## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Prognostic impact of alkaline phosphatase for in-hospital mortality in patients with acute coronary syndrome: a prospective cohort study in China |
| **AUTHORS** | Yu, Tongtong; Jiao, Yundi; Song, Jia; He, Dongxu; Wu, Jiake; Wen, Zongyu; Sun, Na; Duan, Weili; Sun, Zhijun; Sun, Zhaoqing |

## VERSION 1 - REVIEW

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| **REVIEWER** | Jean FERRIERES<br>Toulouse University Hospital<br>Toulouse,<br>France |
| **REVIEW RETURNED** | 28-Aug-2018 |

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| **GENERAL COMMENTS** | The biochemical effect of ALP on vascular calcification, including PPi hydrolysis, increasing passive hydroxyapatite crystal deposition is known. Clinical association between ALP levels and cardiovascular events or mortality in secondary prevention population has been described. Rationally, authors want to describe an association between ALP and mortality in unstable atherosclerosis. It is an interesting work, extending pathophysiological observations to clinical and prognosis observations. However, a few comments should be addressed:<br><br>Major Points:<br>- Authors chose the GRACE score to predict in hospital mortality in ACS, including STEMI and NSTEMI patients. The GRACE score was initially used in NSTEMI patients and secondarily extended to STEMI patients. However, the cut off value for mortality risk prediction using the GRACE score is different for STEMI or NSTEMI patients. The authors must justify why they merged the two samples.<br><br>- The number of STEMI patients in the tertile 3 of ALP seems to be higher than the tertile 1 (774 vs 710 patients). Could the author show the p value for STEMI patients? Each tertile should have the same number of patients and a significant difference could induce a significant bias on mortality. A separated statistical analysis and adjustments for STEMI and NSTEMI patients should be done.<br><br>Minor points:<br><br>Introduction |

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| | - “ALP can catalyse it and promote vascular calcification”: could the authors minimize this affirmation?<br><br>- “new risk factor (…) such ALP”: ALP is not a risk factor of ACS, just a marker<br><br>Methods<br>- Could the authors indicate the mean duration of hospitalization?<br>- Could the authors specify the type of ALP (tissue non-specific?)?<br>- Could the authors indicate the cut off value of clearance to define CKD, and the modality of measurement for serum creatinine?<br><br>Results<br>- Could the authors show the OR and p value for GRACE score in the multivariate analysis?<br><br>Discussion<br>- Could the author discuss a clinical mechanism for the association between ALP and in hospital mortality? In fact, despite adjustments on statistical analysis, cardiac insufficiency, liver injury or kidney failure during hospitalization could increase ALP levels. Thus, authors should moderate the effect of ALP levels on coronary calcification, which is a chronic effect, different from the acute phase of coronary disease.<br><br>- Could the author explain the non-significant difference of GRACE scores in tertiles of APL, despite a significant difference of in hospital mortality? |

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| **REVIEWER** | Alparslan Kurtul<br>Ankara Education and Research Hospital |
| **REVIEW RETURNED** | 04-Sep-2018 |

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| **GENERAL COMMENTS** | This study explored the association between ALP and in-hospital mortality in 6368 ACS patients undergoing PCI. Investigators concluded that in patients with ACS undergoing PCI, ALP was an independent predictor of in-hospital mortality. But, ALP could not improve the prognostic performance of GRACE score. Overall, this study is well conducted but I have major concerns about the article. When I look at the literature, in a previously published study (Ndrepepa G, Holdenrieder S, Xhepa E, Cassese S, Fusaro M, Laugwitz KL, Schunkert H, Kastrati A. Prognostic value of alkaline phosphatase in patients with acute coronary syndromes. Clin Biochem. 2017 Oct;50(15):828-834. doi: 10.1016/j.clinbiochem.2017.05.020. Epub 2017 Jun 1. PubMed PMID: 28579339.), I realized that elevated ALP is an independent predictor for prognosis in patients with ACS. Thus, this article does not present new contributions to the literature. There are also several grammar and vocabulary mistakes, which warrant revision. |

| REVIEWER | Shasha Bai<br>Research Assistant Professor<br>The Ohio State University<br>U.S.A. |
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| REVIEW RETURNED | 17-Oct-2018 |

| GENERAL COMMENTS | The design of the study is straightforward. The primary aim was well defined. Overall the writing of the manuscript is easy to follow. I have some specific comments outlined below:<br><br>1. In the Statistical Analysis section, please specify what "variance analysis" you are referring to.<br><br>2. Table 1 shows comparison of three groups. Does the p-value show overall comparison among all three groups? Why didn't you perform pairwise comparison between neighboring groups with proper multiple comparison adjustment?<br><br>3. How are the final set of predictor variable determined? Through a step-wise or best subset approach? Please add this information.<br><br>4. Please indicate to the readers how to interpret the C-statistic.<br><br>5. The C-statistic for ALP predicting in-hospital mortality is not high (95% CI higher end <0.65), and a C statistic of 0.5 means that the model is no better than predicting an outcome than random chance. Furthermore, the specificity of the optimal cutoff is lower than 0.5. This result suggests to me that ALP is not a great individual predictor of in-hospital mortality, even though the p-value is <0.05. Because of the large sample size, it is likely to find significant association result, but the predictive performance of ALP is not satisfactory. I would reconider the conclusion that "ALP is an independent predictor of in-hospital mortality".<br><br>6. Please add AUC in addition to specificity and sensitivity. |
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## VERSION 1 – AUTHOR RESPONSE

2. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Major Points 1: Authors chose the GRACE score to predict in hospital mortality in ACS, including STEMI and NSTEMI patients. The GRACE score was initially used in NSTEMI patients and secondarily extended to STEMI patients. However, the cut off value for mortality risk prediction using the GRACE score is different for STEMI or NSTEMI patients. The authors must justify why they merged the two samples.)

Response: Thank you for your comment. As you say, the GRACE score could be used in NSTEMI patients as well as STEMI patients, which have also been confirmed by a large number of clinical studies. [1-7] Meanwhile, the Global Registry of Acute Coronary Events (GRACE) (from 1999 to 2009) was an international registry designed to track in-hospital and long-term outcomes of patients presenting with acute coronary syndrome (http://www.outcomes-umassmed.org/grace/). In 2001, the rationale and design of the GRACE project was firstly published on AHJ. [8] The patients hospitalized with acute coronary syndromes (ACS), including the spectrum of conditions from unstable angina to ST segment elevation myocardial infarction, were included in the GRACE project in 14 countries. [8] In 2002, the baseline characteristics, management practices, and in-hospital outcomes of patients hospitalized with acute coronary syndromes in the Global Registry of Acute Coronary Events

(GRACE) was firstly published on AJC. [9] Of 11,543 patients enrolled in the GRACE project, 30% had ST-segment elevation myocardial infarction (STEMI), 25% had non-ST-segment elevation myocardial infarction (NSTEMI), 38% had unstable angina pectoris, and 7% had other cardiac or noncardiac diagnoses. [9] In 2003, the single GRACE score was developed to assess the risk for in-hospital mortality for the entire spectrum of ACS, including unstable angina, ST segment elevation myocardial infarction and non-ST segment elevation myocardial infarction. [10] From then on, a large number of clinical studies also confirmed that the single GRACE score could be used in ACS patients, including unstable angina, ST segment elevation myocardial infarction and non-ST segment elevation myocardial infarction. [11-14] So, our study merged STEMI and NSTEMI.

Reference：

[1] Isilak Z, et al. Comparison of clinical risk assessment systems in predicting three-vessel coronary artery disease and angiographic culprit lesion in patients with non-ST segment elevated myocardial infarction/unstable angina pectoris. Kardiol Pol. 2012;70(3):242-50.

[2] Maciejewski P, et al. Assessment of the prognostic value of coronary angiography in patients with non-ST segment elevation myocardial infarction. Kardiol Pol. 2013;71(2):136-42.

[3] Fu R, et al. CAMI-NSTEMI Score - China Acute Myocardial Infarction Registry-Derived Novel Tool to Predict In-Hospital Death in Non-ST Segment Elevation Myocardial Infarction Patients. Circ J. 2018 Jun 25;82(7):1884-1891.

[4] Kim HK, et al. A new risk score system for the assessment of clinical outcomes in patients with non-ST-segment elevation myocardial infarction. Int J Cardiol. 2010 Dec 3;145(3):450-4.

[5] Koonsiripaiboon E, et al. Validation of the GRACE risk score to predict in-hospital mortality in patients with ST segment elevation myocardial infarction in Thailand. J Med Assoc Thai. 2013 Feb;96 Suppl 2:S139-45.

[6] Yang JH, et al. Prognostic value of admission blood glucose level in patients with and without diabetes mellitus who sustain ST segment elevation myocardial infarction complicated by cardiogenic shock. Crit Care. 2013 Oct 3;17(5):R218.

[7] Barbarash OL, et al. The prognostic value of peripheral artery diseases in patients with ST-segment elevation myocardial infarction. Dis Markers. 2013;35(6):877-82.

[8] The GRACE Investigators. Rationale and design of the GRACE (Global Registry of Acute Coronary Events) Project: a multinational registry of patients hospitalized with acute coronary syndromes. Am Heart J 141(2), 190-199 (2001).

[9] Steg PG, et al. Baseline characteristics, management practices, and in-hospital outcomes of patients hospitalized with acute coronary syndromes in the Global Registry of Acute Coronary Events (GRACE). Am J Cardiol. 2002 Aug 15;90(4):358-63.

[10] Granger CB, et al. Predictors of hospital mortality in the global registry of acute coronary events. Archives of internal medicine 163(19), 2345-2353 (2003).

[11] Tang EW, et al. Global Registry of Acute Coronary Events (GRACE) hospital discharge risk score accurately predicts long-term mortality post acute coronary syndrome. Am Heart J. 2007 Jan;153(1):29-35.

[12] Elbarouni B, et al. Validation of the Global Registry of Acute Coronary Event (GRACE) risk score for in-hospital mortality in patients with acute coronary syndrome in Canada. Am Heart J. 2009 Sep;158(3):392-9.

[13] Lin A, et al. Performance of the GRACE scores in a New Zealand acute coronary syndrome cohort. Heart. 2014 Dec;100(24):1960-6.

[14] Gong IY, et al. GRACE risk score: Sex-based validity of in-hospital mortality prediction in Canadian patients with acute coronary syndrome. Int J Cardiol. 2017 Oct 1;244:24-29.

3. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Major Points 2- The number of STEMI patients in the tertile 3 of ALP seems to be higher than the tertile 1 (774 vs 710 patients). Could the author show the p value for STEMI patients? Each tertile should have the same number of patients and a significant difference could induce a significant bias on mortality. A separated statistical analysis and adjustments for STEMI and NSTEMI patients should be done.)

Response: Thank you for your comment. Indeed, in our study, the number of STEMI patients in the tertile 3 of ALP is significantly higher than the tertile 1 and 2 (774 vs 710 and 723 patients, p=0.001). However, the all individuals were divided into three groups according to the tertile of ALP level on admission. So, each tertile has the same number of patients (tertile 1: 2123; tertile 2: 2123; tertile 3: 2122), but has the different number of STEMI patients (774 vs 710 and 723 patients, p=0.001). In order to correct the bias on mortality, we then adjusted for diagnosis on admission in the multivariate logistic regression.

We also think that a separated statistical analysis and adjustments for STEMI and NSTEMI patients is a wonderful idea. To achieve this goal, we need to carry out the subgroup analysis of STEMI and NSTEMI patients. However, subgroup analyses might be inappropriate in this study from the opinion of the statistical expert, Prof. Liqiang Zheng, the biostatistician from the Department of Clinical Epidemiology, Library, Shengjing Hospital of China Medical University (In the section of “Acknowledgments”, we also thank for his work!). Firstly, subgroup analyses should be determined before the start of the study, not when the results were analyzed [1-3]. In fact, this study did not set subgroup analyses at the beginning. Secondly, usually, subgroup analyses are used in randomised controlled trials. Subgroup analyses in the observational cohort study might lead to overstated and misleading results [1-3]. However, we will carry on a new study with the bigger sample size and subgroup analyses in the future and make the results more reliable. Thank you again!

Reference：

[1] Pocock SJ, Assmann SE, Enos LE, Kasten LE. Subgroup analysis, covariate adjustment and baseline comparisons in clinical trial reporting: current practice and problems.Stat Med. 2002 Oct 15;21(19):2917-30.

[2] Wang R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Statistics in medicine--reporting of subgroup analyses in clinical trials. N Engl J Med. 2007 Nov 22;357(21):2189-94.

[3] Sun X, Ioannidis JP, Agoritsas T, Alba AC, Guyatt G. How to use a subgroup analysis: users' guide to the medical literature. JAMA. 2014 Jan 22-29;311(4):405-11.

4. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Introduction - “ALP can catalyse it and promote vascular calcification”: could the authors minimize this affirmation? - “new risk factor (…) such ALP”: ALP is not a risk factor of ACS, just a marker.)

Response: Thank you for your comment. According to your suggestion, we have corrected them.

5. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Methods - Could the authors indicate the mean duration of hospitalization?)

Response: Thank you for your comment. According to your suggestion, we added a new variable “duration of hospitalization” in Table 1. And, we indicated the mean duration of hospitalization in the

Methods section. The mean duration of hospitalization for the whole cohort, tertile 1, tertile 2 and tertile 3 was 7.5±4.4 days, 7.4±4.1 days, 7.6±4.6 days and 7.5±4.5 days (p=0.871) separately. Thank you again!

6. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Methods - Could the authors specify the type of ALP (tissue non-specific?)?)

Response: Thank you for your comment. According to your suggestion, we specified the type of ALP (tissue-non-specific) in the Methods section.

7. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Methods - Could the authors indicate the cut off value of clearance to define CKD, and the modality of measurement for serum creatinine?)

Response: Thank you for pointing this out. In our study, the patients with chronic kidney failure with dialysis were excluded. We used the variable “serum creatinine” instead of “CKD” (defined as: (140-age)*body weight (kg) / (72*Scr)(mg/dL) for male; (140-age)*body weight (kg)*0.85 /(72*Scr)(mg/dL) for female), since the body weight data of the included patients were seriously incomplete. According to your suggestion, we indicated the modality of measurement for serum creatinine in the Methods section.

8. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Results - Could the authors show the OR and p value for GRACE score in the multivariate analysis?)

Response: Thank you for pointing this out. As we known, the GRACE score was based on 8 variables: age, Killip class, systolic blood pressure, ST-segment deviation, cardiac arrest during presentation, serum creatinine level, positive initial cardiac enzyme findings, and heart rate.[1] However, most of them were also included in the variables entering the multivariate analysis. A serious multicollinearity problem will come, if GRACE score enters the multivariate analysis. [2] OR and p value will be also unbelievable. Multicollinearity arises when at least two highly correlated predictors are assessed simultaneously in a regression model. The adverse impact of multicollinearity in regression analysis is very well recognized and much attention to its effect is documented in the literature [2–6]. So, we did not show the OR and p value for GRACE score in the multivariate analysis.

Reference：

[1] Granger CB, et al. Predictors of hospital mortality in the global registry of acute coronary events. Archives of internal medicine 163(19), 2345-2353 (2003).

[2] Dormann CF, Elith J, Bacher S. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Echography. 2013; 36:27–46.

[3] Farrar DE, Glauber RR. Multicollinearity in regression analysis: the problem revisited. Review of Economics and Statistics. 1967; 49:92–107.

[4] Aiken, LS.; West, SG. Multiple regression: Testing and interpreting interactions. Newbury Park C: SAGE Publications, Inc; 1991. Editor

[5] Gordon RA. Issues in Multiple Regression. The American Journal of Sociology. 1968; 73:592–616.

[6] Mason Charlotte H, Perreault William D Jr. Collinearity, Power, and Interpretation of Multiple Regression Analysis. Journal of Marketing Research. 1991; 28:268–280.

9. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Discussion - Could the author discuss a clinical mechanism for the association between ALP and in hospital mortality? In fact, despite adjustments on statistical analysis, cardiac insufficiency, liver injury

or kidney failure during hospitalization could increase ALP levels. Thus, authors should moderate the effect of ALP levels on coronary calcification, which is a chronic effect, different from the acute phase of coronary disease.)

Response: Thanks for your wonderful comment! According to your suggestion, we have discussed the possible clinical mechanism for the association between ALP and in-hospital mortality in the third paragraph of the Discussion section. We also think the effect of ALP levels on coronary calcification may be the very important. The previous study confirmed the association between serum alkaline phosphatase and coronary artery calcification in CAD patients. [1] Our study also found that there was the significantly higher percentage of moderate or severe calcification in the highest ALP group (7.0% vs 5.2% and 6.6%, p=0.034 for Tertile 3 group vs Tertile 1 and Tertile 2 group) Coronary calcification contributes to atherosclerosis, and is also a predictor of adverse cardiovascular events. [2-4] Although ACS is usually associated with non-calcified fibroatheroma [5], spot calcification still predicted atherosclerotic plaque rupture [6]. Although coronary calcification is a chronic effect, in the acute phase of coronary disease, coronary calcification may hamper the process of re-endothelialization, and be a marker of extensive atherosclerotic disease. [7, 8] Furthermore, severe coronary calcification was an independent predictor of worse prognosis in patients undergoing percutaneous coronary intervention for obstructive coronary artery disease. [7]

Reference：

[1] Panh L, Ruidavets JB, Rousseau H, et al. Association between serum alkaline phosphatase and coronary artery calcification in a sample of primary cardiovascular prevention patients. Atherosclerosis 2017;260:81-86. doi: 10.1016/j.atherosclerosis.2017.03.030 [published Online First: 2017/04/04]

[2] Budoff MJ, Shaw LJ, Liu ST, et al. Long-term prognosis associated with coronary calcification: observations from a registry of 25,253 patients. J Am Coll Cardiol 2007;49(18):1860-70. doi: 10.1016/j.jacc.2006.10.079 [published Online First: 2007/05/08]

[3] Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. The New England journal of medicine 2008;358(13):1336-45. doi: 10.1056/NEJMoa072100 [published Online First: 2008/03/28]

[4] Van Campenhout A, Golledge J. Osteoprotegerin, vascular calcification and atherosclerosis. Atherosclerosis 2009;204(2):321-9. doi: 10.1016/j.atherosclerosis.2008.09.033 [published Online First: 2008/11/15]

[5] Zheng B, Mintz GS, McPherson JA, et al. Predictors of Plaque Rupture Within Nonculprit Fibroatheromas in Patients With Acute Coronary Syndromes: The PROSPECT Study. JACC Cardiovascular imaging 2015;8(10):1180-7. doi: 10.1016/j.jcmg.2015.06.014 [published Online First: 2015/10/21]

[6] Hutcheson JD, Maldonado N, Aikawa E. Small entities with large impact: microcalcifications and atherosclerotic plaque vulnerability. Current opinion in lipidology 2014;25(5):327-32. doi: 10.1097/mol.0000000000000105 [published Online First: 2014/09/05]

[7] Bourantas CV, Zhang YJ, Garg S, et al. Prognostic implications of coronary calcification in patients with obstructive coronary artery disease treated by percutaneous coronary intervention: a patient-level pooled analysis of 7 contemporary stent trials. Heart 2014;100(15):1158-64. doi: 10.1136/heartjnl-2013-305180 [published Online First: 2014/05/23]

[8] Yiu KH, Wang S, Mok MY, et al. Role of circulating endothelial progenitor cells in patients with rheumatoid arthritis with coronary calcification. The Journal of rheumatology 2010;37(3):529-35. doi: 10.3899/jrheum.090782 [published Online First: 2010/01/19]

10. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Minor points: Discussion - Could the author explain the non-significant difference of GRACE scores in tertiles of APL, despite a significant difference of in hospital mortality?)

Response: Thank you for your comment. We also think it is an interesting phenomenon. There may be several explanations. Firstly, although there is the non-significant difference of GRACE scores in tertiles of ALP, tertile 3 group (highest ALP group) still has a tendency towards increasing GRACE scores, compared with tertile 1 and 2 (129.7±36.5 vs 128.7±37.4 and 129.0±35.9). If we further increase the sample size, significant difference maybe show. Secondly, the GRACE score, containing the main traditional factors for cardiovascular disease, was derived in the early 21st century. Since then, increasing amounts of novel factors have been studied; nevertheless, the GRACE score does not contain any of these new factors. It is the limitation of the GRACE score. Lastly, age is the most important weight of the GRACE score. However, there is the non-significant difference of age in tertiles of ALP (62.0±11.3 vs 62.6±11.3 and 62.0±11.4, p=0.229). This may also make the non-significant difference of GRACE scores in tertiles of APL.

11. Response to comment (Reviewer: 2; Reviewer Name: Alparslan Kurtul): (I realized that elevated ALP is an independent predictor for prognosis in patients with ACS. Thus, this article does not present new contributions to the literature.)

Response: Thank you for your comment. As you say, many studies have confirmed the association between higher ALP and the long-term adverse outcome in patients with CAD, [1-3] ACS [4, 5] and ST-segment elevation myocardial infarction (STEMI).[6, 7] However, the role of ALP level in predicting in-hospital mortality in patients with ACS is not as clearly defined. In this study, we aimed to assess whether ALP was a useful clinical parameter to predict in-hospital mortality in patients with ACS undergoing PCI. Also, we hope this article may give the readers the new sight.

Reference：

1. Tonelli M, Curhan G, Pfeffer M et al. Relation between alkaline phosphatase, serum phosphate, and all-cause or cardiovascular mortality. Circulation 120(18), 1784-1792 (2009).

2. Ndrepepa G, Xhepa E, Braun S et al. Alkaline phosphatase and prognosis in patients with coronary artery disease. European journal of clinical investigation 47(5), 378-387 (2017).

3. Nunes JP, Melao F, Godinho AR, Rodrigues JD, Maciel MJ. Plasma alkaline phosphatase and survival in diabetic patients with acute myocardial infarction. Annals of translational medicine 4(11), 210 (2016).

4. Park JB, Kang DY, Yang HM et al. Serum alkaline phosphatase is a predictor of mortality, myocardial infarction, or stent thrombosis after implantation of coronary drug-eluting stent. Eur Heart J 34(12), 920-931 (2013).

5. Ndrepepa G, Holdenrieder S, Xhepa E et al. Prognostic value of alkaline phosphatase in patients with acute coronary syndromes. Clinical biochemistry 50(15), 828-834 (2017).

6. Oh PC, Lee K, Kim TH et al. Prognostic impact of alkaline phosphatase measured at time of presentation in patients undergoing primary percutaneous coronary intervention for ST-segment elevation myocardial infarction. PLoS One 12(2), e0171914 (2017).

7. Huseynov A, Baumann S, Becher T et al. Liver and cholestatic parameters as prognostic biomarkers of in-hospital MACE in patients with STEMI. European journal of clinical investigation 46(8), 721-729 (2016).

12. Response to comment (Reviewer: 2; Reviewer Name: Alparslan Kurtul): (There are also several grammar and vocabulary mistakes, which warrant revision.)

Response: Thank you for your comment. According to your suggestion, we have invited an English native speaker to make a thorough revision of the written English throughout the manuscript.

13. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (1. In the Statistical Analysis section, please specify what "variance analysis" you are referring to.)

Response: Thank you for your comment. According to your suggestion, we have specified it, and "variance analysis" is Kruskal-Wallis H test in the Statistical Analysis section.

14. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (2. Table 1 shows comparison of three groups. Does the p-value show overall comparison among all three groups? Why didn't you perform pairwise comparison between neighboring groups with proper multiple comparison adjustment?)

Response: Thank you for your comment. Yes, the p-value showed overall comparison among all three groups. This study aimed to assess whether ALP was a useful clinical parameter to predict in-hospital mortality in patients with ACS undergoing PCI. A multivariate logistic regression model was used to identify it. Only variables with $p<0.05$ on Logistic univariate analysis (shown at Appendix Table S1), which was performed to evaluate predictors of mortality of all variables, were entered the final multivariate analysis. So, Table 1 has no effect on the conclusion. It just showed the baseline characteristics of the population. We think the p-value showing overall comparison among all three groups may be enough for the readers to understand the tendency among all three groups. Pairwise comparison between neighboring groups with proper multiple comparison adjustment is a perfect idea! We completely agree. However, Table 1 is very big, and pairwise comparison between neighboring groups may make it bigger and more complex to influence the readers' understanding.

15. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (3. How are the final set of predictor variable determined? Through a step-wise or best subset approach? Please add this information.)

Response: Thank you for your comment. According to your suggestion, we have added the information in method section. Logistic univariate regressions were performed to evaluate predictors of mortality of all variables using “Enter”. A multivariate logistic regression model was used to identify independent predictors of mortality using “Forward: conditional”.

16. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (4. Please indicate to the readers how to interpret the C-statistic.)

Response: Thank you for your comment. According to your suggestion, we have added the information in method section.

17. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (5. The C-statistic for ALP predicting in-hospital mortality is not high (95% CI higher end <0.65), and a C statistic of 0.5 means that the model is no better than predicting an outcome than random chance. Furthermore, the specificity of the optimal cutoff is lower than 0.5. This result suggests to me that ALP is not a great individual predictor of in-hospital mortality, even though the p-value is <0.05. Because of the large sample size, it is likely to find significant association result, but the predictive performance of ALP is not satisfactory. I would reconider the conclusion that "ALP is an independent predictor of in-hospital mortality".)

Response: Thank you! Your comment is wonderful! We also agree with your saying: the predictive performance of ALP is not satisfactory! In fact, in addition to the C-statistic, the performance of ALP is also not satisfactory in the multivariate logistic regression model. When ALP was evaluated as a continuous variable, the p value was 0.014 and higher than 0.01. When ALP was evaluated according to three categories, the p value was even as high as 0.032, and much higher than 0.01. So, when we merged ALP and the GRACE score, we failed: ALP could not improve the prognostic performance of GRACE score.

However, the statistical significance was defined as $p < 0.05$. The p values in the C-statistic and the multivariate logistic regression model all met this standard. Based on this, we can conclude that "ALP is an independent predictor of in-hospital mortality". Indeed, ALP is not a good marker to predict in-hospital mortality in patients with ACS undergoing PCI, but may be still a useful one.

18. Response to comment (Reviewer: 3; Reviewer Name: Shasha Bai): (6. Please add AUC in addition to specificity and sensitivity.)

Response: Thank you for your comment. The C-statistic is defined by the area under the receiver operating characteristic (ROC-AUC). Table 3 has showed the C-statistic (AUC), standard error, p value and 95% CI of the GRACE, GRACE+ ALP and ALP performance for the prognosis prediction.

## VERSION 2 – REVIEW

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| **REVIEWER** | Jean FERRIERES<br>Toulouse University hospital,Department of Epidemiology, Unite de Soutien Methodologique ——————————a la Recherche (USMR), Toulouse University Hospital (CHU), 37 Allees Jules Guesde, 31073<br>Toulouse Cedex 7, France |
| **REVIEW RETURNED** | 15-Dec-2018 |

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| **GENERAL COMMENTS** | To the editor:<br>The author's response to our second major point is not appropriate . Despite their explanations, in this observational study, a separated analysis on STEMI and NSTEMI analysis should be done.<br>It is a major issue because this adjustment could change the final results of the study.<br>In the present form, the study is not acceptable for publication. |

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| **REVIEWER** | Shasha Bai<br>Department of Biomedical Informatics<br>The Ohio State University Wexner Medical Center<br>USA |
| **REVIEW RETURNED** | 05-Dec-2018 |

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| **GENERAL COMMENTS** | The revised manuscript has addressed all my editorial comments. |

## VERSION 2 – AUTHOR RESPONSE

Responds to the reviewer's comments:

1. Response to comment (Reviewer: 1; Reviewer Name: Jean FERRIERES): (Major Points: To the editor: The author's response to our second major point is not appropriate. Despite their explanations, in this observational study, a separated analysis on STEMI and NSTEMI analysis should be done. It is a major issue because this adjustment could change the final results of the study. In the present form, the study is not acceptable for publication.)

Response: Thank you for your comment. According your comment, a separated analysis on STEMI and NSTEMI-ACS has been done. Logistic univariate regressions, multivariate logistic regression, C-statistic, the Hosmer-Lemeshow test, the Nagelkerke-R2, Brier scores, IDI and NRI were all made for STEMI and NSTEMI-ACS, separately. The results were present in the Supplementary Files, from Appendix S2 to Appendix S9. The conclusions were also consistent to the main conclusion. In STEMI group (Appendix S2, S4, S6, S7) or NSTEMI-ACS group (Appendix S3, S5, S8, S9), (1) ALP was an independent predictor of in-hospital mortality; (2) the prognostic performance of GRACE score+ ALP was similar to GRACE score, and ALP could not improve the prognostic performance of the original GRACE score model.

## VERSION 3 - REVIEW

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| **REVIEWER** | Jean Ferrieres<br>Department of Cardiology, Toulouse-Rangueil University Hospital (CHU), TSA 50032,1 Avenue du Professeur Jean Poulhes, 31059 Toulouse Cedex 9<br>France |
| **REVIEW RETURNED** | 07-Apr-2019 |

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| **GENERAL COMMENTS** | Thank you for the new statistical analysis. |