Author's response to reviews

Title: Sensitive cardiac troponin I in diabetic patients with and without obstructive coronary artery disease

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Author’s response to reviews: see over
To the Editor:

Thank you for the detailed analysis of the text. The changes in the text are shown below.

Editorial Comment –

Question 1: It is not clear when troponin was measured
Reply 1: Blood samples were collected from patients between January 2011 and March 2012. Blood samples were frozen at -70 degrees Celsius for posterior analysis. Laboratory analysis and troponin determination was done in November 2012.

Question 2: It is not clear why statin were discontinued before measurement.
Reply 2: By the time this study was designed, there was a concern regarding a statin effect in troponin release [1][2]. Now there are several articles showing this interference is probably not significant[3][4].


Question 3: Role of diffuse coronary artery disease in these patients should be highlighted (comment on Diffuse coronary disease: short- and

Reply 3: I agree with you about the importance of diffuse coronary artery disease. The subject was included in the discussion (line 196) with 2 references including the one above:

These two studies did not include multivessel coronary artery disease patients.

It is possible that troponin release is related to more diffuse coronary disease, which is a prognostic factor in coronary artery disease [29] and is considered more common among diabetic patients with triple vessel disease [30], which is the group of patients included our study sample.


Question 4: Given the small sample size, normal distribution should be checked for.

Reply 4: The distribution is not normal. The distribution is skewed to the left, with several values near to the limit of detection of the method. The probability of normal distribution is shown below, as well as the distribution plot:

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>50</td>
</tr>
<tr>
<td>Lowest value</td>
<td>5,9000</td>
</tr>
<tr>
<td>Highest value</td>
<td>28,0000</td>
</tr>
<tr>
<td>Statistical Measure</td>
<td>Value</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>13,3080</td>
</tr>
<tr>
<td>Median</td>
<td>12,0000</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>6,3659</td>
</tr>
<tr>
<td>Coefficient of Skewness</td>
<td>0,6075 (P=0,0718)</td>
</tr>
<tr>
<td>Coefficient of Kurtosis</td>
<td>-0,6943 (P=0,1942)</td>
</tr>
<tr>
<td>D'Agostino-Pearson test for Normal distribution</td>
<td>acceptNormality (P=0,0852)</td>
</tr>
</tbody>
</table>

**Suspected outliers**

None


**95% Reference interval, Right-sided**

**A. Method based on Normal distribution**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper limit</td>
<td>23,7790</td>
</tr>
<tr>
<td>90% CI</td>
<td>21,1967 to 26,3612</td>
</tr>
</tbody>
</table>

**B. Non-parametric percentile method (CLSI C28-A3)**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper limit</td>
<td>25,9000</td>
</tr>
<tr>
<td>90% CI</td>
<td></td>
</tr>
</tbody>
</table>
### Reference interval No CAD

<table>
<thead>
<tr>
<th>Variable</th>
<th>NO_CAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>45</td>
</tr>
<tr>
<td>Lowest value</td>
<td>5,9000</td>
</tr>
<tr>
<td>Highest value</td>
<td>39,0000</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>9,0889</td>
</tr>
<tr>
<td>Median</td>
<td>7,0000</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5,9845</td>
</tr>
<tr>
<td>Coefficient of Skewness</td>
<td>3,5219 (P&lt;0,0001)</td>
</tr>
<tr>
<td>Coefficient of Kurtosis</td>
<td>14,8474 (P&lt;0,0001)</td>
</tr>
<tr>
<td>D'Agostino-Pearson test for Normal distribution</td>
<td>rejectNormality (P&lt;0,0001)</td>
</tr>
</tbody>
</table>

**Suspected outliers**

\[a, b\]
These values were not excluded for the current analysis.

### 95% Reference interval, Right-sided

<table>
<thead>
<tr>
<th>Method</th>
<th>Upper limit</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Method based on Normal distribution</td>
<td>18.9326</td>
<td>16.3718 to 21.4934</td>
</tr>
<tr>
<td>B. Non-parametric percentile method (CLSI C28-A3)</td>
<td>23.0000</td>
<td></td>
</tr>
</tbody>
</table>

![Histogram graph showing frequency distribution for NO CAD values from 5 to 45.](image)

Reply 5: I agree with you about the importance of glycemic control. This subject was included in the discussion (line 229) with 2 references, including the one above:

One unanticipated finding is that serum glucose and hemoglobin A1C concentrations were not different between CAD patients and the control group. However, in the multivariate analysis, serum glucose (with a negative correlation coefficient) was related to CAD in diabetic patients. Hyperglycemia is a risk factor for CAD in epidemiological studies [35]. The unexpected finding of a negative correlation between serum glucose and CAD is probably related to a bias in the study sample, in which the control group had slightly worse, but not statistically significant, glycemic control. Another factor that could explain the lack of difference in glycemic control among CAD and controls is that macrovascular complications do not correlate linearly with HbA1c [36] and the presence of prior vascular disease is one of the predictors of poor cardiovascular outcomes after treatment intensification for insufficient glycemic control [37].


First reviewer –

Major Compulsory Revisions:

Question 1: Authors should better explain why they carried out the comparison only with the diabetic patients with multivessel coronary artery disease. They should specify why patients with single vessel disease (i.e. proximal LAD stenosis) were excluded. Similar considerations regarding the choice to include patients with "proximally " stenosis (study subjects chapter)
Reply 1: There is really need for explanation: proximal multivessel coronary disease is a definition used in several studies which aim to investigate patients with widespread coronary artery disease. Our aim was to compare patients with heavy coronary atherosclerosis burden to patients with no coronary atherosclerosis. That is the reason why patients with single or two vessel disease were excluded.

Question 2: The authors described the limitations of their work in the section "Discussion" I believe that the authors should insert the appropriate section "Limitations"

Reply 2: I agree with the change, a section limitations (line 244) was inserted in the discussion:

Limitations

The troponin kit used in this study is not among the most sensitive tests available, nevertheless, the sensitivity was high enough to show a significant difference between the two groups studied.

Other limitations of this study deserve comment. First, the number of patients is relatively small, and these findings need to be confirmed in larger studies. Second, this is a case-control study that cannot provide information on prognostic implications of subjects with elevated high-sensitivity troponin.

Question 3: This article describes the results of the kit: "Siemens Centaur Ultra". In order to remove any doubt, I advise to utilize the term "level 1 troponin assay" avoiding "high-sensitivity assay".

Reply 3: I thank you for the advice. I have changed the designation to level 1 assay. I have also removed the term high sensitivity from the title.

Minor Essential Revisions:
1. The authors state that "Patients with CAD had a worse metabolic profile" but in TABLE 1 they found statistical significance only in Total Cholesterol i advise to reformulate the previous sentence.
   1. I accept the correction and have changed the sentence: There were no significant differences among the two groups. Patients with CAD had higher concentrations of total cholesterol and LDL cholesterol.

2. I suggest to replace the words "obstructive coronary artery disease" with "chronic coronary artery disease"
   2. I accept the suggestion and have changed the designation, also in the title: Troponin in diabetic patients with and without chronic coronary artery disease
Second reviewer –

**Major Compulsory Revisions:**

**Question 1:** Please, clarify the inclusion criteria of the study, first providing a definition of proximal multivessel disease (2-vessel disease included? Only 3-vessel disease? Left main disease?); second, point out the clinical context (stable CAD /silent ischemia with previous revascularization strategy (PCI/CABG)? stable CAD/silent ischemia without previous revascularization strategies?)

**Reply 1:** I accept the correction and have changed the definitions in inclusion criteria:

**Study subjects**

The study included patients with type 2 diabetes mellitus with angiographically documented proximal multivessel coronary stenosis of >70%, these patients had normal ventricular function and received optimal medical treatment without coronary revascularization.

**Minor Essential revision**

3) Hypertension is one of the exclusion criteria, but appears in the results (Table1). Clarify please.

**Reply 3:** I thank you for the correction. Hypertensive patients whose blood pressure was controlled with medication were included. We have excluded patients with blood pressure higher than 180x100 mm Hg. I have changed the exclusion criteria:

uncontrolled hypertension (systolic blood pressure greater than 180 mm Hg and/or diastolic blood pressure greater than 100 mm hg),

4) Please remove from Discussion technical aspect of troponin determination, adding it in the Methods Section.

**Reply 4:** I have removed the technical discussion to methods (line 134) and have also changed the troponin designation according to another request:

**Cardiac Troponin I**

Cardiac troponin-I was determined using the ADVIA Centaur®TnI-Ultra kit (Siemens Healthcare Diagnostics, NY, USA) in the automated equipment of the same manufacturer. The test is an immunoassay that uses a direct chemiluminescence technology and constant amounts of two monoclonal antibodies. An increased TnI concentration is defined as a value exceeding the 99th percentile of a normal reference population. According to the manufacturers, the detection limit is 0.006 ng/mL, the population reference value at the 99th percentile is <0.04 ng/mL, and the coefficient of variation is <10% at this 99th percentile.

Siemens Centaur ultra is not considered a high-sensitivity assay, instead, it is designated as a contemporary or level 1 assay. The new troponin assays are defined by their ability to detect troponin above the limit of detection in normal individuals and classified according to the percentage of normal individuals detected in four categories: level 1 corresponds to <50% of measurable normal
values, level 2: 50-75%, level 3: 75-95%, and level 4: ≥95%. Only levels two to four are considered high-sensitivity assays [27]. We have used the designation level 1 assay or the general designation: cardiac troponin.

5) **What could this paper add to the clinical practice?**
Reply 5) As I comment in the discussion: This result suggests that troponin release is related to the presence of coronary atherosclerosis and not to any other kind of damage to the heart caused by diabetes mellitus.

The role of troponin as biomarker in chronic coronary artery disease patients is still under debate. Our group is currently investigating a possible impact of chronic troponin elevation in the extent of coronary atherosclerosis and the impact of revascularization in the chronic release of troponin.