PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Diagnostic accuracy of presepsin in predicting bacteremia in elderly patients admitted to the emergency department : prospective study in Japan
AUTHORS	Yoshiro, Imai; Taniguchi, Kohei; Ryo, Iida; Masahiko, Nitta; Kazuhisa, Uchiyma; Akira, Takasu

VERSION 1 – REVIEW

REVIEWER	Juan E Losa Hospital U. F. Alcorcón. Universidad Rey Juan Carlos
REVIEW RETURNED	15-Apr-2019

GENERAL COMMENTS	1.- Nowadays it is not recommended to use the term "systemic inflammatory response syndrome". We are set to use "sepsis" according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). This document should be referenced in the text. 2.- There is at least another study that have analysed the same issue and must be referenced: Leli C, et al. Diagnostic accuracy of presepsin (sCD14-ST) and procalcitonin for prediction of bacteraemia and bacterial DNAaemia in patients with suspected sepsis. Journal of Medical Microbiology (2016), 65, 713–719. 3.- It is not clear how many methods or devices were used to determine presepsin:only one or four? 4.- The authors must revise the odds ratio and CI90% because there is disagreement between tne abstract and the text and table 3 4.- The reviewer provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Amith Shetty Westmead Institute for medical research, Sydney Australia
REVIEW RETURNED	25-Apr-2019

GENERAL COMMENTS	Was the study specifically designed for the research question or is this a analysis conducted on a larger cohort study? The issue a a very low cut-off with increased sensitivity with a PCT with very high cut-off and low sensitivity needs to be further explored. 30-40% of patients with sepsis do not have positive cultures and I would think the primary goal for ED clinicians would be to pick patients at risk of adverse outcomes and not just bacteremia - a composite table comparing presepsisn/ PCT/CRP for adverse outcomes/ mortality/ SOFA sepsis and bacteremia even in such a

	small sample size may be of more value and add weight to presepsin as a biomarker Another option would be to check if a combination biomarker approach could work with presepsin and/or PCT/CRP? The use of Multiple logistic regression model with hazard ratio for independent/ dependent biomarker levels (what were the variables included in model?) is questionable and possibly needs statistical review? Overall a worthwhile study which could add value if revised appropriately - attached file with comments on article

REVIEWER	ANTHONY C ATKINSON London School of Economics, London UK
REVIEW RETURNED	25-May-2019

GENERAL COMMENTS	Report for the authors on 'BMJ Open' submission bmjopen-2018-030421 "Diagnostic accuracy of presepsin in predicting bacteremia in elderly patients admitted to the emergency department" by Yoshino and 5 others Thank you for your carefully written paper. I have a very few comments on statistical matters. As a footnote to Table 3 you explain what CI means. I think you should do something similar for the ± values in Table 1. I assume they are standard errors of the means, but they might be ±2 standard errors ... On p.5 you say "a multivariate logistic regression model was used". I would like more details around p.7, l.26. Indeed you have a binary response and, for each biomarker, you have three risk factors from Table 1; age, sex and biomarker type. You say "presepsin was the only risk factor". Does this mean that age, sex and the other biomarkers had no predictive power? You further say that the three biomarker levels significantly correlate with the SOFA and APACHE II scores and give highly significant numbers. You then give Spearman's rank correlation values. Are these the ones for which you have just given the significance? What is the importance in this investigation of these two scores? Could they not provide additional risk factor assessments, or do they take too long to calculate to be useful in ED? At the bottom of p.6, the list of bacteria is presumably just for interest; it is not clear how this enriches the analysis. Figure 2 is very hard to read at this size. However, the plots don't seem to show a good relationship between the two quantities. In a-1 and, especially, a-2, there seem to be a cluster of points and a line perhaps influenced by two rather different points. Also b-2 seems strange with a virtually horizontal line and two distant points in the centre. Is this why you used Spearman rank correlation values? I thought your English was good and I only spotted a few typos. p.3, l.16-17. Erroneous white space. Also below. l.44. "due to various". p.5, l.40. "A chi-squared" p.6 l.47. "Thee" → "The" p.7, l.17. "0.52) values."

VERSION 1 – AUTHOR RESPONSE

To Reviewer 1
Reviewer Name: Juan E Losa
Reviewer's comment 1
Nowadays it is not recommended to use the term "systemic inflammatory response syndrome". We are set to use "sepsis" according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). This document should be referenced in the text.
Our response to comment 1
Thank you for your valuable suggestion. We reference The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).
Updated definitions of sepsis in 2016. Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated response to infection. The diagnostic criteria for sepsis use SOFA instead of SIRS. (page8 line13-15)

Reviewer's comment 2
There is at least another study that have analysed the same issue and must be referenced: Leli C, et al. Diagnostic accuracy of presepsin (sCD14-ST) and procalcitonin for prediction of bacteraemia and bacterial DNAaemia in patients with suspected sepsis. Journal of Medical Microbiology (2016), 65, 713–719.
Our response to comment 2
Thank you for your comment. We referenced this study.
Leli et al reported that the cutoff value for bacteremia was 843.5 pg/mL for suspected sepsis(2016) in various departments. (page8 line7-9)

Reviewer's comment 3
It is not clear how many methods or devices were used to determine presepsin:only one or four?
Our response to comment 3
Thank you for your suggestion.
The separated plasma of presepsin was collected and stored at −35 °C until analysis. Plasma presepsin levels were determined only by a chemiluminescent enzyme immunoassay (PATHFAST immunoassay analytical system; Mitsubishi Chemical Medience Corporation, Japan),
(page5 line8-11)

Reviewer's comment 4
The authors must revise the odds ratio and CI90% because there is disagreement between the abstract and the text and table 3
Our response to comment 4
We corrected the indicated sentences. Thank you. (page2 line24)

Attachment presepsin_system_appendPDF_proof_hi.pdf
Reviewer's comment (page2 line26)
Today it´s not recommended to use this concept. It´s preferred "sepssis"
Our response to comment
Thank you for your valuable suggestion.
We changed SIRS to sepsis. (page2 line6)

Reviewer's comment (page3 line5) (page3 line6)
Presepsin could be a better biomarker to evaluate bacteremia : with sepsis criteria

Our response to comment
We corrected the sentence as you pointed out. Thank you (page3 line2-3).

Reviewer's comment (page4 line5)
I think it is necessary to reference this sentence. That is: It is demonstrated that CRP and PCT support the diagnosis of sepsis
Our response to comment
Thank you for your suggestion. We add the reference. (page4 line1)
Reviewer's comment (page5 line33)
Was presepsin determined by three different methods or devices?
Our response to comment
Thank you for your suggestion. We used to determine presepsin only one methods.
PATHFAST immunoassay analytical system;Mitsubishi Chemical Medience Corporation, Japan (page5 line10-11)

Reviewer's comment (page7 line8)
mg/L
Our response to comment
We corrected the term. Thank you.

Reviewer's comment (page7 line28)
odds ratio, 8.84; 95%
confidence interval, 1.32–177.09; p = 0.02
As noted in abstract
Our response to comment hazard ratio, 8.84; 95% confidence interval, 0.95–81.79; p = 0.02 (page2 line24)

Reviewer's comment (page8 line17)
superior in this small study, wasn´t it?
Our response to comment
Thank you for your suggestion. We changed it as belows.
Therefore, presepsin was was superior than CRP and PCT in diagnosing bacteremia in elderly patients admitted to the ED. (page7 line24-page8 line2)

Reviewer's comment (page9 line17)
I suggest to do a sensitivity analysis without CNS
Our response to comment
Thank you for your suggestion
If we assume that a CNS positive patient is blood culture negative, the univariate analysis showed no significant differences between the 2 groups in terms of presepsin (p=0.09), PCT (p=0.18) and CRP (p=0.20).
Therefore, I did not mention it.
And, we did not do a sensitivity analysis without CNS
However, because we are targeting the elderly, CNS was included in blood positive.

Reviewer's comment (page9 line24)
Presepsin could be more useful
Our response to comment
Thank you for your suggestion. We changed it as belows.
This cohort study suggested that Presepsin could be more useful in detecting bacteremia in elderly patients admitted to the ED. (page9 line20-21)

Reviewer's comment (Table3)
Our response to comment
We corrected the term. Thank you.

Reviewer's comment (References)
To add
Leli C, et al. Diagnostic accuracy of presepsin (sCD14-ST) and procalcitonin for prediction of bacteraemia and bacterial DNAaemia in patients with suspected sepsis. Journal of Medical Microbiology (2016), 65, 713–719.Singer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA 2016;315: 801-810.
Our response to comment
We added this reference, thank you. (page8 line15)

To Reviewer 2
Reviewer Name: Amith SHetty
Reviewer's comment 1
Was the study specifically designed for the research question or is this a analysis conducted on a larger cohort study?
The issue a very low cut-off with increased sensitivity with a PCT with very high cut-off and low sensitivity needs to be further explored.
30-40% of patients with sepsis do not have positive cultures and I would think the primary goal for ED clinicians would be to pick patients at risk of adverse outcomes and not just bacteremia - a composite table comparing presepsisn/ PCT/CRP for adverse outcomes/ mortality/ SOFA sepsis and bacteremia even in such a small sample size may be of more value and add weight to presepsin as a biomarker
Our response to comment 1
Thank you for your constructive comments. We agree your comments, so added to the discussion. Bacteremia can be identified in about 30% of septic patients and necessitates further diagnostic evaluation. Therefore, the study which the primary outcome would be to pick up patients at risk of adverse outcomes and not just bacteremia should be necessary. (page9 line14-17)

Reviewer's comment 2
Another option would be to check if a combination biomarker approach could work with presepsin and/or PCT/CRP?
Our response to comment 2
Thank you for your suggestion. But we could work a combination biomarker approach.
The combination was not useful because there were few cases.

Reviewer's comment 3
The use of Multiple logistic regression model with hazard ratio for independent/ dependent biomarker levels (what were the variables included in model?) is questionable and possibly needs statistical review?
Our response to comment 3
We clarified explanatory variables and objective variables, thank you.
A multivariate logistic regression analysis model which objective variable was presence of bacteremia, explanatory variable was CRP, PCT, and presepsin was used to identify the influence of CRP, PCT, and presepsin on bacteremia. (page5 line21-22)

Reviewer's comment 4
Overall a worthwhile study which could add value if revised appropriately - attached file with comments on article

Our response to comment 4
Thank you for your insightful comment.

Attachment presepsin_system_appendPDF_proof_hi.pdf
Reviewer's comment (page3 line39)
BSI severity and bacterial load and separate themes
Our response to comment
Thank you for your suggestion. We corrected.
Bacteremia causes bacterial bloodstream infection that is associated with a significant mortality (page3 line16-17)

Reviewer's comment (page3 line44)
please provide reference to support bacteremia rates are higher in elderly and causation?
Our response to comment
Thank you for your suggestion. We added the reference. (page3 line19)

Reviewer's comment (page4 line26)
why not for sepsis or adverse outcomes? we know bacteremia only occurs or is detectable in a modest proportion
Our response to comment
Thank you for your suggestion.
The purpose of this study was to examine the relationship between blood culture positive rate and presepsin.

Reviewer's comment (page4 line47)
if the study design was to predict bacteremia - why the exclusions?
Our response to comment
Thank you for your comment.
terminal stage of malignant cancer, acquired immunodeficiency syndrome or end-stage liver disease were excluded because these diseases affected CRP level.

Reviewer's comment (page5 line56)
include causative factors, coorelation coefficiencts possibly more likely for categorical outcomes where CRP/PCT and presepsisn possibly not interdependent?
Our response to comment
Thank you for your comment.
I am sorry, it is not examined in this research

Reviewer's comment (page6 line8)
more appropriate
Our response to comment
Thank you for your comment. We corrected.
an area under the curve (AUC) differences were assessed with De Long test. (page5 line1-2)

Reviewer's comment (page6 line28)
ethics approval?
Our response to comment
The study protocol was approved by the ethics committee of Osaka Medical College (1585).
We listed ethics approval in the patient and public section from method section. (page6 line11-12)

Reviewer's comment (page7 line24)
is this balanced AUC?

Our response to comment
Yes it is.

Reviewer's comment (page8 line24)
at cut-off of ....
Our response to comment
Thank you for your comment. We added a cut-off value. (page8 line10)

Reviewer's comment (page8 line24)
were these excluded or included? if yes please compare the values in two groups?
Our response to comment
If we assume that a CNS positive patient is blood culture negative, the univariate analysis showed no significant differences between the 2 groups in terms of presepsin (p=0.09), PCT (p=0.18) and CRP (p=0.20).
Therefore, I did not mention it.
However, because we are targeting the elderly, CNS was included in blood positive.

Reviewer's comment (Table 2)
dropping cut-off value can spuriously increase sensitivity at the cost of specificity
PCT value of 15.8 is very high based on past studies and as the ROC suggest - a lower PCT cut-off may have a much higher sensitivity as well?
Our response to comment
Thank you for your constructive comments. We agree your comments, so added to the discussion.
In our study, the PCT cutoff value of 15.8 ng/mL was higher as the ROC suggested than the past study 5. The reason might be also explained by the small sample size and lack of data on patient's medical history.
(page9 line11-13)

To Reviewer 3
Reviewer Name: ANTHONY C ATKINSON
Reviewer's comment 1
As a footnote to Table 3 you explain what CI means. I think you should
do something similar for the ± values in Table 1. I assume they are standard
errors of the means, but they might be ±2 standard errors ...
Our response to comment 1
We explained what 95% CI means.
95%CI: 95% confidence interval as a footnote to Table 3. (page11 line6)
Thank you for your correction.
We explained the ± values in Table 1. We added it as belows.
Continuous variables were presented as mean ± standard errors (SE)
And, we changed the standard errors of the means in the statistical analysis section.
(page5 line18)
Reviewer's comment 2
On p.5 you say "a multivariate logistic regression model was used". I
would like more details around p.7, l.26. Indeed you have a binary response
and, for each biomarker, you have three risk factors from Table 1; age, sex
and biomarker type. You say "presepsin was the only risk factor". Does this
mean that age, sex and the other biomarkers had no predictive power?
Our response to comment 2

Because the number of cases is small, it is not statistically significant if there are many explanatory variables.
Therefore, three biomarkers were examined in this study.
This dose not mean that age, sex and the other biomarkers had no predictive power.
We added the sentence as below.
Because the number of cases was small, so three biomarkers were examined in this study in Comparison between the bacteremia and non-bacteremia groups section
(page7 line15-16)

Reviewer's comment 3
You further say that the three biomarker levels significantly correlate with the SOFA and APACHE II scores and give highly significant numbers. You then give Spearman's rank correlation values. Are these the ones for which you have just given the significance?
Our response to comment 3
The Spearman's rank correlation values with the SOFA and APACHE II scores were higher for presepsin than for PCT and CRP. We believe that presepsin also correlates with severity.

Reviewer's comment 4
What is the importance in this investigation of these two scores? Could they not provide additional risk factor assessments, or do they take too long to calculate to be useful in ED?
Our response to comment 4
In this research, samples were measured outsourced. We do not have PATHFAST immunoassay analytical system. But if the system is purchased, it can be measured in a few minutes.
It is also very useful in emergency outpatients.

Reviewer's comment 5
At the bottom of p.6, the list of bacteria is presumably just for interest; it is not clear how this enriches the analysis.
Our response to comment 5
Thank you for your comment.
It is as you pointed out. I have listed it as a reference.

Reviewer's comment 6
Figure 2 is very hard to read at this size. However, the plots don't seem to show a good relationship between the two quantities. In a-1 and, especially, a-2, there seem to be a cluster of points and a line perhaps influenced by two rather different points. Also b-2 seems strange with a virtually horizontal line and two distant points in the centre. Is this why you used Spearman rank correlation values?
Our response to comment 6
Thank you for your constructive comments. We agree your comments.
In our investigation, The Spearman's rank correlation values was not so important.
So we exclude the figure because everyone is confused.

Reviewer's comment 7
p.3, l.16-17. Erroneous white space. Also below.
l.44. "due to various".
p.5, l.40. "A chi-squared"
p.6 l.47. "Thee" → "The"
p.7, l.17. "0.52) values."
Our response to comment 7
We corrected the term. Thank you.

VERSION 2 – REVIEW

REVIEWER	Juan E Losa Hospital Universitario Fundación Alcorcón
REVIEW RETURNED	27-Jul-2019

GENERAL COMMENTS	1.- The title is too long 2.- Some phrases are not well understood 3.- Some errors must be corrected 4.- English must be reviewed

REVIEWER	ANTHONY C ATKINSON London School of Economics, London UK
REVIEW RETURNED	20-Jul-2019

GENERAL COMMENTS	Report for the authors on revised 'BMJ Open' submission bmjopen-2018-030421.R1 "Diagnostic accuracy of presepsin in predicting bacteremia in elderly patients admitted to the emergency department" by Yoshiro Imai and 5 others Thank you for the revision of your paper. I think all is well on statistical matters and I have only a few comments on typos, mostly in your changes. Unfortunately, your document reached me without page numbers, so I have inserted them by hand. p.1, l.4. Omit 'the'. p.2, l.6. "with fulfilled the sepsis" → "who satisfied the sepsis criteria". p.5, l.17. I was suggesting a footnote to Table 1. Instead you expanded that to Table 3. Here you could say "are presented" and add "in Table 1". l.20. "objective" → "response". l.21. "explanatory variables were". p.7, l.10. ", presepin was the only significant risk factor". l.24. "superior to". P.8, l.12. "Updated definitions ...". This reads like a section heading. "An updated definition of sepsis was given in 2016" and then give a reference. p.9., l.10. "suggested by the ROC was higher than in a previous study 5. l.14. "Therefore a study is needed in which the primary outcome would be to pick up patients at risk of adverse outcomes, not just the presence of bacteremia".

VERSION 2 – AUTHOR RESPONSE

To Reviewer 3

Reviewer Name: ANTHONY C ATKINSON

Reviewer's comment 1

Please state any competing interests or state 'None declared': None

Response: We indicated a statement in the section of Conflict of Interest.

Reviewer’s comment

p.1, l.4. Omit `the'.

p.2, l.6. with fulfilled the sepsis → who satisfied the sepsis criteria.

Response: We have corrected these, accordingly.

Reviewer’s comment

p.5, l.17. I was suggesting a footnote to Table 1. Instead you expanded

that to Table 3. Here you could say “are presented" and add “in Table 1".

Our response to comment:

Response: We corrected by adding columns for odds ratio, 95% CI, and p-value in Table 1 instead of Table 3.

Reviewer’s comment

l.20. “objective" → “response".

l.21. “explanatory variables were".

p.7, l.10. “presepin was the only significant risk factor".

l.24. “superior to".

Response: Thank you for your comments. We have corrected these accordingly.

Reviewer’s comment

P.8, l.12.“Updated definitions .". This reads like a section heading.

“An updated definition of sepsis was given in 2016" and then give a reference.

Response: Thank you for your comment. We have deleted the section, according to the comments of reviewer #1.

Reviewer’s comment

p.9. l.10.“suggested by the ROC was higher than in a previous study 5.

l.14.“Therefore a study is needed in which the primary outcome would be to pick up patients at risk of adverse outcomes, not just the presence of bacteremia".

Response: Thank you for your comments. We have corrected these accordingly.

To Reviewer 1

Reviewer Name: Juan E Losa

Reviewer's comment 1

The title is too long

Response: Thank you for your comment. According to the editor's suggestion, we changed the title to "Diagnostic accuracy of presepsin in predicting bacteremia in elderly patients admitted to the emergency department: a prospective study in Japan".

Reviewer's comment (page2 line10)

Response: Thank you for your correction.

Reviewer's comment (page2 line17)

Response: Thank you for your suggestion. We changed it to "single-center trial in patients who satisfied the sepsis criteria".

Reviewer's comment (page2 line26)

Reviewer's comment (page3 line8)

Reviewer's comment (page3 line49)

Response: Thank you for your comments. We have corrected these accordingly.

Reviewer's comment (page6 line26)

What does this number mean?

Response: It was an Ethics Committee approval number.

Thank you for your comments. We have corrected these accordingly.

Reviewer's comment (page6 line40)

Reviewer's comment (page6 line42)

Our response to comment:

Response: Thank you for your comments. We have corrected these accordingly.

Reviewer’s comment (page6 line54)

in 4 cases (as specified just below)

Response: Thank you for your comments. We have corrected these accordingly.

Reviewer’s comment (page7 line12)

Be careful with this phrase because 6,77 and 45,04 are not very similar values. I would say "were not significantly different"

Response: Thank you for your suggestion. It has been corrected accordingly.

Reviewer’s comment (page7 line26)

odds ratio better than hazard ratio in this case, isn´t it?

Response: Thank you for your suggestion. It has been corrected accordingly.

Reviewer’s comment (page7 line52)

Response: Thank you for your suggestion. It has been corrected accordingly.

Reviewer’s comment (page8 line24-29)

What is this? What does it mean here?

Our response to comment :

Response: Thank you for your suggestion. The section seemed to not mean anything and has been deleted.

Reviewer’s comment (page9 line41)

Reviewer’s comment (page9 line44)

Our response to comment :

Thank you for your corrections and thy have been done.