Author's response to reviews

Title: Prognostic value of Calcium Score and Coronary Flow Velocity Reserve in asymptomatic diabetic patients

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Author's response to reviews: see over
Dear Professor Sicari,

Please find enclosed our revised manuscript entitled “Prognostic value of Calcium Score and Coronary Flow Velocity Reserve in asymptomatic diabetic patients”, (Manuscript ID:2051167963177421) by Dikic M., Tesic M., Markovi Z., et al.

We would like to thank you for your all valuable comments, great efforts, and the time that you spent to make this manuscript better.

We believe that we have answered and discussed all the issues that were raised by the Reviewers. We have carefully re-analyze our data with new statistical analysis and write practically de novo manuscript in regard to Reviewers comments and made all the efforts in order to improve the quality of the manuscript and make this paper acceptable for publication in your respectable journal.

Sincerely,

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Reviewer's report I:

Predictive power of Calcium Score and Coronary Flow Velocity Reserve in asymptomatic diabetic individuals. The question posed by the authors, i.e. whether CFR-LAD and CS (CT based) are predictive of MACCE cardiac events is scientifically reasonable, particularly in asymptomatic diabetic patients. There are anyway several major concerns regarding this manuscript, that unfortunately end up by making it inappropriate to answer the title question:

1) Question or comment: This is something I generally consider minor, but becomes major in this case: English grammar and writing in general are very bad. Singular and plural forms are often associated incorrectly (just as an example, “Patient” instead of “patients” written not once as a mistake, but several times, lacking articles, and few sentences that cannot be understood in the way they are written). And this starts from the abstract which is not correctly written in English. I’m no English “purist”, since English is not my mother language too, so that I can well understand difficulties, but the level of the English in the manuscript is absolutely not sufficient for international scientific readers.

Response: Thank you for this remark. We rechecked English grammar and revised many writing mistakes that we made.
2) Question or comment: The methods and objectives are not well posed on scientific basis, so that it ends up by looking like a “fishing expedition” in search of whatever data come out. Methods, specifically the statistical ones, are not clear, sometimes wrong and measures like “efficiency” of a diagnostic method are cited as the endpoint, which i cannot understand what it means. And the idea to measure the sensitivity and specificity pertains more to the diagnostic field, not properly to the prognostic field.

Response: All the changes that were made to the manuscript are in the red font color.

a) We changed the title of the manuscript from “Predictive power of Calcium Score and Coronary Flow Velocity Reserve in asymptomatic diabetic individuals” to “Prognostic value of Calcium Score and Coronary Flow Velocity Reserve in asymptomatic diabetic patients”

b) Abstract of the manuscript was fully rewritten.

c) Aim of the manuscript was changed from “Aim of our study was to assess the relative predictive value between CS and coronary flow velocity reserve (CFVR) in asymptomatic patients with diabetes” to “Our aim is to assess joint prognostic value of CFVR and CS in asymptomatic DM patients”. Also the Introduction section was also changed in order to explain the rationale of our study.
d) We did new statistical analysis, as you suggested. Also new statistic methodology is written in the methodology section.

e) Inclusion and exclusion criteria were furthermore written as well as follow up data of the patients in the methodology section which was also suggested from the Reviewer 2.

f) Spearman’s correlation of CS and CFVR was removed as presented formerly in Table 4. The results of correlation was written in one sentence in Results section as follows; “There were significant correlations between CFVR LAD and total CS \((r= -0.266, p=0.008)\) as well as with CS of LAD artery \((r= -0.249, p=0.013)\) in DM patients. CFVR and CS did not correlate significantly in non DM patients. There was also no relation between HbA1c and CFVR of LAD in our DM population”.

g) We removed part of the results according to the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of CFVR < 2.0 and CS>200 as you suggested throughout the manuscript, as well as Table 5- diagnostic performance of CS and CFVR measurement. Consequently, we removed ROC curves as previously presented in Figure 3 and Figure 4 in the manuscript. We defined our cut off values of CFVR and CS.

h) We added in Result section as well as new Figure 3 - Kaplan-Meier event-free survival curve in diabetic patients stratified according to the presence of CS<200 or CS≥200 and CFVR<2 or CFVR≥2.
3) **Question or comment:** In the conclusion, starting from the conclusions in the abstract the authors conclude that CFR and CS are associated with MACCE, not mentioning how they associate between them, potentially independ, incremental, synergic or in other ways they relate each other. Consequently figures are not appropriate, since stats methods are not appropriate, in my view. Please refer to published reviews such as for example “Outcomes-Based Validation and Reliability Assessment of Noninvasive Testin Methods and Limitations of Assessing New Noninvasive Tests : Part II:”by Hachamovitch et al In other words, I am sure the authors have scientific competence, and I appreciate some of their work, but the current study is probably not their best piece of work . It looks not well conceived and the manuscript is badly written, not only regarding the English (you can solve this easily by having a mother-tongue researcher correct this) but also for scientific planning and stats methods. I am sure the data could be much more correctly analysed, with very simple standard prognostic stats methods, and hence conclusions be more correct. These data the authors have in their worksheets are interesting, but the study needs to be fully rewritten in its form, structure and content after new stats. I find no reason to write down all the specific grammar mistakes and things to change in each paragraph, because in my view the manuscript is wrong also in its concept and structure. Sorry for that, since i repeat this is my view of the manuscript, and not a judgement regarding the authors, who i would encourage to think and write a fully new manuscript using the raw data they already have.

**Response:** Thank you for suggesting us to do the new statistics. We agree with you that we made in statistic methodology many wrong analyses with unnecessary data that we
either changed or removed from the manuscript. We hope that with the new statistics we simplified prognostic stats methods and consequently presented more correct results of the manuscript. We really appreciate your patience to analyze it.

a) We wrote new conclusion in the Abstract section as well as in Conclusion section of the manuscript:

“Both CS and CFVR provide independent and complementary prognostic information in asymptomatic DM patients. When two parameters are analyzed together, the risk stratification ability improves, even when DM patients are analyzed together with non DM patients. As a result, DM patients with CS≥200 and CFVR<2 had the worst outcome. Consequently, the use of two tests identified subset of patients who can derive the most benefit from the intensive prevention measures.”

b) In order to furthermore present our results, on the beginning of the Discussion section we wrote:

“This study, represents the results of the annual follow up regarding the prognostic value of CS and CFVR in asymptomatic diabetic patients, where abnormal CFVR<2, detected by Doppler echocardiography identified together with CS≥200 subset of patients at higher risk for adverse events. When the two parameters are analyzed together, the risk stratification ability improves, even when DM patients are analyzed together with non DM patients. Thus, our data confirms and expand previous studies, suggesting not only that CFVR and CS have prognostic value when separately analyzed but also that the
combination of the two parameters has additive value and that they are complementary in their power of prediction.”

We also changed more than a 2/3 of the discussion section as it can be seen on the manuscript in red font.

e) As we wrote in the previous Comment#2 we removed ROC curves as previously presented in Figure 3 and Figure 4 in the manuscript, as well from the results section- sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of CFVR < 2.0 and CS>200.

d) In order to find association of selected variables with outcome in DM patients, we assessed Cox proportional hazard model using univariate and stepwise multivariate procedures instead of linear regression analysis what we did previously and presented in Table 6. Thank you for this comment. Thus, univariate and multivariate prognostic predictors of adverse events in diabetic patient are presented in Table 4.

Reviewer's report II:

Dikic et al. have studied the relationships between coronary artery calcium (CAC), coronary flow reserve (CFR) by transthoracic Doppler echo and cardiovascular events (23 events including 8 angina pectoris) during follow-up of approx. one year in 200
asymptomatic individuals. They also aimed to compare the predictive values of CAC and CFR in diabetics (n=101) and non-diabetics (n=99). The conclusion is that “calcium score and CFR have prognostic power for major cardiovascular end-points during one year follow up.”

1) **Question or comment:** General comment: I think that the study is potentially very interesting. However, the conclusion is somewhat disappointing, since we already know that CAC and CFR have prognostic value. As authors state in their aims, the main interests are in combining/comparing CAC and CFR (as done in Figure 2) as well as possible differences in their prognostic value in diabetics and non-diabetics. I would be interested in seeing your results in this view. Currently, the manuscript is badly written and both the methods and results are presented in extremely complex manner. There needs to be a simpler and more focused way to present you data. Some issues are presented below.

**Response:** Thank you for the valuable comments and honest effort to help us to improve this manuscript. We wrote de novo manuscript with many changes in all sections of the paper. As You suggested as well as Reviewer I we did new statistical analysis and removed unnecessary data from results.

a) We wrote new abstract as well as part of the Introduction section in order to make rationale for this paper more clear and also changed the aim of the study. We know that both CS and CFVR have prognostic value when analyzed alone in many papers, but since
CFVR estimates both presence of coronary artery stenosis and microvascular function and CS morphology of the artery we wanted to see joint prognostic value of CFVR and CS in this subset of patients because of broader risk information that was given by CFVR. Thus in the Introduction section we stated:

“CS evaluates underlying coronary atherosclerotic plaque burden and CFVR estimates both presence of coronary artery stenosis and microvascular function. Consequently, CFVR may provide unique risk information beyond the extent of coronary atherosclerosis. Thus, our aim is to assess joint prognostic value of CFVR and CS in asymptomatic DM patients”.

To discuss furthermore our results, on the beginning of the discussion section we wrote:

"This study, represents the results of the annual follow up regarding the prognostic value of CS and CFVR in asymptomatic diabetic patients, where abnormal CFVR<2, detected by Doppler echocardiography identified together with CS≥200 subset of patients at higher risk for adverse events. When the two parameters are analyzed together, the risk stratification ability improves, even when DM patients are analyzed together with non DM patients. Thus, our data confirms and expand previous studies, suggesting not only that CFVR and CS have prognostic value when separately analyzed but also that the combination of the two parameters has additive value and that they are complementary in their power of prediction".

At the end of the manuscript we changed conclusion as follows:
"Both CS and CFVR obtained by MSCT and by transthoracic Doppler echocardiography assessments, respectively, provide independent and complementary prognostic information in asymptomatic DM patients. When the two parameters are analyzed together, the risk stratification ability improves, even when DM patients are analyzed together with non DM patients. As a result, DM patients with CS ≥ 200 and CFVR < 2 had the worst outcome. Consequently, the use of two tests identified subset of patients who can derive the most benefit from the intensive prevention measures - more aggressive control of the risk factors and more frequent follow-up by noninvasive testing".

2) Question or comment: Specific points: I would recommend consulting an English language expert.

Response: We are sorry for the many language mistakes and I, as a corresponding author, am personally responsible for that. We tried to solve them all.

3) Question or comment: I would recommend consulting instructions for authors on the format of Tables. They are too complex. There should not be abbreviations that are not explained. Tables should highlight your main findings rather than present every detail.

Response:
a) We removed Table 4 (Spearman’s correlation) and Table 5 (Diagnostic performance of CS and CFVR measurement) from the text as suggested from the Reviewer 1. The rest of the tables we changed and rearranged according to the instruction for the authors, where we deleted unnecessary statistical data.

b) From Table 2 we removed Framingham risk score and “Assessment of 10-years cardiovascular risk” as well as from Results section of the text.

c) The associations of selected variables with outcomes were assessed using Cox proportional-hazards modeling in new Table 4 with univariate and stepwise multivariate procedures instead of linear regression modeling as formerly presented in Table 6.

4) Question or comment: Abstract: Paragraph starting “Sensitivity of AS … “. I cannot read your text after that – please revise.

Response: We wrote new abstract, thus that paragraph is deleted.

5) Question or comment: Reference 20: There is a more recent version of pretest probability of CAD according to D-F score in European population (Genders et al. EHJ 2011)

Response: Thank you for this remark. We removed both Diamond - Forrester analysis of probability and Framingham risk score from the manuscript.
6) **Question or comment:** Methods: What devices were used for CT and echo?

**Response:** CT device that was used for CS evaluation was SOMATOM Sensation 64, Siemens Medical Solutions, Forchheim, Germany. Echocardiographic studies were performed with an available digital ultrasound system (Acuson Sequoia C256; Siemens Medical Solutions USA, Inc, Mountain View, CA). We added in Method section the brand names of these two devices that were used as suggested by the reviewer.

7) **Question or comment:** Methods: How was follow-up for events carried out? Hospital records? telephone?

**Response:** In the methodology section we added this sentence:

“During a one year follow up of the patients, outcomes were determined from patient interviews at the outpatient clinic, hospital chart reviews, and telephone interviews with patients, their close relatives, or referring physician.”

8) **Question or comment:** Statistics: I cannot evaluate statistics based on your description.

**Response:** We did completely new statistical analysis with description in methodology section as You suggested.
9) **Question or comment:** End-points: Angina pectoris is very vague end-point. How do you define it? It might be reasonable to exclude it.

**Response:** We did not define end points, and also made great mistake not to write and define - new onset of unstable angina. We believe that this is clinically very important, since the patients were asymptomatic on the beginning of the study.

Thus instead of:

“The study population was followed maximum one year for incidence of major cardiovascular and cerebrovascular events (MACCE). As end-points of this study, death, myocardial infarction, stroke, angina, and necessity for percutaneous coronary intervention (PCI), or coronary bypass grafting (CABG) were considered”

we wrote in the methodology section:

“Cardiovascular death, stroke, nonfatal myocardial infarction, new onset of unstable angina and clinically driven percutaneous coronary intervention (PCI), or coronary bypass grafting (CABG) were registered as clinical events. Diagnosis of death from cardiovascular causes were obtained from the results of postmortem examination and detailed hospital documentation. Myocardial infarction was defined by typical symptoms, electrocardiographic evidence, and cardiac enzyme changes, while stroke was defined as rapid onset of focal or global neurological deficit lasting ≥24h or leading to death, with clinical findings supplemented by neurological imaging. Unstable angina was diagnosed on the basis of clinical features of an acute coronary syndrome without diagnostic enzyme changes or need for hospital admission or both.”
Follow-up data were analyzed for the prediction of composite endpoint of major adverse cardiovascular and cerebrovascular events (MACCE).”

10) Question or comment: Results: What is the basis for cut-off values for classification of coronary calcium into 5 categories? The definitions seem non-standard.

Response: Thank you very much for this important remark; we changed classification of coronary CS into standardized and previously presented classification by Elkeles RS et al. and Raggi P et al. to 0–10 Agatston units (AU), 11–100 AU, 101–400 AU; 401–1000 AU and >1000 AU. Thus we changed this cut off values in methodology section as well as in Table 2 and according to these cut-off values we have done statistics.

11) Question or comment: Results: Paragraph starting “There was statistically significant difference in the follow-up to MACCE…” I cannot read/understand your results after this.

Response: We did new statistics as suggested by the Reviewers, thus we wrote new results in more clearly and acceptable manner, thus this sentence was deleted.