Author’s response to reviews

Title: Assessment of Endothelial Function by Brachial Artery Flow Mediated Dilatation in Cardiac Syndrome X Patients

Authors:

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Author’s response to reviews: see over
Explanations to reviewer (1) comments:

1. **Abstract: no conclusion paragraph has been prepared**

   **Explanation:** Conclusion paragraph has been added in the manuscript

2. **It is not clear how the two groups were selected: please detail how the diagnosis of syndrome X was done (no coronary angiography has been performed in the subjects under investigation, apparently). How was the control group selected. Were these healthy volunteers?**

   **Explanation:** In this study, 30 patients with history of angina like chest pain, reported positive on tread mill test and who underwent coronary angiogram and had normal coronaries, were diagnosed as CsX patients. Thirty age matched volunteers who were diagnosed healthy based on ECG and echocardiogram were treated as control.

3. **In line with the previous comment use the conventional definition of syndrome X as described by Maseri and Crea.**

   **Explanation:** The definition of syndrome X described by Maseri and Crea has been adopted in the manuscript.

4. **Endothelial function was not assessed with an automatic system. This is a major flaw in the methodology, since the measurement of endothelial variations has a very high variability due to the very small differences an automatic system, totally operator-independent is mandatory. Please address.**

   **Explanation:** Measurement was performed manually, because automatic tracking software was not available with us. This is the limitation of the study.

5. **The results section of the manuscript is very poor. However, many of the results are actually reported in the discussion section of the manuscript.**

   **Explanation:** In the light of your comments, changes were made and incorporated in the manuscript.

6. **Please address, in the discussion section, the clinical implications of the present results.**

   **Explanation:** we have addressed the clinical implications of the results in the light of literature in discussion section.

7. **The list of references is not appropriate. Please check the studies from Crea et al. Moreover, most of authors’ names are properly reported. Please follow the rules of the journal for references**

   **Explanation:** As per the requirement of manuscript, we have cited references and arranged them as per the journal format.
8. There are several typos throughout the manuscript.

**Explanation:** To the best of our efforts, we have tried to avoid the typos in the manuscript.

9. Due to the nature of the journal you should upload images with sample cases.

**Explanation:** As per the author’s instruction we have uploaded the image JPEG format.

**Explanations to reviewer (2) comments:**

1. **The paper needs to be edited by an English-speaking technical writer.**

   **Explanation:** We have tried to avoid grammatical errors and improve the clarity as much as possible. After effecting changes we have consulted English speaking technical writer and the requested opinion found to be satisfactory.

2. **Abstract: Conclusions are completely missing.**

   **Explanation:** Conclusions were added in the abstract

3. **Introduction, paragraph 2: hypothesis about the link between endothelial dysfunction and CSX disease should be made clearer.**

   **Explanation:** mechanisms linking endothelial dysfunction and Csx was made clearer and presented in the introduction section.

4. **Methods, page 4, lines 13-16: the way brachial FMD and GTN responses were measured should be explained (was the measurement obtained on B-mode images or by doppler signal as declared in this paragraph?).**

   **Explanation:** When a satisfactory transducer position was found, the skin was marked and the arm was kept in the same position throughout the study. A resting scan was obtained. The arterial diameter was measured. All measurements were made on B –mode images. Increased flow was induced by the inflation of a sphygmomanometer cuff placed around the forearm (distal to the scanned part of the artery) to a pressure of 200 mmHg for 4-5min, followed by release. A second scan was performed 90s after deflation of the cuff. The diameter of the artery was measured at the peak of R wave (Corresponding to end diastole).

   Flow-mediated dilatation was calculated, and the average results of the three observations recorded. Flow - mediated dilatation was presented as the percent change from baseline to hyperemia.
15 minutes were allowed for vessel recovery and then a further resting scan was taken, then sublingual glyceryl trinitrate (GTN-200mics /puff) was administered. After 5 min, the last scan was done. ECG was monitored throughout the scans and the artery diameter was measured at the peak of R wave (corresponding to end diastole). An average of 3 values was taken for each measurement.

5. Methods, page 5, lines 3-5: was the probe hand-held? Was the measurement on ultrasound signal performed manually or automatically? Is the adopted method reliable?

Explanation: Handheld probe was used. Measurement was performed manually, because automatic tracking software was not available with us. This method is accurate and reproducible for measuring small changes in arterial diameter (Clermajer et al, 1992) with low rates of interobserver error in measuring FMD (Sorensen et al, 1995 and Celermajer et al, 1998).

6) Methods, page 5, lines 7: the authors declare that a second scan to compute FMD% was performed 90 s after the cuff deflation, but several studies have suggested that the maximal increase in diameter occurs approximately 60 s after release of the occlusive cuff cuff [Corretti et Al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol. 2002 Jan 16;39(2):257-65]. In addition, also measurements at the end of the examination should be recorded in order to show if the final diameter value is different from baseline. Please provide these data.

Explanation: The time of peak response measurement is quite variable, and this necessitates continuous stimulus and diameter measurement for a minimum of 90 seconds post cuff release (Berry et al., 2000). Increase in diameter was maximal (± 5%) from 45 to 90 seconds under all conditions (Ramsey et al, 1995). Majority of earlier studies performed the second scan 60 to 90 seconds after Cuff-release (Clermajer et al, 1992; Lekakis et al, 1998; Motoyama et al,1997; Kidawa et al,2003). To ensure that no anomalies in arterial diameter occur during cuff occlusion and to capture the immediate hyperemic response, it is recommended that post-cuff measurements be initiated ≥10 seconds before cuff release. Although the peak velocity occurs within the first 15 seconds,
the peak vasodilation can be expected to occur 45 to 80 seconds after cuff release and may differ between populations (Black et al, 2008). Therefore, we have performed FMD 90s after the cuff deflation.


M Kidawa, M Krzeminska-Pakula, JZ Peruga, J D Kasprzak, Arterial dysfunction in syndrome X: results of arterial reactivity and pulse wave propagation tests,Heart 2003;89:422–426


7) Results: data about diameter (D) values should be added: are there differences in D values between the two populations? what about the relationship of FMD% with D?

Explanation: FMD and NMD were calculated based on the brachial artery diameter

**CALCULATION OF FMD:**

\[
FMD = \left( \frac{d2-d1}{d1} \right) \times 100
\]

Where, \(d1\) is the brachial artery diameter at baseline; \(d2\) is brachial artery diameter at 90 seconds of cuff release. Changes in the diameter after both interventions i.e., after stress and GTN, was expressed as percentage change from the pre-treatment value. All studies used FMD as the criteria. At each level, we calculated FMD based on brachial artery
diameter. Therefore, we did not maintain the records of brachial artery diameter values. We have compared FMD values between patients and controls and found to be statistically significant and the data is presented in table 1 of the manuscript.

8) Discussion - not well structured and lacking in depth. Conclusions are completely missing. Much more is needed in terms of interpretation of results.

**Explanation:** Discussion is restructured and explained the implications of the results in the light of literature. Conclusions are added at the end of abstract and as last paragraph in discussion section