in COPD patients. No gold standard for clinical or microbiological criteria exist for pneumonia in COPD patients. We agree that CT scan could be useful to help in the diagnosis of pneumonia in this population. However, our study was done in accordance to routine practice and CT scan is not yet recommended. In addition, pneumonia diagnosis criteria in our study are in accordance to previous report Lieberman D et al[1] and Christ–Crain M et al [11] in which 76/302 (25%) of patients with community acquired pneumonia were COPD.

* These two references are added in the Materials Methods page 4


2) Approximately one third of study patients have received antimicrobial treatment before ICU admission. This high rate of prior antibiotic treatment may have influence the results of microbiological examinations. Authors should add a comment on this point in discussion section.

We thank this Reviewer for this comment. It is possible that microbial examinations are affected by prior antibiotic treatment but probably not PCT levels as previously reported and confirmed in our study (refer Result section page 7 and Discussion section
All patients pre-treated with antibiotics at ICU admission had a PCT max higher than 0.25 µg/L (except 2 in the group with PCTmax 0.1 µg/L- 0.25 µg/L). No patient with PCTmax lower than 0.1 had received prior antibiotic. Therefore, it is possible that microbiological documented infections are underestimated in the subgroup of patients with PCT higher than 0.25 µg/L (PCT levels indicating a high probability of the presence of bacterial infection). For these reasons and in the limitations of our study, we hypothesized that a PCT level cut off > 0.1 µg/L should be more appropriate than 0.25 µg/L (previously proposed for non severe lower respiratory tract infection) to predict the probability of a bacterial infection in severe COPD patients with pneumonia in a procalcitonin-based antibiotic strategies.

*A comment is now added in the Discussion section page 9

3) Authors stated that pneumonia was considered bacteriologically confirmed if Gram stain was positive or if a pathogen was cultured at > 105 cfu/ml. Gram stain is inaccurate in predicting positive culture of tracheal aspirate or BAL at significant threshold. In addition, why not using qualitative tracheal aspirate if positive Gram stain is sufficient to confirm the diagnosis? A reference for the threshold of positive tracheal aspirate should be added.

We agree with these comments. As reported in point 1 by this Reviewer and underlined by Reviewer 2 in his comment, there is no gold standard in diagnosing bacterial etiology of COPD exacerbation. For this reason, PCT could be useful. We performed sputum or tracheal aspirate which were bacteriologically processed only if less than 1% of the observed field contained squamous epithelial cells and more than 25 neutrophils. In addition, the pathogen concentration threshold of $10^5$ cfu/ml admitted to confirm bacteriologically pneumonia was in accordance to previous report ref [17,18,19]. All positive cultures of tracheal aspirate had a threshold higher than $10^7$ cfu/ml and any patient with a negative Gram stain had a positive culture. In fact, all pneumonia reported in our study were culture proven cases.

*This point is now clarified in the Materials and Methods section page 4 and references for the threshold added in Reference section


4) Were all pneumonia episodes community-acquired?
Yes, all pneumonia episodes were community-acquired

*This point is now clarified in the Material and Methods section page 3

5) Figure 1: authors stated that in the 3 patients with PCT <0.1 µg/L no bacteria was isolated. Did these patients receive prior antibiotic treatment?
There is no patient with PCTmax lower than 0.1 µg/L that received prior antibiotic.

*Patients who received prior antibiotics were presented with a different colour in Figure 2

6) Minor
page 6, line 1,2, and 4: “invasive” mechanical ventilation.
Agreed and thank you for seeing this
*This point is now corrected

table 1, the number of current smokers in all patients should be corrected (at least 11 patients and not 10)
Agreed and thank you for seeing this.
*This point is now corrected

Reviewer 2: Beat Muller

Major compulsory comments

1) Very small number of patients, especially in the important group of PCT<0.1, in which the authors want to stop antibiotics. Study design is purely observational, all patients received antibiotics, therefore not possible to say that is safe to stop antibiotics early or to not initiate antibiotics. Therefore the conclusions have to be changed.
We thank this Reviewer for this important comment. We agree that the design of our study did not permit to assess the capability of procalcitonin guidance to shorten antibiotic duration in COPD patients with severe pneumonia. For this reason we had use the word “may” to describe the limit to consider withdrawing antibiotics (in future studies). Because it was not considered clear enough for the majority of the peer-reviewers, we decided to completely reformulate the conclusion.
*These points are now clarified in Introduction section page 3 and the conclusions changed in Abstract and Conclusion section page 2 and 10, respectively.

2) A PCT value correlates with the probability of the presence of a bacterial infection, but doesn’t prove the presence or absence of a bacterial infection. This statement should be revised throughout the manuscript (including abstract, page 5)
We thank this reviewer for his suggestion.
*This point is now clarified throughout the manuscript

Minor compulsory comments

1) Page 5: Was there a difference in the distribution of previous antibiotic and steroid use between the 3 PCT groups? Suggest to mention this in the table and/or text. A bit confusing: 11 patients had antibiotics in 24 h prior to ICU admission and 7 in the 30 days (are these exclusive groups?) Please clarify
Patients treated with antibiotic or oral steroid during the previous 30-day period for exacerbation of COPD are exclusive of those receiving antibiotic in 24h prior ICU. All patients pre-treated with antibiotics at ICU admission had a PCT max higher than 0.25 µg/L except 1 in the group with PCTmax 0.1 µg/L - 0.25 µg/L. The majority of patients treated with antibiotic or oral steroid during the previous 30-day period for exacerbation of COPD had PCTmax higher than 0.25 µg/L
*This point is now clarified and details added in Result section in the table 1

2) Page 6: “…PCT levels were not different...”: there seem to be 3 groups, but you have two 2-way-comparisons, so 4 groups? Please clarify.
In this part of the results, we defined 2 groups: one which had received antibiotics and another group which had not received antibiotics. Then we had compared the PCT value (i.e. as a quantitative variable, not with thresholds) and found that the median did not differ significantly.

3) Page 7: confusing to me what you mean with “PCT level <0.1 could indicate...in approximately 10%? But you found no bacteria in all 3?

10% of all patients (3/32) had PCT level <0.1 indicating a low probability of a bacterial infection.
* This point is now clarified in Discussion section page 8

4) You suggest a cut-off of 0.1 to avoid antibiotics to be started, but you don’t test whether this is safe. There are a lot of problems with specificity and sensitivity of bacterial cultures in COPD patients (and LRTIs in general). You have to test this hypothesis first!!

Agreed and thank you. This point has already been discussed. Please refer response to point 1 in major compulsory comments.

5) Conclusion: You could call this a Hypothesis generating observational study.

We thank again this reviewer for this excellent suggestion.
*Conclusion was changed in accordance to the first comment in major compulsory comments

6) What do you mean with sequential PCT testing: only on admission, 6h and 24h or afterwards as well?

As mentioned in Materials and methods section PCT levels were sequentially assessed only on admission and 6 hours and 24 hours after.

Minor discretionary revisions:

1) Why were 34 patients included but PCT only measured in 32?

All blood samples for PCT assessment were centrifuged, decanted, aliquoted, frozen at −80°C and stocked in department of biochemistry until analysed at the end of the study. Unfortunately, samples of two patients were missing.

2) Page 4: suggest to at least mention that you used rtPCR for the viruses, not only refer to the references

* This point is now corrected in Materials and Methods section page 4 and a reference is added in Reference section


3) Reference 3 is in French, therefore-if space permits-you should at least very briefly describe the recommendations or at least what is relevant to the current analysis (e.g all patients treated with antibiotics?)

* This point is now corrected in Materials and Methods section page 4.
4) Table 1: would add previous antibiotics as row

* This point is now added in Table 1

Reviewer 3: Reinhard Berner

Specific comments

1) Page 4, first paragraph “Definition”: The authors state that “…pneumonia was considered bacteriologically confirmed when at least one of the following criteria were present: positive Gram stain in respiratory samples.” Is this really a valid criterion for bacteriologically confirmed pneumonia? In the results section (page 7, second para) the authors report only about patients (n=15) with microbiologically confirmed pneumonia, and give the bacterial species isolated. This might suggest that only culture-proven cases were considered to be microbiologically confirmed? The issue should be clarified.

This reviewer is correct. All pneumonia reported in our study were culture proven cases.

* This point is now clarified in Materials and Methods section page 4

2) Page 7, last paragraph, “Discussion”: The authors state that “…bacteria were detected in more than half the patients with PCT levels suggesting a bacterial infection to be unlikely (PCTmax between 0.1 and 0.25 /l)…”, and conclude that “…a PCT cut off below 0.25 /l for antibiotic use may not be appropriate…” . Although probably true in a clinical setting of COPD patients admitted to the ICU for severe pneumonia (of note in this study 12 out of 32 patients died!); the number of patients in this study does not allow such a conclusion. This conclusion is based on the interpretation of the PCT and bacteriological results in only 4 (!) patients. In addition, it seems not really appropriate to talk about “more than half of the patients” when 4 out of 7 are meant. By the way, the microbiological results in these patients are not given; these findings, however, would be of interest.

We agree with this comment. We are now aware that the small number of included patients does not allow definitive conclusion as mentioned in the Discussion section page. Thank you for this comment. For this reason and according to comments of the Reviewer 2 (refer response to point 1 in major compulsory comments), we have changed conclusion in Abstract and Conclusion section. In addition, details of microbiological results according to the range of PCT are done in Figure 1.

* Abstract and Conclusion section page 2 and page 10, respectively were changed to take in consideration this Reviewer comment. Details of microbiological results according to the range of PCT are done in Figure 1.

3) Page 8, third para, “Discussion”: The second conclusion is also remarkable. The authors state that “…this study confirms the importance of a sequential PCT levels assessment…”. Although the statement per se probably is also true, it is based again only on data of three from five patients who crossed from PCT <0.1 /l to 0.1-0.25 /l. This is weak evidence for a conclusion which might be rather cost-expensive.

Agreed. We think however, that this study underline the importance to early reassess PCT levels specifically when the first measurement is low (i.e. PCT < 0.1 µg/L) to avoid
mistake about the probability of bacterial infection. This point should have important implications for further studies assessing procalcitonin-based antibiotic strategies.

* This point is now corrected in Discussion section page 9

Reviewer 4: Pierre-Emmanuel Charles

Major compulsory revisions

1) First of all, as acknowledged by the authors, the low sample size (n=34) is the main weakness of the study. As a result, groups including less than 4 patients are compared each other, making elusive any conclusion. Regarding this point, the authors’ conclusions should be far more cautious (in the main text as in the abstract). For example, a PCT <0.1 ng/ml was found in only 3 patients included in the study. Although none of them had microbiological evidence for a bacterial infection, it is inappropriate to conclude “antibiotic treatment may be withdrawn early in this subset of patients because of the low likelihood of infection”

This reviewer is correct and we thank this Reviewer for this comment. According to comments of Reviewer 2 and 3 (refer responses point 1 R2 and point 2 R3), we have changed the conclusion in Abstract section and Conclusion section in the main text, as appropriate.

2) Second, the lack of positive culture does not mean that bacterial infection in unlikely given the low reliability of the usual diagnostic tools. Similarly, positive cultures of respiratory samples do not mean bacterial pneumonia. It is however well known that PCT elevation is more pronounced when bacteria are recovered. I believe that the authors should be more cautious regarding this point.

This reviewer is again correct. This point has been already discussed. Please refer responses to points 1 and 3 of Reviewer 1 and point 1 of Reviewer 3.

3) Third, the objectives of the study do not clearly appeared. It is in the introduction section that the main goal of the study was to describe PCT variations in the COPD patient admitted to the ICU with suspected pneumonia. Instead of this, the authors has classified the patients according to the different ranges of PCT values used by Muller et al in their interventional studies (ProCAP, ProCOLD). The present study should therefore be described as a “validation” study that aims at validating the PCT cut-off values proposed by these authors in a peculiar subset of patients with suspected pneumonia since those included in the abovementioned studies were probably less severely ill and not necessarily COPD.

The aim of this pilot study was to assess PCT levels at ICU admission and their relation with bacterial infection in COPD patients with severe pneumonia and to assess whether PCT thresholds predicting probability of bacterial infection previously reported by Muller at all could be efficient in our cohort patient.

* This point is now clarified in Introduction section page 3

Another way to present the data would have been to actually describe PCT values according to the microbiological findings and to determine cut/off values through the construction of ROC curves.

While we understand this reviewer suggestion, we disagree. It is easier to establish ROC curves with a gold standard to assess sensibility, specificity and predictive values. However, this does not exist in this setting. Our pilot work was closer to clinical practice,
while cut-off validation with ROC curves would require proven pneumonia and proven absence of pneumonia (and without prior antibiotic therapy).

Actually, COPD patients included in the present study were more likely to have received antibiotics and steroids prior to ICU admission, factors known to influence PCT behaviour in sepsis

Studies focused on this topic (e.g influence of steroids on PCT in COPD with CAP) are scarce and to our knowledge, there is no evidence that steroids influence the time course of PCT in sepsis. Recently, de Kruif et al reported that increasing doses of corticosteroid did not inhibit the release of PCT during human endotoxemia. In addition, recently, Perren et al reported that in COPD patients with pneumonia and treated with steroids, the slopes over 7 days of PCT are nearly parallel decline compared to those with pneumonia but without COPD. Moreover, the majority of patients treated with antibiotic or oral steroid during the previous 30-day period for exacerbation of COPD had PCTmax higher than 0.25 in our study (Results added in table 1) and PCT levels were not different between patients receiving or not steroids prior ICU admission, PCTmax (p=0.76), as reported in Results section page 7.


Minor essential revisions:

1)Introduction (p3, 2nd §): the main finding of the previously published study about the use of PCT in the patients with AECOPD should be provided (ref 12)
We disagree with this minor suggestion. In the previous article, the population differed as compared with the population investigated in this study. For this reason, we decided to present the data separately. We agree however, that our previous study should be referenced in this work. Interested readers could easily obtain the results with free-access to the full text.

2)What about Legionella sp pneumonia? Has urinary antigen testing been performed in these patients since it has been published that PCT elevation could be lower in this setting than in the patients with Streptococcus pneumoniae pneumonia. If not, one could not exclude that patients without ant microbiological documentation has atypical pneumonia and that antibiotics were required despite low PCT. This point should be clarified.
We thank this reviewer for this comment. Search for bacteria included legionella sp pneumonia. For all patients, a serological diagnostic for antibodies to Legionella pneumophila was performed by indirect immunofluorescence, associated with a detection of Legionella pneumophila serogroup 1 antigen in urine samples.
* This point is now clarified in Materials and Methods section page 4

3)Conclusion (p.9): the last sentence (“...PCT guidance to shorten antibiotic duration”) should be modified since this is not the issue addressed in the present study.
Agreed. This major point is well taken and shared by most of the reviewers. Thank you. Please refer to response to point 1 in major compulsory comments of Reviewer 2. 
* Abstract and Conclusion section page 2 and page 10 respectively were changed to take in consideration this Reviewer comment

4) English copyediting is required
This reviewer is correct when he hypothesized that English is not our primary language. For this reason, we had already sent our previous manuscript to a professional copyediting paid-services (San Francisco Edit).
* Because the manuscript had already been copyedited and because other reviewer who also read this manuscript quoted the quality of written English as “Acceptable”, we consider this reviewer’s suggestion not relevant.

5) Suppress Figure 1
Disagreed, since other reviewers asked for details in this Figure

6) ROC curves
Please, refer to Reviewer 4, point 3