Peer Review File

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Reviewer A

The authors attempt to add to the literature that catheter-based treatment of ISR is associated with peri-procedure MI if neointimal tissue represents neoatherosclerosis.

However, the manuscript needs a lot of work.

1) In the Introduction and Discussion, the authors mix up catheter-based treatment of ISR and catheter-based treatment of de novo lesions some of which are not even in the coronary arteries. Conversely, they ignore other previous publications specifically addressing catheter-based treatment of ISR, neoatherosclerosis (OCT or NIRS), and peri-procedure MI. As presented, the context is utterly confusing to this reviewer which makes me think that it is confusing to the authors as well.

Response: We thank the reviewer for pointing out this issue. As far as we are concerned, the investigation between periprocedural myocardial injury (PMI) and neointimal characteristics identified by OCT in patients with ISR was few. PMI was mainly reported in stable coronary artery disease. Therefore, we aimed to investigate the relationship between PMI and neointimal characteristics of ISR by using OCT.

2) There were too many univariate predictors of PMI, and many of them are obviously concordant. In addition, most of them were not significant in Table 1 or Table 2 nor did they have historic context to be included as univariate predictors. For just one example, LRP and ISNA are identical.

Response: We thank the reviewer for pointing out this issue. In our study, we included univariate predictor of PMI based on clinical importance, scientific knowledge, and predictors identified in previously published articles. Hence the univariate predictors seemed too many. Although the ratio of LRP and ISNA was the same, the LRP and ISNA were in different patients.

3) Please provide inter- and intra-observer variability for the OCT analysis.

Response: We thank the reviewer for pointing out this issue. Indeed, the OCT images in our study were mainly analyzed by an experienced physician (Zhijiang Liu). The other veteran observer would provide the final decision only when doctor Zhijiang Liu was not sure about the OCT images analyzed. Therefore, we could not provide inter- and intra-observer variability.

4) In a recent publication (doi: 10.1007/s10554-023-02956-1), the current authors reported "A total of 216 patients with 216 lesions who underwent optical coherence tomography (OCT) before interventional procedure for late drug-eluting stent ISR were enrolled and divided into NA and non-NA groups based on OCT findings" What happened to the missing 66 patients between the two studies?

Response: In our study, we consider that the high-sensitivity troponin T (hs-cTnT) levels are time-dependent in patients with acute myocardial infarction (AMI), 56 patients with AMI were excluded (49 patients were included in the study doi: 10.1007/s10554-023-02956-1). Another 27 patients included in the resent study (doi: 10.1007/s10554-023-02956-1) were excluded for its missing data of hs-cTnT after PCI.

Reviewer B

the paper on ISR and PMI, is a nice and extensive work.

As mentioned by the authors, there are some limitations (retrospective, subgroup etc..)

There is one thing I miss:

manipulation of the ISR with balloons/other devices can cause the troponin leak.

In the univariate analysis, one should check for the relationship between number of balloons (devices) used, and if available, number of balloon inflations and balloon/stent pressure. If no relation is found, the multivariate remains as it is and stands.

Response: We thank the reviewer for pointing out this issue. We investigated the relationship between the number of balloons used, the maximal balloon inflation pressure, balloon/stent pressure and the occurrence of PMI. The results showed that there was no relation between the variables and PMI.

Reviewer C

Comment for the authors

In this manuscript, the author reported the association between periprocedural myocardial injury and neointimal characteristics in patients with in-stent restenosis. The authors concluded that intra-intimal microvessels and intra-stent plaque rupture detected by OCT were independently associated with periprocedural myocardial injury. The topic is relatively interesting and the result is acceptable. However, I have some general comment to help further improve the manuscript.

General comments:

The previous studies have shown that the presence of OCT-based TCFA was associated with periprocedural myocardial injury in in-stent restenosis. Moreover, another study reported that the presence of the microvessels detected using OCT was associated with the incidence of TCFA. However, in this cohort, there was no significant difference between the presence of TCFA and the occurrence of PMI. Why was the TCFA not associated with the predictor of PMI in this cohort ?

Response: We thank the reviewer for pointing out this issue. The co-authors also agree with the reviewer's opinion that TCFA may be associated with the occurrence of PMI. But in our investigation, no correlation was found between TCFA and PMI. The most possible reason may be the limited sample size.

Specific comments

1. Please add the information of the incidence of slow flow and DES/DCB diameter

and length in angiography characteristics.

Response: The incidence of slow flow and DES/DCB diameter and length in angiography characteristics were shown in the Table 2 and the results were indicated with yellow highlighted.

2. Inter- and intra-observer agreement for OCT findings (TCFA, microvessels, Macrophage, etc) are not mentioned. Please mention them in the manuscript.

Response: We thank the reviewer for pointing out this issue. Indeed, the OCT images in our study were mainly analyzed by an experienced physician (Zhijiang Liu). The other veteran observer would provide the final decision only when doctor Zhijiang Liu was not sure about the OCT images analyzed. Therefore, we could not provide inter- and intra-observer variability.

3. I think it would be better to reduce the variables to the more important ones in Table

Response: We thank the reviewer for pointing out this issue. As the reviewer's advice, we reduced the variables in the final Table 4.

Reviewer D

The manuscript may be of interest to the readers of the journal .

There are however few issues that should be resolved :

- Periprocedural myocardial injury frequently occurs in complex procedures . In this

analysis no variables related to this important factor were reported . How long did the procedures last in the two groups ? Were there anatomical differences between the two groups (e.g. bifurcation lesions et..) that could have led to procedural difficulties in some patients ? How much contrast medium was used ? Were more inflations needed to dilate restenosis lesions in the PMI group? More stents were used (51% versus 42% , though the difference was not statistically significant) in the PMI group : was it a sign of angiographic deterioration after initial lesions dilations ?

Response: We thank the reviewer for pointing out this issue. We investigated the relationship between variables above the reviewer indicated and PMI. The procedural time was 85.5±31.5 (min) in the non-PMI group, and 78.9±26.1 (min) in PMI group. 230±65 (ml) contrast medium was used in the non-PMI group, and 230±52 (ml) in PMI group. The use of balloon between the two groups was similar. There was no significant statistic difference in maximal balloon pressure between the two groups. In our investigation, 82 (58.6%) patients had lipid-rich plaque, the procedural process (catheter or guide wire) may disrupt the lipid-rich plaque causing the occurrence of PMI.

- The authors state that patients with acute myocardial infarction were not included in the study. However this is hard to believe considering that almost 50% of patients with PMI had either red (21%) or white thrombus (40%) and that plaque rupture was observed in 60% of PMI patients. Please provide an explanation for these surprising findings.

Response: We thank the reviewer for pointing out this issue. In our study, the definition of silent ischemia, stable AP or unstable angina pectoris (UAP) mainly based on the diagnosis of the electronic medical record systems at discharge. Actually, the ratio of UAP was underestimated. We found either red or white thrombus was mainly in UAP patients. And the prevalence of lipid-rich plaque was high. Maybe the procedural process disrupts the lipid-rich plaque causing the occurrence of thrombus.

- The detection of microvessels is quite challenging: a single OCT pullback contains 300–500 image frames and this manual analysis can be subject to inter-and intra-observer variability so that deep learning methods have been proposed to overcome these shortcomings (see doi: 10.3390/bioengineering9110648)

Response: We agree with the reviewer's opinion. The accurate detection of microvessels would be difficult sometimes. In our study, the OCT images were analyzed manually. We believe deep learning methods must overcome the shortcomings in the future.

- Please report inter-observer and intra-observer variability for the various OCT parameters used for this analysis.

Response: We thank the reviewer for pointing out this issue. Indeed, the OCT images in our study were mainly analyzed by an experienced physician (Zhijiang Liu). The other veteran observer would provide the final decision only when doctor Zhijiang Liu was not sure about the OCT images analyzed. Therefore, we could not provide

inter- and intra-observer variability.

- NTproBNP was significantly different between the two groups but not included in the multivariable analysis (Table 4).

Response: We thank the reviewer for pointing out this issue. We investigated NTproBNP in the multivariable analysis furtherly, The results showed that NTproBNP was not associated with PMI.

Reviewer E

Shen et al. have demonstrated the relationship between neointimal characteristics of in-stent restenosis (ISR) evaluated with OCT and periprocedural myocardial injury (PMI) from their registry.

The reviewer has some comments that needs to be cleared before the publication.

Major Comments

1, The biggest drawback of the manuscript is the definition of "in-stent plaque rupture". Is there any previous manuscript that defined in-stent plaque rupture? The reviewer believe that if there are in-stent plaque rupture occurs in the patient, the patient should have ACS symptoms. However, the patient characteristics demonstrates that more than 7o% of the patients were silent ischemia or stable AP, that does not make no sense clinically. In addition, the representative picture of the in-stent plaque rupture in panel C of Figure 1 can be a wire injury. Therefore, the

reviewer is not convinced to the result of the current manuscript.

Response: Previous studies reported in-stent plaque rupture (see doi: 10.1177/10760296221146742 and 10.1093/eurheartj/ehv205). We thank the reviewer for pointing out this issue. We divided patients into silent ischemia, stable AP or unstable angina pectoris (UAP) mainly based on the diagnosis of the electronic medical record systems at discharge. According to the reviewer's proposal, we re-analyzed each patient's inpatient data, and after discussion with department specialists, we found that the proportion of UAP was underestimated. The actual ratio of UAP was 51.4% and in-stent plaque rupture (71.6%) was mainly found in UAP patients. In fact, not all plaque rupture would have ACS symptoms. A previous study reported that nonculprit plaque ruptures were observed in 66.7% patients with SAP (see doi: 10.1161/ATVBAHA.116.307891). We are sorry that the representative picture makes the reviewer misunderstand. We re-selected the typical OCT image, exhibited in figure 1.

2, Another concern is that the methodology of the OCT imaging analysis. That would be great if the authors could take 1 plaque characteristics from the culprit lesion (or most narrowed section).

Response: We thank the reviewer for posing this suggestion. We had found the representative OCT images from the culprit lesion.

3, Volume of the lipid rich plaque needs to be measured and compared in the two

groups.

Response: The volume of the lipid rich plaque between the two groups had been analyzed. The result showed that the volume of the lipid rich plaque in patients with PMI was larger than those without PMI. the result was shown in table 3 and was indicated with yellow highlighted.

4. Lesion length would be another important predictor for PMI, could you check t?

Response: We investigated the lesion length between the two groups. The result indicated that there was no association between lesions and PMI. This has been described in the Table 2 and indicated with yellow highlighted text.

Minor comments

1, Panel A of Figure 1 seems like in-stent calcification, not a homogeneous. Please find the better image.

Response: We thank the reviewer for pointing out this issue. According to the reviewer's suggestion, we found a more representative homogeneous neointima image and replaced it.

2, The diagnosis of lipid rich plaque, in-stent neoatherosclerosis, and cholesterol crystals were somewhat overlapped and it would be almost impossible to differentiate these plaque types.

Response: We agree with the reviewer's opinion. In fact, it was difficult to

distinguish the lipid rich plaque and in-stent neoatherosclerosis. Sometimes, they coexisted in the same patient. What's more, in-stent neoatherosclerosis included the neointimal calcification. However, it may be not difficult to distinguish the lipid rich plaque, in-stent neoatherosclerosis and cholesterol crystals. The cholesterol crystals were defined as thin, high signal intensity, linear regions that do not present confluent punctuate regions.

Reviewer F

Critique: Assuming the OCT and interpretations are accurate, indicated the types of coronary stent lesions most likely to lead to PMI. However several questions need to be answered:

a. What is the correlation between the OCT images and the actual histopathology of the types of in-stent stenosis?

Response: Previous study (doi: 10.1093/ehjci/jet162) investigated the correlation between the OCT images and the histopathology of in-stent stenosis in swine. The researchers compare different OCT image characteristics with different in-stent stenosis neointimal tissue types analyzed by histology. They found the neointimal was thicker in heterogeneous and layered pattern compared with the homogeneous pattern. And the fibrin deposits were more commonly seen in the heterogeneous pattern. Peri-strut inflammation was most frequently found in the layered pattern compared with homogeneous and heterogeneous ones.

b. What is the sensitivity, specificity and predictive value of finding actual "plaque rupture" and "plaque microvessels" by OCT? Although many references cited purporting to identify these structures with in vivo imaging techniques (including IVUS), none seem to document pathology verification. In the absence of such verification, not clear that the current study adds much to our understanding of the pathology of stent stenosis and PMI.

Response: Previous study (doi: 10.3390/bioengineering9110648) indicated that the sensitivity of plaque microvessels identified automatically by OCT was 99.5 ± 0.3%, specificity was 98.8 ± 1.0%, and accuracy was 99.1 ± 0.5%. While it would be hard to identify the structure when using manual method. But We believe that with the emergence of new detection technologies, the detection accuracy of plaque microvessels and plaque rupture will definitely be achieved.

c. What is the minimal size microvessel that can be seen by OCT and how determined that this is not part of a necrotic core with loose, lipid material, or a possible intramural hematoma? Of note, some of the references cited refer instead to "micro-channels" again don't seem to have clear pathology correlation.

Response: The lateral resolution of OCT ranges from 20 to 90 μm (see doi: 10.1016/j.jcin.2009.06.019). Therefore, microvessels size greater than or equal to 20 μm can be detected by OCT theoretically. We defined microvessels as non-signal tubulo luminal structures without a connection to the vessel lumen recognized on more than three consecutive cross-sectional OCT images. We think the necrotic core

always accompany with plaque rupture. Hence, we analyzed the consecutive cross-sectional OCT images. When no plaque rupture was detected, the tubulo luminal structures were defined as microvessels then.

d. What is the inter-observer variability in determining features seen by OCT? Who is "an experienced physicians (L.Z.J)" an author (did not see listed).

Response: We thank the reviewer for pointing out this issue. Indeed, the OCT images in our study were mainly analyzed by an experienced physician (Zhijiang Liu). The other veteran observer would provide the final decision only when doctor Zhijiang Liu was not sure about the OCT images analyzed. Therefore, we could not provide inter- and intra-observer variability.

e. Can the OCT technique (putting a catheter into a small stent) itself disrupt the plaque causing plaque rupture?

Response: We thank the reviewer for pointing out this issue. We agree with this view. In our study, 82 (58.6%) patients had lipid-rich plaque, we also think the procedural process may disrupt the lipid-rich plaque causing plaque rupture.

f. Many of these questions could be answered by animal experimentation or ex-vivo studies of human stented arteries, with clear correlation with pathology. If this has been done, then it should be referenced with the above questions explained. If not then speculation needs to be admitted in these interpretations.

Response: Previous animal experimentation had compared different OCT morphological characteristics with different in-stent neointimal tissue in swine model of restenosis (doi: 10.1093/ehjci/jet162). The researchers found that fibrous connective tissue was mainly deposited in the homogeneous neointimal, while fibrin was principally deposited in the heterogeneous pattern.

g. Does the size of the lesion (degree of stenosis) have an effect on PMI incidence? One can imaging that the tighter the stenosis, the more likely such instrumentation is likely to dislodge in-stent material with downstream embolization causing PMI.

Response: We thank the reviewer for pointing out this issue. In my opinion, it seemed reasonable that the higher degree of stenosis, the higher incidence of PMI. But in our analysis, there was no correlation between degree of restenosis and PMI. The possible reason may be the limited sample size.

h. Although statistical significance of finding the in-stent lesions with microvessels or plaque rupture being more associated with PMI, there is much overlap in the groups in this regard, and not clear what the clinical ramifications of such findings are.

Response: In this study, we found microvessels and in-stent plaque rupture were associated with PMI. But there may be a long way that these findings were used in clinical.

i. Is there a rationale for why lesions with microvessels (assuming they are such) should be more likely to produce PMI?

Response: Previous study showed that microvessels density was associated with coronary plaque progression. The microvessels was associated with the incidence of TCFA and higher high-sensitivity C-reactive protein levels. Therefore, the higher density of microvessels means the instability of the plaque. The procedural process (catheter or guide wire) may disrupt the instable plaque causing the occurrence of PMI.

Additional comments: The OCT images presented are of low resolution and appear to contain variable imaging artifacts (e.g. radial lines projecting outward). Does this affect the interpretation? Hard for this reviewer to see what's clearly present/absent (e,g, minimal heterogenity in fig 2a?).

Response: We are sorry to that the resolution of the OCT images was too low. We have replaced it with a high-resolution image. In fact, the radial lines projecting outward doesn’t affect the interpretation.