Peer Review File

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Reviewer A:

The premise that coronary microvascular dysfunction (CMD) is likely to present in a high proportion of patients with recurrent symptoms after PCI is solid. However, the study design and the methods used in this study are inadequate to test that hypothesis for reasons below:

Comment 1: There is inherent bias in a retrospective study that included subjects who had both SPECT and angiography, which would be considered a higher risk study.

Reply 1: As the reviewers cautioned, patients may be at potential risk if they undergo both coronary angiography and SPECT. However, not all patients with recurrent chest pain after PCI received SPECT in our clinical work. For CAD patients who had previously undergone complete revascularization, if symptoms were considered ischemia related during outpatient visit, admission evaluation and coronary angiography should be reviewed if necessary. If coronary angiography did not reveal severe stenosis, we could not rule out the possibility of CMD in these patients, so we communicate with them for SPECT evaluation and inform the risks. We retrospectively collected the clinical data of these patients for analysis. This study is not a prospective design. As the reviewers mentioned, there were certain deviations in the selected patients, and the positive rate of abnormal blood flow quantification was higher after the patients' self-report and doctors' screening, which is the limitation of this study. **Changes in the text:** We have modified our text as advised (see Page 6, line 109-110 and Page 16, line 326-328).

Comment 2: There is no mention of how often SPECT was performed prior to angiography. If the vast majority of subjects had positive qualitative SPECT studies as a reason for angiography, then again you have selected for a population more likely to have ischemia.

Reply 2: We express our regret for our negligence of the details of the sequence of coronary angiography and SPECT in selected patients. As mentioned above, SPECT MPI is not a routine test for CAD patients who had received complete revascularization. If the patients had no need for revascularization, SPECT was used to evaluate the possibility of CMD (The flow of participants shown in Fig. 1.). We aimed to discuss the possibility and possible implications of CMD diagnosis with minimal interference from severe epicardial vascular stenosis. As the reviewer mentioned, selected patients has certain deviation, increasing abnormal blood flow quantitative positive rate, which is the limitations of this study.

Changes in the text: We have modified our text as advised (see Page 6, line 109-110 and Page 16, line 326-328).

Comment 3: There is no description of how myocardial blood flow is measured by SPECT. The validation of this technique has not been widely accepted and there is a known non-linear relationship between Tc-MIBI uptake and flow during hyperemia (see work by Glover and Beller).

Reply 3: Myocardial blood flow is measured by MyoFlowQ 1.0.2 (Beijing Larkcloud Biomedical, Beijing, China) workstations. The dynamic list mode data were transferred to MyoFlowQ workstation and automatically re-binned into 18 frames consisting of 10×10 s, 5×20 s, 2×60 s, 1×280 s frames.The regions of interest for input function and myocardial radioactivity sampling were automatically or manually set to obtain the dynamic curve and fitting curve of the left ventricular blood pool and left

ventricular myocardium, and to calculate the rest MBF (rMBF) and stress MBF (sMBF) of the left ventricle (LV),. MFR was then obtained, calculated by the ratio of sMBF to rMBF. We have cited previous publications by our team that elucidated the methodology applied, so no further description has been made in this article. Indeed, myocardial uptake and blood flow in MIBI had a not completely linear relationship, and there was a certain plateau in the condition of high blood flow, which caused the underestimate of the high blood flow area, which is the limitation of conventional tomography image. Fortunately, the image aimed to show the low blood flow area, which might also cause the underestimate and missed diagnosis of mild ischemia. In the quantitative blood flow analysis of MIBI, the low myocardial uptake rate of MIBI was first regression, and the linear relationship of reduction was corrected.

Changes in the text: We have cited previous publications by our team that describe in detail the methodology used as advised (see Page 7, line 136, and see Page 18, line 385, reference10).

Comment 4: If prevalence of microvascular dysfunction in those with post-PCI chest pain is the goal, then the denominator of 102 patients is incorrect. You have excluded patients who needed revascularization.

Reply 4 : We express our apologies for our incorrect writing and we haven't made this point any clearer. MFR was unable to evaluate CMD concomitant with coronary stenosis requiring revascularization, although such cases do exist. We aimed to evaluate the prevalence and characteristics exhibited by CMD in patients with recurrent chest pain who received PCI before, but CAG reexamination identified no sever coronary stenosis. We have made correction in accordance with the reviewer's comments.

Changes in the text: We have modified our text as advised (see Page 4, line 63-65 and Page 16, line 334-337).

Comment 5: Many of these patients who were included in the study that had CMD also had obstructive CAD in another territory. Therefore, you do not know that symptoms had anything to do with CMD.

Reply 5: It is really true as reviewer mentioned that there were indeed some cases with obstructive coronary arteries. At the first step, patients with severe stenosis requiring revascularization were excluded from the screening. At the second step, in screened patients, we excluded vessels with stenosis ≥50% and MFR<2.0 at the vascular level, because we could not identify this coronary artery and its supplying area with the possibility of CMD by MFR. However, if coronary artery stenosis ≥50% but MFR >2.0, we consider that there was no ischemia in the area supplied by this coronary artery. Thus, for CMD patients, even if there was obstructive CAD with MFR >2.0 in another area, we did not consider it as the cause of the patient's symptoms, and CMD might be the potential cause.

Changes in the text: We have added this part in discussion as advised (see Page 13, line 257-267).

Comment 6: Why is it important to make a conclusion that SPECT-based MBF is different according to CMD status if SPECT was used to determine CMD status?

Reply 6: We express our regret for we haven't made the language any clearer. Although interventional IMR is the gold standard for the diagnosis of CMD, it is rarely used in actual clinical practice due to various reasons, and it is also rarely used in the author's cardiovascular hospital, which is not practical. Accordingly, CMD diagnosis and evaluation should be conducted based on non-invasive imaging. MPI is the earliest application in quantitative myocardial blood flow (MBF), represented by PET, whereas it is not universal due to low equipment penetration rate and high price. With the recent

advent of CZT detector cardiac SPECT dynamic quantitative imaging, its quantitative MBF is well consistent with PET and will have a significant effect on in this aspect to solve part of the clinical diagnosis problems. Thus, quantification of MBF and MFR with CZT dynamic SPECT imaging are very important for CMD typing and diagnosis, but unfortunately, it does not solve all CMD typing. We have re-written this part in discussion part.
Changes in the text: We have modified our text as advised (see Page 16, line 335-337).

Comment 7: Vital signs (HR and BP) at the time of the imaging is always necessary for microvascular assessment since work and load determine flow requirement.
Reply 7: Vasodilators, unlike positive inotropic agents or exercise load, measure MFR by measuring endothelium-dependent dilation ability of vascular smooth muscle and calculating peak MFR load. At this time, due to the amplification effect of vascular drugs, blood pressure is slightly decreased and heart rate is slow. At this time, heart rate and blood pressure cannot reflect the working condition of the heart. This is different from positive inotropic drugs and exercise load. Therefore, the stress blood flow of vasodilating drugs is not required to be corrected, while the rest blood flow measurement method has been corrected according to RPP (the product of heart rate and blood pressure, namely the effect of the heart at rest, when RPP≥1000, the coefficient of 10000/RPP was used for correction, which is an international practice).
Changes in the text: we have modified our text as advised (see Page 7, line 143).
Special thanks to you for your good comments.

Reviewer B:
The authors demonstrated that almost 50% of post-PCI patients with recurrent chest pain suffer from CMD. They also showed that increased BMI and LDL-C were independent predictors of CMD. Chest pain without significant coronary artery stenosis could be a common problem in clinical settings. This paper would be interesting and meaningful to the physicians in this viewpoint.
Dear reviewer: I am very grateful to your comments for the manuscript. According with your advice, we amended the relevant part in manuscript. Some of your questions were answered below.

Comment 1: According to Figure 1, patients with≥50% stenosis were excluded. It is a bit confusing that the patients with "residual coronary stenosis" were included in the 102 patient group with the final analysis in Table 2. Please clarify the inclusion and exclusion criteria of the study population.
Are there patients with stenosis ≥50% and MFR ≥2.0? If so, please show the features of the patients. It would be intriguing for the readers.
Reply 1: We express our apologies for we haven't made this point any clearer. At the first step, patients with severe stenosis requiring revascularization were excluded from the screening. At the second step, in screened patients, we excluded vessels with stenosis ≥50% and MFR<2.0 at the vascular level, because we could not identify this coronary artery and its supplying area with the possibility of CMD by MFR. However, if coronary artery stenosis ≥50% but MFR >2.0, we consider that there was no ischemia in the area supplied by this coronary artery. For patients with stenosis ≥50% and MFR ≥2.0, no matter in CMD or control, we did not consider it as the cause of the patient's symptoms.
Changes in the text: We have added this part in discussion as advised (see Page 12-13, line 257-267).

Comment 2: Did the authors include the patients without stenosis in the main coronary arteries but with

stenosis of the side branches, such as diagonal branch and large obtuse marginal branch? How did the stenosis of side branches impact myocardial flow reserve?

Reply 2: Thank you for your comments. We express our regret for we haven't made this point any clearer. The patients without stenosis in the main coronary arteries but with severe stenosis of the side branches, such as diagonal branch and large obtuse marginal branch, will lead to increase of myocardial blood flow (MBF) in this corresponding territory, especially in stress MBF. MFR is the ratio of stress to rest MBF. With the decrease in stress MBF, MFR goes down. In our study, we excluded vessels with stenosis ≥50% and MFR<2.0 at the vascular level (no matter in the main coronary arteries or the side branches), because we could not identify this coronary artery and its supplying area with the possibility of CMD by MFR. If coronary artery stenosis ≥50% but MFR >2.0, there was no ischemia in the area supplied by this vessel.

Changes in the text: We have added this part in discussion as advised (see Page 8, line 153 and Page 12-13, line 257-267).

Comment 3: Is there any difference in myocardial flow reserve between culprit or non-culprit coronary arteries? Could myocardial flow reserve be affected by injury associated with PCI?

Reply 3: We appreciate your good comments. We apologize for having not made this point clearer. Included patients were those who still had intermittent chest pain symptoms during outpatient review at least 3 months after PCI. Frequent coronary angiography is undesirable for patients if necessary. Of course, this does not include emergency department visits for persistent chest pain. We considered whether PCI could damage the microvascular function of culprit coronary arteries. At the vascular level, 50.8%(60/118) of vessels received PCI in the CMD group, which showed no statistical difference compared with the control. From the results of our study, no difference was found in MFR between culprit or non-culprit coronary arteries, and PCI had no effect. Indeed, it is not excluded that microvascular dysfunction may occur in the short term after PCI (such as within one week), which should be studied in depth.

Changes in the text: We have added this part in discussion as advised (Page 12, line 250-256).

Comment 4: Myocardial flow reserve could be affected by blood pressure and heart rate. Please add the data of hemodynamics during the stress test.

Reply 4: Vasodilators, unlike positive inotropic agents or exercise load, measure MFR by measuring endothelium-dependent dilation ability of vascular smooth muscle and calculating peak MFR load. At this time, due to the amplification effect of vascular drugs, blood pressure is slightly decreased and heart rate is slow. At this time, heart rate and blood pressure cannot reflect the working condition of the heart. This is different from positive inotropic drugs and exercise load. Therefore, the stress blood flow of vasodilating drugs is not required to be corrected, while the rest blood flow measurement method has been corrected according to RPP (the product of heart rate and blood pressure, namely the effect of the heart at rest, when RPP≥1000, the coefficient of 10000/RPP was used for correction, which is an international practice).

Changes in the text: We have added this part in method as advised(see Page 7, line 143-147).

Comment 5: According to the conclusion and the discussion, the authors described “the risk of CMD is relatively high.” Please clarify which data the authors compared to in this study.

Reply 5: We apologize for having not made this point clearer. According to this study, the proportion of CAD coexisted with CMD in post-PCI patients with recurrent chest pain of 49.0%, and at the vascular level, the proportion of CMD accounted for 43.1%, is relatively higher than patients with non-obstructive coronary artery disease (NOCA) chest pain in our previous study which reported that 30% patients had CMD.

Changes in the text: We have modified our text as advised and cited previous publications by our team (see Page 11, line 231, reference17).

Comment 6: According to the result section, T wave inversion was associated with CMD. Please mention it in the discussion section.

Reply 6: T wave represents the "repolarization" of the ventricle, and T wave inversion is a common electrocardiography (EKG) manifestation in clinic, which indicates the possibility of myocardial ischemia. According to the results of this study, the proportion of T wave inversion was 44% in the CMD group and 19.2% in the control. As indicated by the scatter diagram, the majority of CMD patients reduced MFR in three coronary arteries. Based on the possibility of diffuse myocardial ischemia, T-wave inversion might be one of the EKG manifestations, which could be the underlying cause.

Changes in the text: We have added this part in discussion as advised (see Page 13, line 268-275).