PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The effect of simvastatin on inflammatory cytokines in community-acquired pneumonia: a randomised, double-blind, placebo-controlled trial
AUTHORS	Viasus, Diego; Garcia-Vidal, Carolina; Simonetti, Antonella; Dorca, Jordi; Llopis, Ferran; Mestre, Mariona; Morandeira-Rego, Francisco; Carratala, Jordi

VERSION 1 - REVIEW

REVIEWER	Salvador Bello Servicio de Neumología Hospital Universitario Miguel Servet Zaragoza (Spain)
REVIEW RETURNED	10-Aug-2014

GENERAL COMMENTS	1. It is supposed that patients with hepatic disease or with serum creatin kinase high level were also excluded. Please, explain this issue. 2. Regarding cytokines, the authors performed two comparisons: a) Simvastatin vs placebo groups after 48 h of hospitalisation, and b) Between initial and after 48 h values in patients of each group separately. Figure 2 shows that there were differences in both groups, but this finding seems to go rather unnoticed. Please, include figures of cytokine levels and make a table. 3. In discussion section, the authors should extend some explanations about relationships between their study and some others, especially those from Kruger (similar results) and Novak (different results), approaching the possible causes.

REVIEWER	Richard G. Wunderink Northwestern University Feinberg School of Medicine
REVIEW RETURNED	23-Aug-2014

GENERAL COMMENTS	6/9/10. Primary outcome of time to clinical stability clearly and appropriately justified in the introduction. However, no report of TCS in the actual results. I suspect this will also not be significantly different but should at least be reported. A trend favoring statins gives greater justification for a multicenter trial. More importantly, preliminary data like this is critical to power a larger multicenter study with this endpoint. 7. Probably inappropriate to compare medians of groups as primary analysis for change in cytokines with such a large range at baseline. Would recommend changing primary analysis to a comparison of the median change from baseline (T0-T48h). Since the numbers are so

	limited, an additional figure showing the change in cytokine levels from T0 to T48 would be helpful. Clearly the main weakness of the study is that it is underpowered to answer the question. What was the original power analysis and anticipated sample size prior to initiation of the study? 11. The discussion does not address the elephant in the room until the last sentence. A clinical trial designed with the exclusion criteria used in this study is likely difficult, if not impossible, to recruit adequate numbers, even if multicenter. It is likely also clinically of limited relevance - if only a small minority of patients can be enrolled, this is less likely to be used clinically. 12. Major limitations are not addressed: a) frequent use of corticosteroids. This will have a significant effect on change in cytokine levels, particularly the ones chosen. This is one of the major limitations of the study and compounds the #1 issue of inadequate power. To help understand this effect, the patients on steroids could be marked differently in the additional Figure suggested above. b) time to first antibiotic dose is relatively long (median > 4 hours in both groups and 75% > 4 hours in the placebo group). This may complicate analysis of cytokine levels c) It appears that this group is not very ill with only one on vasopressors and one ICU admission. If the benefit of statins is on cytokine response, the benefit is likely best seen in the more severely ill. The number of patients on mechanical ventilation should be included in the demographic table. Use of one of the ICU admission scores (ATS/IDSA minor criteria, Espana score, REA-ICU, or SMART-COP should be added as well) 1. Figure 1. 44 patients are not accounted for in the excluded group 2. How was the duration of 4 days for the statin chosen? If the alternative explanation of benefit on CV plaque stabilization is viable, longer duration may be needed. The rationale for the duration should be included in the methods section. 3. Statins are used routinely in patients taking other medications that affect CYP3A4 with appropriate monitoring. Why were these patients excluded for this trial? A more extensive justification for this should be given than that they are metabolized by CYP3A4. 4. I am a little concerned about the AST levels at 48 hours with a difference at a p level of 0.08. In an underpowered study like this, that level is concerning. The actual levels should be included in the manuscript. How many had an increase of > 2x baseline?

REVIEWER	Jose Bordon Providence Hospital, Washington, D.C. US
REVIEW RETURNED	01-Sep-2014

GENERAL COMMENTS	This study address a major question in an attempt to modulate inflammation by using simvastatin in relation to TCS. Unfortunately, the study design and manuscript organization do not follow the standards. There are some inconcistency in the study aims and primary aim points in the abstract and manuscript M&M. Study aims, objectives and endpoints do have to be consistent.

	In abstract the text: "However, the trial was stopped because enrolment was much slower than originally anticipated and the study would not have been completed in a reasonable period of time" is part of results in the abstract and of limitations in the discussion section; no where else. The abstract results should address the TCS as primary end point. Abstract conclusion: The use of simvastatin, 20 mg once daily for four days, since hospital admission did not reduce the levels of inflammatory cytokines in hospitalised patients with CAP. This is a strong statement for the study limitations in relation to the small sample size. Instead, the authors should say they were unable to find a reduction of the blood cytokines levels….. Introduction section, the text: "In this study, we hypothesized that statin therapy given to hospitalised patients with CAP would reduce the time to clinical stability and the concentration of inflammatory cytokines. Primary end-point of this trial was the time from hospital admission to clinical stability" is clear and should be kept consistently along the manuscript and abstract. Page 10 End points needs to be revised to consistency. Similarly to abstact conclusion, the discussion first and last paragraph need to be corrected as indicated previously.

REVIEWER	Dr Victoria Allgar University of York
REVIEW RETURNED	06-Oct-2014

GENERAL COMMENTS	There was no detail of the original power calculation. This is required. The trial was stopped early, but no reasons were given and it is not clear without the original power calculation what the % recruitment was. Table 1 details the baseline characteristics, in line with CONSORT baseline characteristics should not be compared statistically, so the p-values should e removed. Primary end-point of this trial was the time from hospital admission to clinical stability - I could not see this data in the paper. Table 2 details the enrolment and 48 hour serum cytokine concentrations and PaO2/FiO2. This is useful descriptive data but the statistical tests should take account of the paired nature of the data. An ANCOVA analysis would be more useful here, comparing the two groups looking at 48 hour values, adjusting for baseline. The Wilcoxon signed-rank test was used to compare two related measurements - this is not appropriate as this looks within group, rather than between group. There are flaws in the study data in terms of the approach to analysis and lack of detail on the power calculation and reasons for early trial closure.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name
Salvador Bello
Institution and Country Servicio de Neumología
Hospital Universitario Miguel Servet
Zaragoza (Spain)
Please state any competing interests or state 'None declared': None declared

We greatly appreciate the comments made by the reviewer.

1. It is supposed that patients with hepatic disease or with serum creatin kinase high level were also excluded. Please, explain this issue.

Patients with chronic hepatic disease or with high serum creatine kinase levels (this was not measured as a requisite for the initial administration of simvastatin) at admission were not excluded. Importantly, no patient with hepatic cirrhosis was recruited in the study.

2. Regarding cytokines, the authors performed two comparisons: a) Simvastatin vs placebo groups after 48 h of hospitalisation, and b) Between initial and after 48 h values in patients of each group separately. Figure 2 shows that there were differences in both groups, but this finding seems to go rather unnoticed. Please, include figures of cytokine levels and make a table.

Information about the two comparisons performed in citokynes are described in the Table 2 (simvastatin vs placebo groups at admission and after 48 h of hospitalisation) and Figure 2 (between initial and after 48 h values in patients of each group separately). In addition, we have performed the ANCOVA analysis (Table 2) and a comparison of the median change from baseline (T0-T48h) (Table 3).

3. In discussion section, the authors should extend some explanations about relationships between their study and some others, especially those from Kruger (similar results) and Novak (different results), approaching the possible causes.

Following the reviewer recommendation, we have added new information regarding relationship between our study and the others (Page 14, line 13 and Page 15, line 15).

Reviewer: 2

Reviewer Name
Richard G. Wunderink
Institution and Country Northwestern University Feinberg School of Medicine
Please state any competing interests or state 'None declared': None declared

We are grateful to the comments noted by the reviewer.

6/9/10. Primary outcome of time to clinical stability clearly and appropriately justified in the introduction. However, no report of TCS in the actual results. I suspect this will also not be significantly different but should at least be reported. A trend favoring statins gives greater justification for a multicenter trial. More importantly, preliminary data like this is critical to power a larger multicenter study with this endpoint.

Following the reviewer recommendations, we have added the results of the analysis about TCS (Page 12, lines 11-12).

7. Probably inappropriate to compare medians of groups as primary analysis for change in cytokines with such a large range at baseline. Would recommend changing primary analysis to a comparison of the median change from baseline (T0-T48h). Since the numbers are so limited, an additional figure showing the change in cytokine levels from T0 to T48 would be helpful.

Please see response 2 from the reviewer 1.

8. Clearly the main weakness of the study is that it is underpowered to answer the question. What was the original power analysis and anticipated sample size prior to initiation of the study?

We have added information about original power analysis and anticipated sample size (Page 11, lines 5-8).

11. The discussion does not address the elephant in the room until the last sentence. A clinical trial designed with the exclusion criteria used in this study is likely difficult, if not impossible, to recruit adequate numbers, even if multicenter. It is likely also clinically of limited relevance - if only a small minority of patients can be enrolled, this is less likely to be used clinically.

We have added some sentences about this limitation of the study (Page 16, lines 20-21).

12. Major limitations are not addressed:
a) frequent use of corticosteroids. This will have a significant effect on change in cytokine levels, particularly the ones chosen. This is one of the major limitations of the study and compounds the #1 issue of inadequate power. To help understand this effect, the patients on steroids could be marked differently in the additional Figure suggested above.

Following the reviewer recommendation, we have added the results of the analysis in patients using and not using corticosteroids (Page 12, lines 18-20).

b) time to first antibiotic dose is relatively long (median > 4 hours in both groups and 75% > 4 hours in the placebo group). This may complicate analysis of cytokine levels

We agree with the reviewer comment. However, no significant differences were observed between study groups regarding this topic. We have added a sentence regarding this issue in the limitations of the study (Page 16, lines 15-17).

c) It appears that this group is not very ill with only one on vasopressors and one ICU admission. If the benefit of statins is on cytokine response, the benefit is likely best seen in the more severely ill. The number of patients on mechanical ventilation should be included in the demographic table. Use of one of the ICU admission scores (ATS/IDSA minor criteria, Espana score, REA-ICU, or SMART-COP should be added as well)

We have added the number of patients on mechanical ventilation, and ATS/IDSA minor criteria to the Table 1.

1. Figure 1. 44 patients are not accounted for in the excluded group

We have corrected the figure.

2. How was the duration of 4 days for the statin chosen? If the alternative explanation of benefit on CV plaque stabilization is viable, longer duration may be needed. The rationale for the duration should be included in the methods section.

We have included information about the rationale for the duration of 4 days for simvastatin in the methods section (Page 10, line 1-4).

3. Statins are used routinely in patients taking other medications that affect CYP3A4 with appropriate monitoring. Why were these patients excluded for this trial? A more extensive justification for this should be given than that they are metabolized by CYP3A4.

We have added data regarding this issue (Page 16, lines 4-6).

4. I am a little concerned about the AST levels at 48 hours with a difference at a p level of 0.08. In an underpowered study like this, that level is concerning. The actual levels should be included in the manuscript. How many had an increase of > 2x baseline?

We have added the Table 3.

1. The authors appropriately attempt to address the potential role of statins as immunomodulatory agents. However, alternative explanations for potential benefit are not discussed, including stabilization of cardiovascular system to avoid acute CV events associated with CAP and potential antiviral and antibacterial effects

Information about the effects of statins in endothelial is already discussed. In addition, following the reviewer recommendation, we have added other information about alternative explanations for potential benefit of statins in this context (Page 16, lines 13-16).

Reviewer: 3

Reviewer Name
Jose Bordon
Institution and Country Providence Hospital, Washington, D.C. US
Please state any competing interests or state ‘None declared’: None declared

We appreciate the reviewer´s comments.

This study address a major question in an attempt to modulate inflammation by using simvastatin in relation to TCS. Unfortunately, the study design and manuscript organization do not follow the standards. There are some inconcistency in the study aims and primary aim points in the abstract and manuscript M&M. Study aims, objectives and endpoints do have to be consistent.

We really appreciate the reviewer's comments about the organization of the manuscript. We have followed the suggested recommendations.

In abstract the text: "However, the trial was stopped because enrolment was much slower than originally anticipated and the study would not have been completed in a reasonable period of time" is part of results in the abstract and of limitations in the discussion section; no where else. The abstract results should address the TCS as primary end point. Abstract conclusion: The use of simvastatin, 20 mg once daily for four days, since hospital admission did not reduce the levels of inflammatory cytokines in hospitalised patients with CAP. This is a strong statement for the study limitations in relation to the small sample size. Instead, the authors should say they were unable to find a reduction

of the blood cytokines levels…..

Following the reviewer recommendations, we have rewritten the abstract.

Introduction section, the text: "In this study, we hypothesized that statin therapy given to hospitalised patients with CAP would reduce the time to clinical stability and the concentration of inflammatory cytokines. Primary end-point of this trial was the time from hospital admission to clinical stability" is clear and should be kept consistently along the manuscript and abstract.

We have modified the sentences to keep consistently along the manuscript.

Page 10 End points needs to be revised to consistency.

We have clarified the information regarding the study end points (Page 10, lines 13-14).

Similarly to abstract conclusion, the discussion first and last paragraph need to be corrected as indicated previously.

We have corrected the first and last paragraphs of the discussion (Pages 14 and 17).

Reviewer: 4

Reviewer Name
Dr Victoria Allgar
Institution and Country University of York
Please state any competing interests or state ‘None declared’: None declared

We thank you for the comments.

There was no detail of the original power calculation. This is required. The trial was stopped early, but no reasons were given and it is not clear without the original power calculation what the % recruitment was.

Please see response 8, reviewer 2. In addition, we have added information dealing with the reasons for low recruitment (Page 12, line 2-3).

Table 1 details the baseline characteristics, in line with CONSORT baseline characteristics should not be compared statistically, so the p-values should be removed.
Following the reviewer recommendation, we have deleted the p-values from Table 1.

Primary end-point of this trial was the time from hospital admission to clinical stability - I could not see this data in the paper.

Data about time from hospital admission to clinical stability was added (Page 12, lines 11-12).

Table 2 details the enrolment and 48 hour serum cytokine concentrations and PaO2/FiO2. This is useful descriptive data but the statistical tests should take account of the paired nature of the data. An ANCOVA analysis would be more useful here, comparing the two groups looking at 48 hour values, adjusting for baseline. The Wilcoxon
signed-rank test was used to compare two related measurements - this is not appropriate as this looks within group, rather than between group.

We have added the ANCOVA analysis (Table 2).

There are flaws in the study data in terms of the approach to analysis and lack of detail on the power calculation and reasons for early trial closure.
We really appreciate the reviewer's comments about the study analysis. We have followed the suggested recommendations.

VERSION 2 – REVIEW

REVIEWER	Richard G. Wunderink Northwestern University Feinberg School of Medicine
REVIEW RETURNED	13-Nov-2014

GENERAL COMMENTS	The authors have adequately addressed my previous concerns. I have no further comments/recommendations

REVIEWER	Salvador Bello Servicio de Neumología Hospital Universitario Miguel Servet Zaragoza (Spain)
REVIEW RETURNED	25-Nov-2014

GENERAL COMMENTS	After having been taken into consideration the reviewer´s suggestions, the manuscript has been significantly improved. According to my opinion, it can be regarded for publication.