PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Predicting value of white blood cell and total bilirubin on clinical outcomes in patients with ST-elevation myocardial infarction following percutaneous coronary intervention: a cohort study
AUTHORS	Munire, Tuxun; Zhao, Qian; Yang, Xiang; Liu, fen; Shan, Chun Fang; Zhou, Xin Rong; Song, Ning; Ajiguli, Waisiding; Zhang, XueHe; Gulandanmu, Aihemaiti; Yang, Yi-Ning; Li, Xiao-Mei

VERSION 1 – REVIEW

REVIEWER	Alexander E Berezin Senior consultant and Professor of Therapeutic Unit of Internal Medicine Department, State Medical University, Zaporozhye, Ukraine
REVIEW RETURNED	24-Jun-2019

GENERAL COMMENTS	Authors reported good written manuscript with clear aim and interesting results. Authors included STEMI patients rrquired to be treated with PCI and found that the combination of WBC and TB may be an independent predictor for in-hospital outcomes in patients with STEMI than single detection. This is intriguing finding, while I see several items needed to be explained. 1. Study design should be reported as flow chart. Sample size calculation is required to be discussed, so, please, give the calculation with an appropriate formula. Drop-out probability should be calculated also. 2. In-hospital mortality was too high, please, report clear explanation what causes were. 3. Please, report why authors did not use troponin levels and other biomarkers that are recommended generaly to preeict clinical outcomes and to use the levels of them to get comparissons. 4. MANCOVA method is recommended to the purpose that is indicated in the manuscript. Please, report it and put the data in the section Results and Discussion. 5. C-statistic is required to compare AUC for numerous biomarkers

REVIEWER	Prof. Dr. Franz-Josef Neumann University Hospital Freiburg Bad Krozingen, Cardiology
REVIEW RETURNED	01-Jul-2019

GENERAL COMMENTS	Munire and co-workers investigated white blood cell count and total bilirubin for prediction of major adverse cardiac events (MACE) in patients with ST-elevation myocardial infarction (STEMI) undergoing primary PCI. They report that white blood cell count in combination with bilirubin is associated with early complications, but not with long-term outcome.

	Specific Comments: The authors need to acknowledge that there are a number of studies demonstrating an association of white blood cell count with outcome in patients with ischaemic heart disease. Among the many markers of oxidative stress the authors chose bilirubin. The association of bilirubin with oxidative stress is less well established than that of other markers. It remains unclear to me why the authors picked this variable. The authors need to clarify whether white blood cell count and bilirubin were entered into the model as separate variables or whether they were collapsed into one variable according to a specific equation. In the legend of figure 2 the authors state that the two groups were defined by a threshold for the combination of white blood cell count and bilirubin without clarifying this threshold. This needs to be corrected. In addition, figure 2 should show the numbers at risk. I do not understand the odds ratio of 0.0 in table 3. How did the authors select the independent variables for the multivariate analysis? MACE is not well defined (“etc.” is not sufficient and needs to be explained). The authors may consider seeking professional help to improve the language.

REVIEWER	Anggoro Budi Hartopo Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada - Dr. Sardjito Hospital, Yogyakarta, Indonesia
REVIEW RETURNED	02-Sep-2019

GENERAL COMMENTS	The manuscript provide the evidence that combined WBC and TB value increased the prediction of in-hospital MACE, however in long-term MACE the value is not increased the prediction capacity.While this finding is exciting, there are several concerns should be address by authors: 1. The out-of hospital MACE did not clearly defined, while in-hospital MACE were a composite of cardiogenic death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction, etc.What are etc? 2. Authors state the criteria of successful PCI as TIMI flow 3 and stenosis <50%. The data regarding this result should be presented in the table between MACE and non MACE. 3. Authors draw ROC curve with binomial outcome of mortality, while later use the value of cut-off WBC and TB to predict in hospital and out of hospital MACE. Author should be coherent that the design of ROC curve and cut-off value determination use similar outcome (i.e. MACE other than mortality). 4. Please clearly defined "combined" value . 5. The discussion should be added by the explanation of mechanism increased inflammatory and oxidative markers during STEMI affects the myocardial function.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

1. Study design should be reported as flow chart. Sample size calculation is required to be discussed, so, please, give the calculation with an appropriate formula. Drop-out probability should be calculated also.
Reply: Thank you for this suggestion. We've perfected the flow chart of our study design in the RESULTS. (Figure 1. The flowchart of study design with including and excluding procedures.)

The sample content calculation formula in this research is
During June 2012 and June 2017, we consecutively recruited 647 adult STEMI patients who underwent primary PCI. Of these patients 32 were excluded according to the exclusion criteria. A total of 615 STEMI patients (525 males and 90 female) with an average age of 58 years were included in this study (line 3-5, page 10). Based on the sample size of the current study, the power of the research results using Power and sample size calculation is 81.2%..(line 17-20, page 9).

2. In-hospital mortality was too high, please, report clear explanation what causes were.
Reply: In our study there were 17 (2.8%) patients occurred death in-hospital among the 615 STEMI patients, comparable to the China's national level. The in-hospital mortality of STEMI in China between 2001 and 2011 was 7.0%. 2017 ESC reported that the in-hospital mortality rate of STEMI is as high as 4%-12%. So in our study the prevalence of in-hospital mortality was not high.(line20-24, page 15).

[1]Li J, Li X, Wang Q, et al. Lancet 2015; 385(9966): 441-51.

3. Please, report why authors did not use troponin levels and other biomarkers that are recommended generaly to predict clinical outcomes and to use the levels of them to get comparissons.
Reply: Thank you for this suggestion. We acknowledge that troponin is currently the most specific biochemical indicator for the early diagnosis of ACS, which can reflect the number of myocardial cell necrosis. Although the value of troponin in STEMI differential diagnosis cannot be replaced, but the use of troponin concentrations to estimate the clinical prognosis is not necessarily reliable, needs further study confirms. And, more importantly, As we know, troponin test fee will be more expensive and testing requirements for basic-level hospitals is too high, not all hospitals have this condition, while white blood cell and bilirubin, common and fast acquired biomarkers in routine blood tests, can be detected by most hospitals because of low test cost. We are more focused on the clinical prognostic value of white blood cell and bilirubin in patients.(line24-30, page 15).

4. MANCOVA method is recommended to the purpose that is indicated in the manuscript. Please, report it and put the data in the section Results and Discussion.
Reply: Thank you for this suggestion. In our study the differences between MACE group and non-MACE group were evaluated using Student’s unpaired t test and Chi-square test. The stepwise multivariable logistic regression was used to analyze all potential influencing factors associated with in-hospital MACE. We consider that maybe there is no need to use the MANCOVA method.

5. C-statistic is required to compare AUC for numerous biomarkers.
Reply: Thank you for this suggestion. The C-statistic has added in the Result.

Reviewer: 2

1.The authors need to acknowledge that there are a number of studies demonstrating an association of white blood cell count with outcome in patients with ischaemic heart disease.

Reply: Thank you for this suggestion. There is indeed a lot of literature on the relationship between leukocytes and prognosis of ischemic heart disease. In the discussion section of our paper, we have listed relevant literatures for discussion(line 19-26, page 13), but we think STEMI is a disease both with inflammatory and oxidative stress injury. As such we suggest that combining WBC and TB has a stronger predictive power for in-hospital MACE than individual markers, was of particular clinical importance for the subset of patients with STEMI at admission.

[2]Kyne L , Hausdorff J M , Knight E , et al. Am Heart J 2000, 139(1):94-100.
[3]Kojima S, Sakamoto T, Ishihara M, et al.Ann Med 2004, 36(2):153-160.
[4]Nunez, E J . Heart 2005, 91(8):1094-1095.
[5]Çiçek G, Açıkgöz S K, Yayla Ç, et al.Cardiol J 2016, 23(3):225-235.

2. Among the many markers of oxidative stress the authors chose bilirubin. The association of bilirubin with oxidative stress is less well established than that of other markers. It remains unclear to me why the authors picked this variable.
Reply: Thank you for this suggestion. In recent years, more and more attention has been paid to the role of bilirubin in the pathophysiology of major cardiovascular diseases such as myocardial infarction, atherosclerosis and cardiovascular complications of diabetes. As an antioxidant in the body, bilirubin is involved in the occurrence and development of myocardial infarction and plays an anti-oxidative role. Studies have shown when STEMI occurs, acute myocardial ischemia and hypoxia activates the stress process of the body, producing oxygen free radicals and oxides as well as infarction-related inflammatory factors, which significantly increases the activity of Heme oxygenase-1(HO-1)and ultimately leads to increased bilirubin. Some studies have proposed that bilirubin concentration in AMI patients will increase, which has the effect of antioxidant stress and can be used as a biological indicator to predict clinical prognosis, so we picked this variable aim to predict adverse clinical outcomes(line 15-23, page 14). Besides, we have no data about indicators related to oxidative stress and HO-1 enzyme activity, which is limitation of our study.(line 4-6, page 16)

[6] Wei S, Mao L, Liu B, et al. Herz, 2014, 39(3):384-389.
[7] Erkan A, Ekici B, Ugurlu M, et al. Herz, 2014, 39(6):711-715.
[8] Vítek L.Front Pharmacol, 2012, 3:55.
[9]Gul M, Uyarel H, Ergelen M, et al. Am J Cardiol, 2013, 62(18):C20-C21.

3. The authors need to clarify whether white blood cell count and bilirubin were entered into the model as separate variables or whether they were collapsed into one variable according to a specific equation.
Reply: Thank you for this suggestion. In the multivariable logistic regression, the white blood cell count and bilirubin were entered into the model which was collapsed into one variable according to a specific equation.(line 27, page 10)

4. In the legend of figure 2 the authors state that the two groups were defined by a threshold for the combination of white blood cell count and bilirubin without clarifying this threshold. This needs to be corrected. In addition, figure 2 should show the numbers at risk.
Reply: We are sorry for this negligence, it has been corrected in the “RESULTS” (line2-8, page11).The figure2 has been modified.

5. I do not understand the odds ratio of 0.0 in table 3.
Reply: We are sorry for this mistake, it has been corrected. The odds ratio for constant term in table 3 was 0.002.

6. How did the authors select the independent variables for the multivariate analysis?

Reply: Thank you for this suggestion .In our study, We first performed univariate logistics analysis, and in the multivariable logistic regression, we select the traditional risk factors for atherosclerosis, including age, body mass index (BMI),gender, smoking, hypertension, diabetes, low-density lipoprotein cholesterol, we also chose the variables with positive study structure in single factor analysis such as combined equation, left ventricular ejection fraction(LVEF), culprit vessels, also includes some variables which can affect outcomes and has already identified by the researchers based on the literature and clinical work, such as heart rate, creatine kinase isoenzymes(CK-MB).(line 17-22, page 11).

7. MACE is not well defined ("etc." is not sufficient and needs to be explained).
Reply: Thank you for this suggestion. In-hospital endpoints (1) In-hospital mortality; (2) Major adverse cardiovascular events (MACE) during hospitalization including cardiac death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction.
Long-term follow-up endpoints: MACE including cardiac death, angina pectoris readmission, non-fatal myocardial infarction, malignant arrhythmia (ventricular tachycardia and ventricular fibrillation), severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization.(line 18-24, page 8).

8. The authors may consider seeking professional help to improve the language.

Reply: Thank you very much, we have thoroughly checked the revised MS to improve the language and correct grammatical errors.

Reviewer: 3

1. The out-of hospital MACE did not clearly defined, while in-hospital MACE were a composite of cardiogenic death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction, etc.What are etc?

Reply: Thank you for this suggestion.Thank you for this suggestion. In-hospital endpoints (1) In-hospital mortality; (2) Major adverse cardiovascular events (MACE) during hospitalization including cardiac death, cardiogenic shock, malignant arrhythmia (ventricular tachycardia, ventricular fibrillation), severe cardiac insufficiency, non-fatal myocardial infarction.
Long-term follow-up endpoints: MACE including cardiac death, angina pectoris readmission, non-fatal myocardial infarction, malignant arrhythmia (ventricular tachycardia and ventricular fibrillation), severe cardiac insufficiency (cardiac III-IV level), stent restenosis, target vessels reascularization.(line 18-24, page 8).

2. Authors state the criteria of successful PCI as TIMI flow 3 and stenosis <50%. The data regarding this result should be presented in the table between MACE and non MACE.
Reply: Thank you for this suggestion. The data has added in the Table 1. The TIMI flow grade after PCI in non-MACE group was higher than MACE group. It is explained in the discussion section (line15-20, page 15).

3. Authors draw ROC curve with binomial outcome of mortality, while later use the value of cut-off WBC and TB to predict in hospital and out of hospital MACE. Author should be coherent that the design of ROC curve and cut-off value determination use similar outcome (i.e. MACE other than mortality).

Reply: We apologize for the inaccurate description. ROC curve and cut-off value were determined the in-hospital mortality. In order to comprehensively evaluate the prognostic effect of this combined equation, the predictive value for in-hospital MACE were also analyzed.

4. Please clearly defined "combined" value .
Reply: Thank you for this suggestion, in our study , we combined WBC and TB used logistic regression,and get the regression model : Logit (P) =-8.00+0.265 WBC+0.077 the TB, so the "combined" value refers to Logit (P).(line 1-2, page 11).

5. The discussion should be added by the explanation of mechanism increased inflammatory and oxidative markers during STEMI affects the myocardial function.
Reply: Thank you for this suggestion. We have added some discussion about explanation of mechanism increased inflammatory and oxidative markers. (line9-18 page 13) (line 2-14, page 14).

VERSION 2 – REVIEW

REVIEWER	Alexander Berezin Medical University of Zaporozhye, Ukraine
REVIEW RETURNED	12-Oct-2019

GENERAL COMMENTS	Authors reported good-written and well-structured paper with clear aim and interesting results. It was single-center, prospective cohort study, in which a total of 615 STEMI patients after PCI were enrolled. MACEs were determined and analyzed in accordance to circulating biomarkers, sch as bilirubin and WBC, for 36 month period. Authors found that the combination of WBC and TB may be an independent predictor for in-hospital 20 outcomes in patients with STEMI than single detection. However, there are several items need to be explained. 1. Ethical declaration should be extended. Please add IRB name and approval date. Therefore, procedure of getting an agreement should be described carefully. 2. Several data, such as GRACE, TIMI, should be reported as median and upper/low limits or interquartile range, but not as mean =/- SEM, otherwise a clinical sense of the the parameters is missed. Please, check and correct. 3. Because it was prospective study, authors should not just report sample size calculation formula to assay whether findings for end point are able to be taken into consideration for further analysis, but they should give an example of the calculation with drop-out and screen failure percent if needed.. 4. Standard echocardiographic procedure should describe as B-mode with LVEF assay methods. Please, check and correct. 5. Because PCI and EchoCG were not performed by blinded specialists, please, report whether authors of the article have carried out the procedures. 6. Section Results. Please, give a comparison between new predictive model and traditional predictive model based on peak troponins and clinical criteria. Use, please, C-statistic to compare.

REVIEWER	Prof. Dr. Franz-Josef Neumann University Hospital Freiburg Bad Krozingen, Cardiology
REVIEW RETURNED	23-Oct-2019

GENERAL COMMENTS	Thank you for considering my comments. Nevertheless, there are issues remaining. Although the authors now mention the independent variables in the logistic regression models, the rationale for choosing these variables is not completely clear to me. Specifically, we need to know whether these variables were defined prospectively or whether the variables were selected post-hoc during the process of developing the logistic regression models. The authors now give the definition of MACE. It is not clear to me how the component of “angina pectoris readmission” can be relevant to the in-hospital events that the authors are addressing. Moreover, the time frame is too short to look at “stent restenosis” which also was a component of the endpoint MACE. I appreciate that the authors give more reason for choosing bilirubin as an important independent variable. However, they should address other markers of oxidative stress as well, at least when discussing their findings.

REVIEWER	Anggoro Budi Hartopo Universitas Gadjah Mada, Cardiology and Vascular Medicine
REVIEW RETURNED	21-Oct-2019

GENERAL COMMENTS	The manuscript is improved. There are some minor revisions: 1. Abstract: Methods: please describe the MACE Conclusion: "may be" should be changed into "is an" 3. Is TB, DB, IB measured by The automatic blood analysis equipment 4 (Beckman LH750/DXC800 automatic blood analyzer)? 4. Use statistics term such as mean as a substitute of average (page 15, line 12) 5. Conclusion: "may be" should be changed into "is" . 6. Many typography and grammar errors. Please correct.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Authors reported good-written and well-structured paper with clear aim and interesting results. It was single-center, prospective cohort study, in which a total of 615 STEMI patients after PCI were enrolled. MACEs were determined and analyzed in accordance to circulating biomarkers, such as bilirubin and WBC, for 36 month period. Authors found that the combination of WBC and TB may be an independent predictor for in-hospital 20 outcomes in patients with STEMI than single detection. However, there are several items need to be explained.

1.Ethical declaration should be extended. Please add IRB name and approval date. Therefore, procedure of getting an agreement should be described carefully.

Reply: Thank you for your question. The study protocol was first approved in December 2014 by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China)

(approval ID: 20141201-03) and ethics review was conducted in January 2017 (approval ID:20141201-03-1701A) (page6, line5-8). All patients provided written informed consent.

2 Several data, such as GRACE, TIMI, should be reported as median and upper/low limits or interquartile range, but not as mean =/- SEM, otherwise a clinical sense of the the parameters is missed. Please, check and correct.

Reply: Thank you for your advice. We have made modifications in the Table1 and gave description in the result (page11, line18)

3. Because it was prospective study, authors should not just report sample size calculation formula to assay whether findings for end point are able to be taken into consideration for further analysis, but they should give an example of the calculation with drop-out and screen failure percent if needed.

Reply: Thank you for your advice. In our study, 583 patients or family members was complete in follow-up, 15 patients were lost to follow-up and the loss rate was 2.5%. Based on the sample size of the current study, the power of the research results using Power and sample size calculation is 81.2% (page10, line19-20; page13, line3-4).

4. Standard echocardiographic procedure should describe as B-mode with LVEF assay methods. Please, check and correct.

Reply: Thank you for your advice. All patients were assessed by transthoracic echocardiography within 48 h after primary PCI Standard echocardiographic views were acquired and analyzed by two experienced cardiologists who were unware of grouping information. Left ventricular ejection fraction (LVEF) was measured by B mode echocardiography.We have made modifications in the manuscript.(page7, line8-11).

5. Because PCI and EchoCG were not performed by blinded specialists, please, report whether authors of the article have carried out the procedures.

Reply: In our study , angiograms were independently reviewed by two interventional cardiologists who were blinded of patients' information in the Cardiac Center of the First Affiliated Hospital of Xinjiang Medical University. The authors of this article were not involved in the PCI treatment of these patients. (page8, line3-6)

6. Section Results. Please, give a comparison between new predictive model and traditional predictive model based on peak troponins and clinical criteria. Use, please, C-statistic to compare.

Reply: Thank you for your advice. We have made modifications in the Table3 and we made a comparison between new predictive model and traditional predictive model based on the high-sensitivity troponin T (hs-TnT). The recommended cut-off value for peak hs-TnT on the ROC curve was 0.87μg/ml and it had 85.2% sensitivity and 77.8% specificity in predicting in-hospital mortality (AUC=0.894 95% CI: 0.831-0.961) (page12, line9-15).

Reviewer: 2

Thank you for considering my comments. Nevertheless, there are issues remaining.

1.Although the authors now mention the independent variables in the logistic regression models, the rationale for choosing these variables is not completely clear to me. Specifically, we need to know whether these variables were defined prospectively or whether the variables were selected post-hoc during the process of developing the logistic regression models.

Reply: Thank you for your question. In the multivariable logistic regression, we included a total of 12 indicators. To avoid the influence of traditional factors on the results, we select the traditional risk factors for atherosclerosis, including age, body mass index (BMI), gender, smoking, hypertension, diabetes and low-density lipoprotein cholesterol. We also choose age, diabetes, hypertension, heart rate because they are included in the TIMI risk score, with left ventricular ejection fraction (LVEF), culprit vessels and combine detection, has clinical significance in the univariate analysis. And we choose CK-MB, because CK-MB represents the area of myocardial necrosis and we considered there is a relationship between CK-MB and the poor prognosis of patients. (page12, line21-29)

2.The authors now give the definition of MACE. It is not clear to me how the component of “angina pectoris readmission” can be relevant to the in-hospital events that the authors are addressing. Moreover, the time frame is too short to look at “stent restenosis” which also was a component of the endpoint MACE.

Reply: Thank you for your question. In our study angina pectoris readmission and stent restenosis are attributed to long-term MACE. After the primary PCI treatment, if the patient experienced angina pectoris again after discharge and was readmitted due to angina, we treat it as an MACE. Stent restenosis can be defined as 50% re-stenosis of the lumen diameter after stent implantation by angiography, if stent restenosis is found during follow-up this will count as an MACE.

3.I appreciate that the authors give more reason for choosing bilirubin as an important independent variable. However, they should address other markers of oxidative stress as well, at least when discussing their findings.

Reply: Thank you for your question. We recognize that there have many antioxidants and markers which can represent oxidative reactions in vivo, we have address other markers of oxidative stress in the discussing part. (page15, line16-30, page16, line1)

Reviewer: 3

The manuscript is improved. There are some minor revisions:

1. Abstract:

Methods: please describe the MACE

Conclusion: "may be" should be changed into "is an"

Reply: Thank you for your advice. We have made modifications. (page2, line10-13, 22)

2. Is TB, DB, IB measured by The automatic blood analysis equipment 4 (Beckman LH750/DXC800 automatic blood analyzer)?

Reply: We are sorry for this negligence. TB, direct bilirubin, indirect bilirubin were measured by Hitachi 7060 automatic biochemical analysis and the automatic blood analysis equipment (sysmex XE-5000 automatic blood analyzer) was used for WBC in our hospital testing center. (page7, line3-6)

3.Use statistics term such as mean as a substitute of average (page 15, line 12)

Reply: Thank you for your advice. We have made modifications. (page10, line4)

4. Conclusion: "may be" should be changed into "is" .

Reply: Thank you for your advice. We have made modifications. (page17, line28)

5. Many typography and grammar errors. Please correct.

Reply: Thank you very much, we have thoroughly checked the revised MS to improve the language and correct grammatical errors.

VERSION 3 – REVIEW

REVIEWER	Alexander E Berezin State Medical University of Zaporozhye, Yukraine
REVIEW RETURNED	16-Nov-2019

GENERAL COMMENTS	Authors reported revised version of the article according to reviewers' comments. The revised version can be accepted for processing further.

REVIEWER	Franz-Josef Neumann University Hospital Freiburg Bad Krozingen, Cardiology
REVIEW RETURNED	27-Nov-2019

GENERAL COMMENTS	Thank you for considering my comments. From my side, there is only one question remaining: Did you define the independent variables in the logistic regression models prospectively or were they selected post-hoc during the process of developing the logistic regression models? Please, add a specific statement to the methods section.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1

Authors reported revised version of the article according to reviewers' comments. The revised version can be accepted for processing further.

Reply: I am glad to hear that the revised version can be accepted for processing further. Thanks for your comments on my manuscript.

Reviewer: 2

Thank you for considering my comments. From my side, there is only one question remaining:

Did you define the independent variables in the logistic regression models prospectively or were they selected post-hoc during the process of developing the logistic regression models? Please, add a specific statement to the methods section.

Reply: Thank you for your advice. We add the statement “The multivariate analysis controlled for all factors with significant associations emerging from the univariate analysis and the traditional risk factors for atherosclerosis” in the Statistical Analysis.(page10,line13-14)