Endothelin-1 as predictor of major adverse cardiovascular events in chronic coronary syndrome patients undergoing coronary intervention [version 2; peer review: 1 approved with reservations, 1 not approved]

Trisulo Wasyanto, Ahmad Yasa, Nimas Ayu

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Abstract

Background: Major adverse cardiovascular events (MACE) are predicted to be low in chronic coronary syndrome (CCS) patients who have undergone percutaneous coronary intervention (PCI). Endothelin-1 has been considered a pro-inflammatory biomarker and suggested as a novel prognostic indicator in CCS. The objective of this research was to prove endothelin-1 as predictor of MACE within 1-year evaluation in CCS patients undergoing PCI.

Methods: This research was an analytic observational study with a cohort design. The participants were CCS patients who had undergone PCI. Endotelin-1 levels were checked before the patient underwent PCI. Occurrences of MACE were observed within 1 year. The comparison between normally distributed continuous data was performed with a T-test, and the Mann-Whitney test was used for not normally distributed data. A comparison between categorical data was performed with the Chi-square test. The cut-off point of endothelin-1 levels to predict MACE was analyzed by receiver operating characteristics (ROC).

Results: Participants in this study were 63 patients. Six patients experienced MACE within 1 year (9.5%) and 57 patients were included in the non-MACE group (90.5%). Mann Whitney T test showed there were significance differences in endothelin-1 levels from the two groups (p=0.022). The ROC curve showed cut off point the endothelin-1 is 4.07 ng/dl with a sensitivity of 83.3%, specificity of 75.4% and accuracy of 76.2% for the occurrence of MACE. Based on the area under curve (AUC) value and the accuracy of this study, endothelin-1 was able to detect MACE within 1 year of follow-up.

Conclusions: Endothelin-1 can be used as predictor of MACE within 1-year evaluation in CCS patients undergoing coronary intervention.
Introduction
Coronary heart disease (CHD) is a type of heart disease caused by the narrowing of the coronary arteries due to the atherosclerosis process. CHD can be divided into acute coronary syndrome (ACS) and chronic coronary syndrome (CCS).1–3 The diagnosis of CCS includes identification of risk factors for atherosclerosis, clinical evaluation, and supporting examinations.4,5

Endothelin-1 is derived from endothelial cells and several studies have reported its associated with endothelial dysfunction.6–8 Endothelial dysfunction has been reported as an atherosclerotic risk factor associated with future cardiovascular events,9–11 and therefore has been considered a pro inflammatory factor12–14 and suggested as a novel prognostic indicator in ACS. However, its role in predicting cardiovascular events in stable coronary artery disease is unclear.15–17 Endothelin-1 in the cardiovascular system is produced not only by vascular endothelial cells but also by vascular smooth muscle cells, cardiomyocytes, and fibroblasts.18–20 Levels of endothelin-1 in blood plasma are very low under normal conditions, but the levels increase 100 times higher when the vascular wall shows increased cellular activity.21–23 Endothelin-1 is a potent endogenous vasoconstrictor produced primarily by the vascular endothelium.24–26 As a vasoconstrictor it contributes to increased tone in atherosclerotic coronary arteries and is involved in endothelial dysfunction, inflammation, and vascular remodeling.27–29

A meta-analysis by Windecker et al. reported a reduction in mortality and incidence of acute myocardial infarction (AMI) with revascularization vs. medical therapy alone, in CCS patients when revascularization was performed with a coronary artery bypass graft (CABG) or a new generation of drug-eluting stent (DES) instead of the earlier bare metal stent (BMS) or balloon angioplasty alone.30 In patients with stable coronary artery disease, an initial fractional flow reserve (FFR)-guided PCI strategy was associated with a significantly lower rate of the primary composite end point of death, myocardial infarction, or urgent revascularization at five years than medical therapy alone. Patients without hemodynamically significant stenosis had a favorable long-term outcome with medical therapy alone.31

Methods
Ethics and consent
The protocol of the research was approved by the Medical and Health Research Ethics Committee of Dr. Moewardi Hospital Surakarta Indonesia No. 71/II/HREC/2021 on November 12, 2021. Participants provided signed informed consent to participate.

Participants
The design of the research was a prospective cohort study conducted in December 2021 – December 2022. The population were patients diagnosed with chronic coronary syndrome (CCS) who underwent cardiac catheterization (PCI) and were admitted in the intensive cardiovascular care unit (ICVCU) and cardiology ward of Dr. Moewardi Hospital, Surakarta, Indonesia. This study recruited 63 patients, consisting of 46 (73%) male patients and 17 (27%) female patients. We included subjects with a diagnosis of chronic coronary syndrome aged between 30 and 75 years old. We excluded subjects with acute myocardial infarction (AMI), previous history of PCI, severe heart valve abnormalities, history of chronic heart failure with New York Heart Association (NYHA) class ≥II, chronic renal failure, hepatic cirrhosis, and malignancy; with concomitant infection and sepsis; with concomitant acute stroke and acute inflammatory state (such as acute arthritis and pericarditis) during hospitalization; and acute heart failure. All subjects gave signed informed consent to participate in the study.

Procedure
Upon admission, before the patient underwent catheterization, a peripheral antecubital venous blood sample was obtained from each subject during the supine position. The blood sample was centrifuged at 4000 r.p.m. for 20 minutes and stored at −80°C in a freezer until analysis for endothelin-1 measurement. Endothelin-1 was detected and quantified
with endothelin-1 immunoassay Quantikine® ELISA kit (R&D Systems, Minneapolis, USA) according to manufacturer procedure instructions (CV%: 23). The ELISA method was performed once by a skilled technician in the clinical pathology laboratory at Dr. Moewardi Hospital Surakarta Indonesia.

The subjects’ clinical data were collected during hospitalization. The treatments for subjects was at the discretion of attending cardiologists, without any interference of this research. Subjects were observed from admission until one year after hospital discharge for the occurrence of major adverse cardiac events (MACE). After the patient was discharged from the hospital, they are asked to carry out routine check-ins every month (if there are no complaints), or immediately check-in if there are complaints. If it is time for a check-in and the patient does not attend, they will be contacted by telephone or if necessary, a visit to the patient’s home is made. The adverse cardiac event was the composite of cardiac death, acute heart failure, cardiogenic shock, reinfarction, and resuscitated ventricular arrhythmia. Cardiac death was fatal due to cardiac disease. Acute heart failure was defined as the occurrence of signs/symptoms of congestion and the use of intravenous diuretics. Cardiogenic shock was defined as the signs of reduced peripheral perfusion and the use of vasopressors drugs. Reinfarction was defined as the recurrent chest pain, recurrent ST-segment elevation, and an elevation of cardiac enzymes. Resuscitated ventricular arrhythmia was the return of spontaneous circulation after resuscitation for lethal arrhythmias.

Analysis

For statistical analysis, SPSS 26.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. The subjects were divided into two groups based on the presence of adverse cardiac events. The normal distribution was tested with the Kolmogorov-Smirnov test. The comparison between normally distributed continuous data was performed with Student’s T-test, while the Mann–Whitney test was used for not normally distributed continuous data. A comparison between categorical data was performed with the Chi-square test. A receiver operating characteristic (ROC) curve was designed to determine the cut-off point of endothelin-1 level to predict adverse cardiac events. A univariate and multivariable analysis with logistic regression test were performed to determine the independent predictor of an adverse cardiac event. A p value < 0.05 was set as statistical significance.

Results

This study was conducted in the emergency room, polyclinic, intensive cardiovascular care unit (ICCU), and cardiac care ward, clinical pathology laboratory, and cardiac catheterisation laboratory hospital. 63 samples from patients with CCS were obtained. The 63 patients were then monitored for one year for the development of major adverse cardiovascular events (MACE). None of the patients had chronic total occlusion (CTO). The results of this study listed in Table 1.

For variables related to coronary angiography results, 28 (44.4%) persons involved one coronary vessel, 16 (25.4%) persons involved two vessels, and 19 (30.2%) person involved three vessels. None of the patients has chronic total occlusion (OTC). The characteristic variable descriptions and coronary angiography are presented in Table 2.

Of the 63 patients in the study, six patients experienced MACE within 1 year (9.5%), and 57 patients were included in the non-MACE group (90.5%). MACE occurred in the evaluation of six patients (9.5%), in the form of acute myocardial infarction, heart failure, and death in two (33.3%), one (16.6%), and three (50%) patients, respectively. In male patients who underwent MACE, two patients experienced AMI, one patient experienced heart failure, and two patients died. Only one female patient experienced MACE due to death. Two patients were not routinely monitored for evaluation, due to busyness of the patients, insurance issues, or geography.

Endothelin-1 levels generally ranged from 2.15 pg/ml to 6.90 pg/ml, with a mean of 3.66 pg/ml and a standard deviation of 1.09 pg/ml (3.66 ± 1.09 pg/ml). In the non-MACE sample group, endothelin-1 levels ranged from 2.15 pg/ml to 6.90 pg/ml, with a mean of 3.59 pg/ml and a standard deviation of 1.09 pg/ml (3.59 ± 1.09 pg/ml). In the MACE sample group, endothelin-1 levels ranged from 3.52 pg/ml to 5.84 pg/ml, with a mean of 4.37 pg/ml and a standard deviation of 0.78 pg/ml (4.37 ± 0.78 pg/ml). The description and testing of endothelin-1 level variables are presented in Table 3.

The calculation of endothelin-1 levels as a predictor of 1-year MACE variables was done by using the receiver operating characteristic (ROC) curve. The area under the curve (AUC) for the ROC curve on the incidence of MACE for the variable endothelin-1 level as a predictor was 0.785. The interpretation of the variable endothelin-1 level can detect the incidence of MACE well. Based on the ROC curve, the cut-off point value of the endothelin-1 level variable was 4.07 ng/dl, and the occurrence of MACE can be detected from the endothelin-1 level variable with a sensitivity rate of 83.3% and a specificity rate of 75.4% and a diagnostic accuracy rate of 76.2%. The results of sensitivity, specificity, and diagnostic accuracy are presented in Table 4.

The relationship between endothelin-1 levels and 1-year MACE (cut-off point = 4.07) are presented in Table 5.
<table>
<thead>
<tr>
<th>Variable Description</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical condition</strong></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.11 ± 3.81</td>
</tr>
<tr>
<td>Heart Rate (x/min)</td>
<td>73.25 ± 12.52</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td></td>
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<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.77 ± 1.68</td>
</tr>
<tr>
<td>Leukocytes (mcg/L)</td>
<td>8.37 ± 2.18</td>
</tr>
<tr>
<td>Platelets (mcg/L)</td>
<td>270.44 ± 78.81</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>30.40 ± 12.09</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.15 ± 0.32</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>72.86 ± 21.47</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>6.78 ± 1.68</td>
</tr>
<tr>
<td>Fasting Blood Sugar (mg/dL)</td>
<td>109.21 ± 42.49</td>
</tr>
<tr>
<td>Blood sugar 2 hours post prandial (mg/dL)</td>
<td>140.59 ± 56.19</td>
</tr>
<tr>
<td>Uric Acid (mg/dL)</td>
<td>6.83 ± 1.87</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>158.04 ± 45.79</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>103.92 ± 35.05</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>39.02 ± 9.73</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>152.32 ± 67.47</td>
</tr>
<tr>
<td><strong>Echocardiographic parameters</strong></td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>48.30 ± 4.59</td>
</tr>
<tr>
<td>TAPSE (cm)</td>
<td>2.07 ± 0.27</td>
</tr>
<tr>
<td><strong>Coronary angiography results</strong></td>
<td></td>
</tr>
<tr>
<td>Left Main</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>1 Vessel</td>
<td>28 (44.4%)</td>
</tr>
<tr>
<td>2 Vessel</td>
<td>16 (25.4%)</td>
</tr>
<tr>
<td>3 Vessel</td>
<td>19 (30.2%)</td>
</tr>
</tbody>
</table>

Note: BMI = Body mass index; CHD = Coronary heart disease; TAPSE = Tricuspid annular systolic excursion.
The statistical test results of the relationship between endothelin-1 levels and MACE with a probability of \( p = 0.008 \) indicate that the relationship is significant at a 1% significance level (\( p \leq 0.01 \)). The odds ratio reached 15.36, with a 95% confidence interval of 1.65 to 142.84, indicating that the relation between endothelin-1 levels and MACE was truly significant.

<table>
<thead>
<tr>
<th>Table 2. Variable description of research characteristics.</th>
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<tbody>
<tr>
<td><strong>Quantitative variables</strong></td>
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<tr>
<td></td>
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<tr>
<td><strong>Demographics</strong></td>
</tr>
<tr>
<td>Age (years)(^a)</td>
</tr>
<tr>
<td>Sex(^b)</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>BMI (kg/m(^2))(^a)</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
</tr>
<tr>
<td>Hypertension(^b)</td>
</tr>
<tr>
<td>DM(^b)</td>
</tr>
<tr>
<td>Smoking(^b)</td>
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<tr>
<td>Dyslipidemia(^b)</td>
</tr>
<tr>
<td><strong>Echocardiographic</strong></td>
</tr>
<tr>
<td>LVEF(^b)</td>
</tr>
</tbody>
</table>

Note: DM = Diabetes mellitus; BMI = Body Mass Index.
\(^a\)Independent t-test (numerical data fulfills normality assumptions).
\(^b\)Chi-squared test/Fisher exact test (nominal categorical data).

<table>
<thead>
<tr>
<th>Table 3. Relation of endothelin-1 level and MACE occurrences.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Endothelin-1</td>
</tr>
</tbody>
</table>

Note: Mann Whitney T-test (numerical data does not meet the assumption of normality).
*significant at the 5 percent significance level.

<table>
<thead>
<tr>
<th>Table 4. Sensitivity, specificity, diagnostic accuracy relation of endothelin-1 level and MACE.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Examination</strong></td>
</tr>
<tr>
<td>Endothelin-1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 5. Relation of endothelin-1 levels and 1-year MACE (cut-off point = 4.07).</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Endothelin-1</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Note: Chi-square test/Fisher exact test.
**Significant at 1% significance level.

The statistical test results of the relationship between endothelin-1 levels and MACE with a probability of \( p = 0.008 \) indicate that the relationship is significant at a 1% significance level (\( p \leq 0.01 \)). The odds ratio reached 15.36, with a 95% confidence interval of 1.65 to 142.84, indicating that the relation between endothelin-1 levels and MACE was truly significant.
significant (convincing). The ROC curve on MACE events for variable endothelin-1 levels as a predictor resulted in an AUC value of 0.785 with an accuracy rate of 76.5%. All of this demonstrates that endothelin-1 levels are a good predictor of MACE within a year.

Discussion

MACE or major adverse cardiovascular event is often used as a composite outcome of an observational study. Various definitions of MACE show different components in each study; some define MACE into three, four of five components. From all studies, it can be concluded that the most common components are acute myocardial infarction, stroke, and death. In addition, there were 15 studies that included heart failure as a component of MACE.

Zhou’s study included 3154 patients with stable CHD who were followed for 24 months. This study showed that endothelin-1 levels were associated with major cardiovascular events in CHD patients who were not revascularized. Endothelin-1 plays a prognostic role in stable CHD patients. An increase in endothelin-1 levels caused vasoconstriction and decreased coronary blood flow, thus causing and exacerbating myocardial ischemia. Haug’s study showed that endothelin-1 production increases in conditions where there are atherosclerotic plaques in the coronary arteries. Endothelin-1 release stimulates smooth muscle cell proliferation in a paracrine or autocrine manner, which may contribute to the development of coronary artery disease.

In our study, MACE occurred in six patients in the study sample. The low incidence of MACE could be due to several reasons. Firstly, the level of patient compliance with treatment was quite good at the 1-year follow-up. In addition, 63 patients underwent percutaneous coronary intervention (PCI). PCI is associated with a significant reduction in the primary composite risk of death, acute myocardial infarction, and urgent revascularization within five years, when compared with medical therapy alone.

In one retrospective cohort study, it was demonstrated that sex has an impact on subsequent adverse cardiovascular outcomes among patients over 60 years of age with atherothrombotic disease.

Research by Hata J and Kiyohara Y, 2013 in Asia reported that adverse cardiovascular events including ACS, all strokes, vascular procedures, and death in hospital were significantly lower in female patients than in males, both with univariate and multivariate analysis. Traditional atherosclerotic risk factors are age, hypertension, dyslipidemia, diabetes mellitus, and smoking.

In contrast to some previous meta-analyses, Zimmermann et al’s 2019 meta-analysis of 2400 subjects reported that reduced MACE was confirmed in patients undergoing PCI procedures, showing significant reductions in cardiac death and MI after a median follow-up 33 months with fractional flow reserve (FFR)-guided PCI vs. medical therapy (hazard ratio 0.74, 95% CI 0.56-0.989, P=0.041).

The ROC curve on MACE events for variable endothelin-1 levels as a predictor resulted in an AUC value of 0.785 with an accuracy rate of 76.5% in our study, which showed that endothelin-1 can act as a predictor for major cardiovascular events within 1 year. Diagnostic test research will be improved if the AUC value is close to 1. Criteria for interpreting the AUC value are as follows: >0.5-0.6 = very weak, >0.6-0.7 = weak, >0.7-0.8 = moderate, >0.8-0.9 = good, >0.9-1 = very good. The following criteria are used to interpret the accuracy score categories: 50-60% is very weak, 60-70% is weak, 70-80% is medium, 80-90% is strong, and 90-100% is very strong. This can be interpreted to mean that endothelin-1 levels can detect the occurrence of MACE in patients with CCS after a 1-year follow-up.

The limitations of this study were that it was only conducted in one center, and two patients were not routinely monitored for evaluation, due to busyess of the patients, insurance issues, or geography. Thus, the follow-up of patients cannot be fully monitored properly. Another limitation of this study is the small sample size, which is clearly insufficient to accurately describe the situation.

Conclusion

Endothelin-1 can be a predictor of major adverse cardiovascular events within 1 year in patients with CCS who had coronary intervention.

Data availability

Underlying data

Data are not able to be made publicly available due to the hospital’s confidentiality and patient privacy policies. Readers and reviewers who wish to access the data (underlying source data and analysis software output) should contact the corresponding author (trisulo.wasyanto@staff.uns.ac.id).
References

Open Peer Review

Current Peer Review Status: 🟢 🠲

Version 2

Reviewer Report 18 July 2023

https://doi.org/10.5256/f1000research.152526.r184679

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Josip A. Borovac
University Hospital of Split, Split, Croatia

The authors did try to address my concerns in their rebuttal letter, however, I do not think this was performed sufficiently. The paper still has significant limitations and ambiguities that raise a high publication concern.

This second review only confirms that the changes undertaken were not sufficient to warrant indexing, as many of the points were not addressed thoroughly and large uncertainties regarding the analysis performed and sample size are still present.

Obviously, the authors cannot change or improve the methodology and numbers for their study because the study has already been performed and has fixed flaws that cannot be corrected at this stage.

Nevertheless, in this current form, I hold that the paper should not be considered for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Acute coronary syndrome, heart failure, interventional cardiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 07 July 2023

https://doi.org/10.5256/f1000research.152526.r184678
Ahmed Shawky Elserafy
Ain Shams University, Cairo, Cairo Governorate, Egypt

Most of the comments provided by the reviewers were answered. However, the number of subjects and low number of events cannot be statistically used to conclude a relationship between endothelin-1 and MACE.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Cardiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Trisulo Wasyanto

Thank you very much for the comments. In fact, our study already exceeded the minimum sample size required to make results statistically significant. Sample size was calculated using Open Epi Software Version 3, to calculate the sample size in tests that compared the two means with reference sources for differentiation and standard deviation of groups 1 and 2 according to a previous study.

**Competing Interests:** No competing interests
In the Abstract section, authors present data on ROC curve and provide sensitivity and specificity info, however, they do not make explicit statement to what does this sensitivity and specificity refer to. It might be implicative this is MACE, however, that should be clearly stated.

The number of patients examined is low, as well as the number of events – only six patients experienced MACE. Do the authors think this is enough to provide validity in their observations? This is a major limitation to this study.

I am not sure that endothelin-1 as a biomarker is able to detect MACE within 1-year follow-up, this is not substantiated with data – number of events was extremely low.

Predictive value of endothelin-1 for these purposes is even more burdened by the heterogeneity of events contained within the MACE outcome – so what does the endothelin-1 measure? Two patients had AMI, one patient had heart failure, and two death events. Even if the endothelin-1 was predictive of adverse events, we do not know for which one. Similarly, this would challenge the pathophysiology of these events and authors would need to make a significant burden of proof to show how would this pathophysiologically relate to the events.

Again diagnostic power of this test cannot be substantiated at the event rate of 6 patients – with such a low number of events, albeit, so heterogeneous as well, statistical associations might be due to chance.

Author state that this was a prospective cohort study, how did they come up with such a sample size? Did they perform any a-priori testing of the study size and sample size. This needs to be thoroughly elaborated since it is important from methodological standpoint.

Exact timing on when endothelin-1 levels were sampled needs to be provided.

Similarly, why before PCI and why not after PCI sampling – authors would need to provide sound rationale for this.

Did any of the patients in your study had CTO? This needs to be disclosed.

What about other biomarkers measured in this cohort? Why no examination of association of endothelin-1 with troponin or NT-proBNP?

Periprocedural and stenting dana would need to be provided – we only have information on stented vessel number distribution.

Please change "ureum" to "urea" in the table.

Since authors followed and monitored patients for 1-year pharmacotherapy at discharge is a very potent and important modifier of outcomes. Authors did not provide any information on post-discharge pharmacotherapy which is a major limitation of these results. Even more, authors mentioned good compliance of patients as one of the potential reason of having low MACE, however, they provide no specificities of such treatment.
What were indications to revascularize these patients? We need to know more on who were these patients and why was revascularization initiated.

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly

**Is the study design appropriate and is the work technically sound?**
No

**Are sufficient details of methods and analysis provided to allow replication by others?**
No

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
No

**Are the conclusions drawn adequately supported by the results?**
No

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Acute coronary syndrome, heart failure, interventional cardiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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**Author Response 03 Jul 2023**

**Trisulo Wasyanto**

**Responses (2)**

**AUTHOR RESPONSE**

**Comment 1**
In the Abstract section, authors present data on ROC curve and provide sensitivity and specificity info, however, they do not make explicit statement to what does this sensitivity and specificity refer to. It might be implicative this is MACE, however, that should be clearly stated.

**Responses 1**
Thank you, you are right. This is indeed the sensitivity, specificity and accuracy of the MACE based on the ROC curve. We have confirmed this in the abstract.

**Comment 2**
The number of patients examined is low, as well as the number of events – only six patients experienced MACE. Do the authors think this is enough to provide validity in their observations? This is a major limitation to this study.

I am not sure that endothelin-1 as a biomarker is able to detect MACE within 1-year follow-up, this is not substantiated with data – number of events was extremely low.

**Response 2**

Our study met the minimum sample size required to make the results valid. Sample size was calculated using Open Epi Version 3 software to calculate sample size in a test that compared two means with reference literature for differentiation and standard deviation of groups 1 and 2 according to a previous study by Abdelrazek et al., 2020.

**Comment 3**

Predictive value of endothelin-1 for these purposes is even more burdened by the heterogeneity of events contained within the MACE outcome – so what does the endothelin-1 measure? Two patients had AMI, one patient had heart failure, and two death events. Even if the endothelin-1 was predictive of adverse events, we do not know for which one. Similarly, this would challenge the pathophysiology of these events and authors would need to make a significant burden of proof to show how would this pathophysiologically relate to the events.

Again diagnostic power of this test cannot be substantiated at the event rate of 6 patients – with such a low number of events, albeit, so heterogeneous as well, statistical associations might be due to chance.

**Response 3**

In one study, endothelin-1 levels were associated with major cardiovascular events in coronary heart disease (CHD) patients who were not revascularized. Increased endothelin-1 causes vasoconstriction and decreases coronary blood flow thereby causing and exacerbating myocardial ischemia. In another study, it showed that endothelin-1 production increased in conditions where there was atherosclerotic plaque in the coronary arteries. Endothelin-1 release stimulates smooth muscle cell proliferation in a paracrine or autocrine manner, thereby contributing to the development of coronary artery disease.

Endothelin-1 originates from endothelial cells and according to several studies, is associated with endothelial dysfunction. Endothelial dysfunction has been reported as an atherosclerotic risk factor associated with future cardiovascular events. Therefore, endothelin has been considered as a proinflammatory factor. Endothelin-1 acts as a vasoconstrictor which plays a role in increasing atherosclerotic coronary artery tone.

Endothelin-1 is involved in endothelial dysfunction, inflammation, and vascular remodeling.

**Comment 4**

Author state that this was a prospective cohort study, how did they come up with such a sample size? Did they perform any a-priori testing of the study size and sample size. This needs to be thoroughly elaborated since it is important from methodological standpoint.

**Response 4**
Sample size was calculated using Open Epi software version 3 to calculate the sample size in a test that compared two means with reference sources for differentiation and standard deviation of groups 1 and 2 according to a previous study by Abdelrazek et al., 2020.

**Comment 5**
Exact timing on when endothelin-1 levels were sampled needs to be provided. Similarly, why before PCI and why not after PCI sampling – authors would need to provide sound rationale for this.

**Response 5**
Blood samples were taken when the sample population met the inclusion criteria and before coronary angiography was performed. Because endothelin 1 is a biomarker that detects pro-inflammatory factors, blood sampling after PCI would confound the study results.

**Comment 6**
Did any of the patients in your study had CTO? This needs to be disclosed.

**Response 6**
In our study none of the patients had CTO. OK, we will include this in the manuscript. Since none of the patients had CTO, this may have resulted in a low MACE.

**Comment 7**
What about other biomarkers measured in this cohort? Why no examination of association of endothelin-1 with troponin or NT-proBNP?

**Response 7**
The aim of this study is to prove that endothelin-1 can act as a new predictor of MACE within 1 year in CCS patients undergoing coronary intervention. Due to cost and insurance limitations, we did not compare endothelin 1 and other cardiac biomarkers.

**Comment 8**
Periprocedural and stenting dana would need to be provided – we only have information on stented vessel number distribution.

**Response 8**
The patient underwent PCI with stent implantation using DES according to the size of the blocked coronary artery.

**Comment 9**
Please change "ureum" to "urea" in the table 1.

**Response 9**
Thank you, we've changed it to urea in Table 1.

**Comment 10**
Since authors followed and monitored patients for 1-year pharmacotherapy at discharge is
a very potent and important modifier of outcomes. Authors did not provide any information on post-discharge pharmacotherapy which is a major limitation of these results. Even more, authors mentioned good compliance of patients as one of the potential reason of having low MACE, however, they provide no specificities of such treatment.

**Response 10**
All patients have been educated for control and come to our heart polyclinic every month. Patient compliance with taking medication at home is one of the factors we cannot control. This is one of the limitations of the research. Patients are treated with standard drugs according to Guideline Directed Medical Therapy, namely in the form of double antiplatelet, Beta blockers, ACE inhibitors or Angiotensin receptor blockers, and MRA as indicated.

**Comment 11**
What were indications to revascularize these patients? We need to know more on who were these patients and why was revascularization initiated.

**Response 11**
1. Patients with suspected CHD, symptoms of stable angina and/or dyspnea.
2. Patients with new heart failure, left ventricular dysfunction, and suspected CHD.
3. Patients with or without symptoms, stable condition of duration <1 year after the incident ACS.
4. Asymptomatic subjects, where CHD is detected on examination.

**Competing Interests:** No competing interest

Reviewer Report 09 June 2023

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Ahmed Shawky Elserafy

Ain Shams University, Cairo, Cairo Governorate, Egypt

Very nice manuscript actually and a sound hypothesis.

The introduction is clear; however, I would remove the word “earlier DES (bare metal stent)” and just keep it bare metal stents.

The methods were constructive but when comparing the patients that had a MACE to the non-MACE group was very faulty as the numbers are not close enough for a statistical analysis.

The results were clearly presented; however, the amount of contrast, number of balloon
dilatations, stents are not included which can increase inflammation and increase mortality. Also, what is the reason for some of the patients being transferred to the CCU? Were they including those who suffered a MACE? What was the LVEF comparison between the two groups? Most literature don't classify occurrence of heart failure as MACE. Why did you do that? And when did the MACE occur in those 6 patients? Very early on or later?

The manuscript is a good idea with a clear hypothesis and constructed in a very good manner and minor language and grammar mistakes. I would recommend extensive revision before accepting this manuscript.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 03 Jul 2023
Trisulo Wasyanto

Responses (1)
AUTHOR RESPONSE

Comment 1
The introduction is clear; however, I would remove the word “earlier DES (bare metal stent)” and just keep it bare metal stents.
Response 1: Thanks so much for the comments. We revise it according to your suggestion.

Comment 2
The methods were constructive but when comparing the patients that had a MACE to the non-MACE group was very faulty as the numbers are not close enough for a statistical analysis.
The results were clearly presented; however, the amount of contrast, number of balloon dilatations, stents are not included which can increase inflammation and increase mortality. Also, what is the reason for some of the patients being transferred to the CCU? Were they including those who suffered a MACE? What was the LVEF comparison between the two groups? Most literature don't classify occurrence of heart failure as MACE. Why did you do that? And when did the MACE occur in those 6 patients? Very early on or later?

Response 2: Thanks so much for the comments. The objective of this research was to prove endothelin-1 as predictor of MACE within 1-year evaluation in CCS patients undergoing PCI. So our study was to see the occurrence of MACE in CCS patients who underwent PCI and was associated with high endothelin 1 levels (≥4.07) compared to low endothelin 1 levels (<4.07), from various clinical variables such as demographic variables and accompanying risk factors.

Some patients after procedures are transferred to the CCU due to complex procedures involving intervention of more than or equal to 2 coronary arteries, or there are periprocedural complications. They are not included in the MACE category in our study.

MACE or Major Adverse Cardiovascular Event is a major cardiovascular event that is commonly used as a composite outcome of observational studies.

Various definitions of MACE show different components in each study. Based on several studies, some defined MACE into 3, 4, or 5 components. From all studies, it can be concluded that the most commonly found components are acute myocardial infarction, stroke, and death. In addition, there are some studies that include heart failure as a component of MACE.

According to AHA (American Heart Association), MACE is defined as a composite of all causes of death; myocardial infarction, stroke, heart failure requiring hospitalization and revascularization, including percutaneous coronary intervention or coronary artery bypass graft. We included heart failure as an indication for hospitalization as it occurred in the sample population referring to several literatures. MACE may occur in the late phase, or during outpatient.

We compare LVEF on the incidence of MACE (+) and MACE (-) with no significant difference in the result and these are added to Table 2 (to view this table please open the link below).
https://tinyurl.com/table2research

Competing Interests: No competing interest
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