PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	High-sensitivity C-Reactive Protein as a Predictor of In-hospital Mortality in Cardiovascular Disease Patients at an Emergency Department: a retrospective cohort study
AUTHORS	Yoshinaga, Ryo; Doi, Yasufumi; Ayukawa, Katsuhiko; Ishikawa, Shizukiyo

VERSION 1 – REVIEW

REVIEWER	Paul Ridker Brigham and Women's Hospital, Boston, MA Dr. Ridker is listed as a co-inventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease and diabetes that have been licensed to Seimens and AstraZeneca.
REVIEW RETURNED	17-Dec-2016

GENERAL COMMENTS	Yoshinaga et al present data from a retrospective cohort of ED patients in Japan entering with acute cardiovascular symptoms, and demonstrate that hsCRP levels are a predictor of both cardiac and non- cardiac in-hospital mortality. The data confirm the utility of hsCRP in this setting in Japan, an interesting issue since, in general, hsCRP levels are lower in Japan than in the West (see for example data from the Hisiyama study). 1. Can the authors compare the utility of hsCRP to other widely used biomarkers such as troponin? 2. Can the authors control for confounding by issues such as obesity? 3. Can the authors present in graphical form a distribution plot of hsCRP levels among those who do and do not die in hospital, perhaps stratified by cardiac and non-cardiac causes? 4. The references are selective and do not cite many of the major papers in this field. See as examples very large data-bases of hsCRP levels measured in acute ischemia in the PROVE-IT (NEJM 2005) and IMPROVE-IT (Circulation 2015) trials. 5. As noted above, the reference cut-off in Japan is lower than that in the West (see page 7). 6. Please remove the term “multivariable adjusted” from table 1 as this is very misleading. According to the legend, this is adjusted for age, sex and WBC only. 7. Is there another way to display the data of Figure 2? This gives

	the false impression that total rates are the same in each group. 8. There is abundant data about hsCRP predicting all-cause mortality in different settings that should be addressed here. See for example Clin Chem 2008;54:234-7 9. How might these data be relevant for ongoing clinical trials of inflammation inhibition as a method to reduce cardiovascular event rates?

REVIEWER	Dietmar MJ Krause Department of Medical Informatics, Biometry and Epidemiology, Ruhr-University of Bochum, 44780 Bochum, Germany
REVIEW RETURNED	19-Apr-2017

GENERAL COMMENTS	This retrospective cohort study adds to the body of knowledge regarding the prognostic value of hs-CRP using the data of a large cohort of ED patients. As the authors state in the introduction and discussion, there are many studies with focus on the prognostic value of CRP in CVD. Thus, the description of the background section of the abstract should be amended. In the Methods and Results part of the abstract, the AR scale should be clarified In the last point of the Strength and Limitation section, is should be mentioned that there is no absence of confounders (I think there are many....), but that they are not taken into account. In the second section of the introduction, the last sentence is not clear to me. In the statistical analysis part, multivariable instead of multivariate should be used as was done in the tables. The meaning of multivariable is defined in the tables but not in the text. It should be mentioned that multivariable just means the WBC is added in the adjustment. In the discussion, it should become clear whether a non-CVD cause of death was regarded as misclassification or a complication of a well classified CVD. Furthermore, one result is that the addition of WBC in the adjustment does not substantially change the HRs; this should be discussed.

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewer 1: Professor Paul Ridker

Comment 1
Can the authors compare the utility of hsCRP to other widely used biomarkers such as troponin?
Comment 2
Can the authors control for confounding by issues such as obesity?

Responses 1 and 2
We felt that confounders and covariates other than age, sex and WBC could not be adjusted in this study, because the numbers of patients in whom troponin was measured was quite small, and because other data about their physical condition such as BMI, hemodynamics and co-morbidities were not available due to our system's use of handwritten data.

Comment 3

Can the authors present in graphical form a distribution plot of hsCRP levels among those who do and do not die in hospital, perhaps stratified by cardiac and non-cardiac causes?

Response 3
In accord with this suggestion, we have presented in graphical form a distribution plot of hs-CRP levels in the revised Figure 2 and described this in the Results section as follows (page 9):

"Figure 2 shows a box plot of the distribution of hs-CRP levels in the surviving patients and the CVD-death and Non-CVD death groups. There were significant differences in the hs-CRP levels among the three groups ($p<0.001$, respectively). The association was unchanged even after Bonferroni adjustment. The median hs-CRP value was 1.7 mg/l in the surviving group, 3.1 mg/l in the CVD death group, and 32.1 mg/l in the Non-CVD death group."

We have also added text to the Statistical Analysis section as follows (page 7):

"For the distribution plot of hs-CRP levels in the surviving patients, CVD-death and Non-CVD death groups, we examined hs-CRP values in each group by performing an analysis of variance (ANOVA). Because the distribution of hs-CRP values was skewed, the hs-CRP levels were natural log-transformed for the statistical analyses."

We also added the following text to the Discussion section (page 12):

"In addition, the median hs-CRP value was the highest in the Non-CVD death group, at 32.1 mg/l."

Comment 4
The references are selective and do not cite many of the major papers in this field. See as examples very large data-bases of hsCRP levels measured in acute ischemia in the PROVE-IT (NEJM 2005) and IMPROVE-IT (Circulation 2015) trials.

Response 4
In accord with this suggestion, we have cited the papers.

Comment 5
As noted above, the reference cut-off in Japan is lower than that in the West.

Response 5
In accord with this suggestion, we have now described this issue in the Discussion as follows (page 10):

"Although hs-CRP levels are much lower in Japanese populations compared to Western populations, our present findings confirmed the utility of measuring the initial hs-CRP in ED settings in Japan."

Comment 6
Please remove the term "multivariable adjusted" from table 1 as this is very misleading. According to the legend, this is adjusted for age, sex and WBC only.

Response 6
As suggested, we have replaced the term "multivariable adjusted" with the wording "age-, sex-, and WBC-adjusted" in the Statistical Analysis section, the Results section, and Tables 1 and 2.

Comment 7
Is there another way to display the data of Figure 2? This gives the false impression that total rates

are the same in each group.

Response 7
As suggested, we have revised Figure 2 as Table 3, and to avoid giving a false impression, we have added the following text to the Discussion section (page 12):

"Although the actual number of non-CVD deaths was not very large, non-CVD deaths accounted for 37.5% of the total deaths among the patients with hs-CRP levels ≥33.3 mg/l."

Comment 8
There is abundant data about hsCRP predicting all-cause mortality in different settings that should be addressed here. See for example Clin Chem 2008;54:234-7

Response 8
In accord with this suggestion, we have described this issue in the Discussion as follows (page 10):

"In addition, several studies have examined the utility of hs-CRP for predicting all-cause mortality in different settings."

Comment 9
How might these data be relevant for ongoing clinical trials of inflammation inhibition as a method to reduce cardiovascular event rates?

Response 9
In accord with this suggestion, we have described this issue in the Discussion as follows (page 11):

"An evaluation of inflammatory risk in CVD patients should thus be routinely performed to identify high-risk patients in need of additional close monitoring. Although the results of the present study suggested that hs-CRP was a strong predictor of cardiovascular mortality, and several studies have indicated the value of determining the CRP level in CVD patients, CRP itself is be unlikely to provide an effective target for intervention and is known to be a downstream surrogate inflammatory marker. Moving upstream in the inflammatory cascade from CRP to IL-6 and IL-1 might provide novel therapeutic opportunities to reduce the cardiovascular event rate. The results of ongoing clinical trials of inflammation inhibition (such as those of the phase II trial data on canakinumab, a human monoclonal antibody that targets IL-1β) are worthy of attention."

Manuscript: "High-sensitivity C-Reactive Protein as a Predictor of In-hospital Mortality in Cardiovascular Disease Patients at an Emergency Department," by Yoshinaga et al.

Responses to Reviewer 2: Professor Dietmar MJ Krause

Comment 1
Please state any competing interests or state ‘None declared': None declared

Response 1.
As suggested, we have added a Competing Interests section, on page 14.

Comment 2
As the authors state in the introduction and discussion, there are many studies with focus on the

prognostic value of CRP in CVD. Thus, the description of the background section of the abstract should be amended.

Response 2
As you suggested, we have amended the background (Objective) section of the Abstract as follows (page 2):

"We investigated whether serum high-sensitivity C-reactive protein (hs-CRP) levels measured in an emergency department (ED) are associated with in-hospital mortality in patients with cardiovascular disease (CVD)."

Comment 3
In the Methods and Results part of the abstract, the AR scale should be clarified

Response 3
As per your suggestion, we have added the absolute risk scale in the Abstract as follows (page 2):

"The absolute risk (AR) of in-hospital mortality increased significantly with the hs-CRP levels (<3.0 mg/l: AR 7.0, 95%CI 6.4–7.6; 3.1–5.4 mg/l: AR 9.6, 95%CI 7.9–11.3; 5.5–11.5 mg/l: AR 11.2, 95%CI 9.4–13.0; 11.6–33.2 mg/l: AR 12.3, 95%CI 10.5–14.1; and ≥33.3 mg/l: AR 19.9, 95%CI 17.6–22.2)."

Comment 4
In the last point of the Strength and Limitation section, is should be mentioned that there is no absence of confounders (I think there are many....), but that they are not taken into account.

Response 4
In accord with this suggestion, we have described this issue in the Strengths and Limitation section as follows (page 3):

" The limitations of our study are (1) the single-center nature of the study (i.e., one teaching hospital) and (2) the confounders such as haemodynamics, comorbidities and other laboratory data could not be investigated."

Comment 4
In the second section of the introduction, the last sentence is not clear to me.

Response 4
We have rewritten the last sentence in the second section as follows (page 4):

"Several studies have also observed that elevated CRP predicts the prognosis of CVD patients at the acute stage."

Comment 5
In the statistical analysis part, multivariable instead of multivariate should be used as was done in the tables. The meaning of multivariable is defined in the tables but not in the text. It should be mentioned that multivariable just means the WBC is added in the adjustment.

Response 5
Thank you for your suggestion. As you and another reviewer suggested, we have replaced the term "multivariable adjusted" with the wording "age-, sex-, and WBC-adjusted" in the Statistical Analysis section, the Results section, and Tables 1 and 2.

Comment 6
In the discussion, it should become clear whether a non-CVD cause of death was regarded as misclassification or a complication of a well classified CVD.

Response 6
In this study, causes of death were investigated by referring to the death certificates, as has been done in many epidemiological studies. Therefore, as you pointed out, non-CVD death causes might include a misclassification and/or a complication of a well-classified CVD. We have described this issue in the Discussion section as follows (page 12):

"However, a non-CVD death cause might be regarded as a misclassification or a complication of a well-classified CVD."

Comment 7
One result is that the addition of WBC in the adjustment does not substantially change the HRs; this should be discussed.

Response 7
As you suggested, we have described this issue in the Discussion section as follows (page 11):

"In addition, the addition of the WBC in the adjustment did not substantially change the HRs in this study. This might be caused by the presence of leukopenia, i.e., a low WBC count. Although both the WBC count and CRP are used as inflammatory biomarkers, patients with leukopenia as the result of a severe inflammatory response or immune suppression might have poorer prognoses."

VERSION 2 – REVIEW

REVIEWER	Dietmar MJ Krause Department of Medical Informatics, Biometry and Epidemiology, University of Bochum, D-44780 Bochum, Germany
REVIEW RETURNED	12-Aug-2017

GENERAL COMMENTS	Only three minor comments: The scale of AR (I guess "%") is not defined in the abstract. "WBC" is explained in the tables but not in the abstract or in the main text. In the second line of the results-section "per cent" may be replaced by "%"

VERSION 2 – AUTHOR RESPONSE

Responses to Reviewer 2: Professor Dietmar MJ Krause

Comment 1
The scale of AR (I guess "%") is not defined in the abstract.
Response 1

As suggested, we have revised the the result section of the Abstract as follows (page 2):

“The in-hospital mortality increased significantly with the hs-CRP levels (<3.0 mg/l: 7.0%, 95%CI=6.4–7.6; 3.1–5.4 mg/l: 9.6%, 95%CI=7.9–11.3: 5.5–11.5 mg/l: 11.2%, 95%CI=9.4–13.0; 11.6–33.2 mg/l: 12.3%, 95%CI=10.5–14.1; and ≥33.3 mg/l: 19.9, 95%CI=17.6–22.2).”

Comment 2
"WBC" is explained in the tables but not in the abstract or in the main text.
Response 2
As you suggested, we have amended the result section of the Abstract as follows (page 2): But in the main text, WBC was explained in the Study design, setting and population of the Patient and Method section (page5, line19).

" This association remained unchanged even after adjustment for age, sex and white blood cell count (WBC) and withstood Bonferroni adjustment for multiple testing."

Comment 3
In the second line of the results-section "per cent" may be replaced by "%"
Response 3
As suggested, we have replaced the term "Fifty-two per cent" with the wording "52%" in the second line of the result-section,