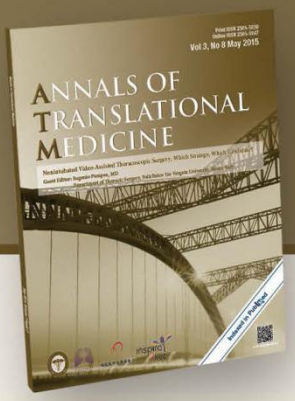
ANNALS OF TRANSLATIONAL MEDICINE



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Comment 1: **********
Reply 1: **********
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Peer Review File

Article information: http://dx.doi.org/10.21037/atm-20-2337

Reviewer A

1. The text of the manuscript should be corrected by a native English speaker.
Reply 1: We regret there were problems with English. The paper has been carefully revised by a professional language editing service to improve the grammar and readability.
Changes in text: We checked all through the paper and modifications are from line 21 to line 748.
.
2. More detailed description of the statistical analysis should be introduced into the text of manuscript.
Reply 2: The reviewer is right. We added detailed description of the statistical analysis in methods and figure legends.
Changes in the text: We have modified our text as advised, see Page 13, line 267-271; Page 12, line 256-259; Page15, line 289; Page 32, line 696; Page 38, line 747. Supplementary, line 5.
3. Statistical significance (p-value) should be provided in the Table 1, and marked in the Figure 4, and Figure S1.
Reply 3: Thanks for reviewer's suggestion. We have added p-value in the Table 1 (Page 14, line 285), and marked in the Figure 4 (Page 38, line 743) and Figure S1(supplementary, line 3). We also made modification in methods description and figure legends.
Changes in the text: We have added p-value in the Table 1 (Page 14, line 285), and marked in the Figure 4 (Page 38, line 743) and Figure S1(supplementary, line 3). We also made modification in methods description and figure legends, see Page 13, line 267-271; Page 12, line 256-259; Page 15, line 289; Page 32, line 696; Page 38, line 747. Supplementary, line 4-8.

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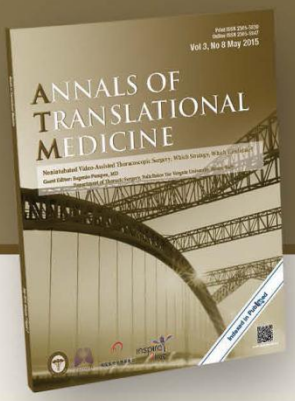

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4. ROC curve analysis should be performed to assess the diagnostic value of 18 exosome miRNAs.
Reply 4: We are extremely grateful to Reviewer A for pointing out this problem. We have performed ROC curve analysis to asses the diagnostic value of the 18 exosomal miRNAs. The AUC of the healthy group was 0.93, 0.75 for the CAD group, and 0.87 for the AMI group indicating that the 18 biomarkers can determine healthy individuals and AMI patients with high accuracy, but not adaptive for predicting CAD patients. The sensitivity of the prediction was 86% for the healthy group and 85% for the AMI group. The specificity of the prediction was 85% for the healthy controls and 75% for the AMI patients. See Figure 3C in Page 36, line725.
Changes in text: We added some description in methods, results and figure legends. See Page 2, line 38-40; Page 13, line 262-265; Page 21, line 424-431; Page 23, line 479-482; Page 37, line 736-739.
5. Data obtained from RNA sequencing for individual miRNA should be validated using RT-qPCR method.
Reply 5: The reviewer's suggestion is right. Quantification of miRNAs in Small RNA sequencing is technically not so accurate because in the process of library construction small RNAs are not fragmented, and the same miRNAs will amplify with bias in the PCR. Therefore, we need RT-qPCR to validate the result of quantification of individual miRNA. However, in this research, we used unique molecular identifier ( UMI ) to overcome the over amplification problem and the quantification of our study is accurate. we also tried qPCR using TaqMan™ Small RNA Assays (Thermo Fisher). Because small RNA is very short, we used to design one specific primer and the one was general primer which leading to the specificity of PCR is very bad. In our RT-qPCR experiment, we found two or more false priming band in the PCR product. We think RT-qPCR is not so practical for our study. But in the future, we may use better solutions to substitute qPCR, like microfluid digital qPCR to solve this problem. However, it's a pity that we don't have the
resources now.
Changes in the text: We also discussed this point in discussion part. See Page 26, line 533-547.

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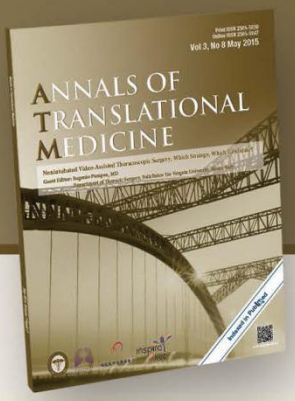

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Reviewer B

This manuscript suggests that Exosomal miRNAs As Potential Diagnostic Biomarkers For Acute Myocardial Infarction Using Next-Generation Sequencing, and revealed 18 exosome miRNAs that may be promising and effective candidates in the development of highly sensitive, noninvasive biomarkers for early AMI diagnosis. There are some issues that need to be addressed during the revision process to make the paper accepted for publication.

1. The author needs to check the proteins which are not expressed in exosomes to confirm the purity of the exosomes.
Reply 1: Thanks for reviewer B' suggestion. We checked one protein named Calnexin , which is an endoplasmic reticular protein, was only detected in cell lysates but not in exosomes by western blot experiment , verifying that the high purity of the isolated exosomes without contamination of high abundance intracellular contents. See Figure 1B, in Page 32, line 686. We also adjusted in the text.
Changes in the text: We renewed Figure 1B (Page 32, line 686) and Figure legends, added methods and results. Page 8, line 161-173; Page 15, line 291-300; Page 32-33, line 689-693.
2. The TEM and exosome-specific marker of exosomes should be provided.
Reply 2: The reviewer is right. We performed TEM to the exosomes, see Figure 1A in Page 32, line 686. We also checked two exosome-specific markers, TSG101 and CD9, with positive band in the western blot experiment, see Figure 1B in Page 32, line 686.
Changes in the text: We renewed Figure 1A and 1B (Page 32, line 686) and Figure legends, added methods and results. Page 8, line 153-160. Page 15, line 291-293. Page 32, line 687-689.
3. What are the diagnostic criteria for CAD and AMI, and the inclusion and exclusion criteria for the research subjects?

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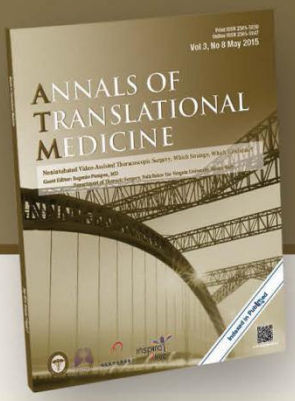

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Reply 3: The diagnostic criteria for AMI: AMI, including STEMI or NSTEMI, was diagnosed according to the (ESC/ACC/AHA/WHF) Fourth Universal Definition of Myocardial Infarction (2018). there is acute myocardial injury with clinical evidence of acute myocardial ischemia and with detection of a rise and/or fall of cTn values with at least 1 value above the 99th percentile URL and at least 1 of the following: Symptoms of myocardial ischemia; New ischemic ECG changes; Development of pathological Q waves; Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology.
The diagnostic criteria for CAD:
CAD, also referred as coronary heart disease, is the term associated with an inadequate supply of blood to the myocardium due to obstruction of coronary arteries, usually from atherosclerosis. The diagnosis is based on clinical characteristics such as angina, chest discomfort, dyspnea on exertion combined with ECG, cardiac image and angiography evidence, with negative detection of biomarker for myocardial injury or infarction.
In the paper, AMI, including STEMI or NSTEMI, was diagnosed according to the (ESC/ACC/AHA/WHF) Fourth Universal Definition of Myocardial Infarction (2018).CAD, was diagnosed according 2014 ACC/AHA/AATS/PCNA/SCAI/STS Guideline for the Diagnosis and Management of Patients With Stable Ischemic Heart Disease. For AMI group we included 55 patients admitted into the chest pain center of Union Hospital from (2019.1-2019.6). For CAD group we included 26 patients requiring CABG surgery admitted in Department of Cardiovascular Surgery of Union Hospital from (2019.1-2019.6). For health control group, we collected 37 health control from medical examination center who have no cardiovascular disease history. Patients associated with valvular disease, cardiogenic shock, myocarditis, severe infections or renal failure were excluded from the study.
Changes in the text: Page 5-6, line 95-123.

4. When were AMI patient's blood collected after myocardial infarction

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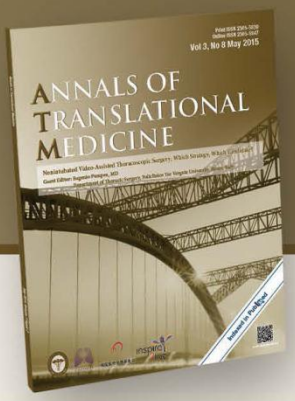

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Reply 4: It takes 2-8 hours for most patients to go to the chest pain center from onset of symptoms. At the chest pain center, after laboratory tests, examinations and initial treatment, patients diagnosed with acute myocardial infarction were admitted to hospital and the blood sample were collected and plasma were prepared. Patients in the hospital are rechecked myocardial enzymes such as cTNI every day to dynamically observe the development of the disease. We will check day 2 and day 3 TNI and other tests and examinations to make sure correct diagnosis and excluded some cases not meeting the inclusion criteria. After included in the study, the plasma was used in the study.
Changes in the text: Page 6, line 113-123.

5. The results should be identified among larger samples, for example, the expression levels of exosomal miRNAs among CAD, AMI, and healthy groups.

Reply 5: The reviewer is right. This Study was a pretrial of a larger cohort. In the next, we may plan to perform larger samples study. But patients with CAD or AMI in this season due to COVID-19 are really few. And the cooperation hospital is located in Wuhan, so we are limited for the larger sample resources until now, possibly till the end of the year. This is the reason why we decided to publish this paper with smaller cohort first. Next step, we will use a new technology to move on to the next larger cohort study.
Changes in the text: Page 26-27, line 547-551.

6. Exosomal miRNAs as biomarkers, AUC area, specificity, sensitivity should be detected.

Reply 6: Thanks for the reviewer's comment. We have performed ROC curve analysis to asses the diagnostic value of the 18 exosomal miRNAs. The AUC of the healthy group was 0.93, 0.75 for the CAD group, and 0.87 for the AMI group indicating that the 18 biomarkers can determine healthy individuals and AMI patients with high accuracy, but not adaptive for predicting CAD patients. The sensitivity of the prediction was 86% for the healthy group and 85% for the AMI group. The specificity of the prediction was 85% for the healthy controls and 75% for the AMI patients. See Figure 3C in Page 36, line725.

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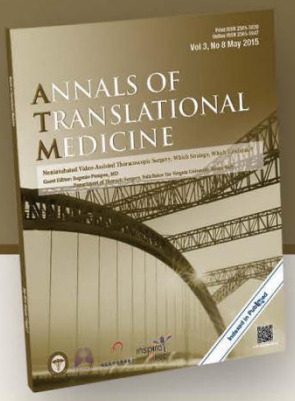

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Changes in text: We added some description in methods, results and figure legends. See Page 2, line 38-40; Page 13, line 262-265; Page 21, line 424-431; Page 23, line 479-482; Page 37, line 736-739.

7. Limitations of this study should be discussed in Discussion part.

Reply 7: Thanks for the reviewer's advice. There are several limitations of this study, for example, there are four low abundance miRNAs of the 18 biomarker miRNAs, may not be consistently detected in the large cohort tests due to differences in sequencing depth; qPCR experiments are not performed to validate the quantification of the 18 miRNAs expression; the cohort is a little small; the cost of next generation sequencing is high. We discussed these points in the discussion part.

Changes in the text: Page 24, line 485-493; Page 26-27, line 533-554.

8. The manuscript still contains some sentences that are not grammatically correct.

Reply 8: We regret there were problems with English. The paper has been carefully revised by a professional language editing service to improve the grammar and readability.

Changes in the text: We checked all through the paper and modifications are from line 21 to line 748.