Peer Review File

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Reviewer Comments

Comment 1: By a multivariate analysis, authors identified that hormonal receptor (HR) status significantly associated with pathological complete response (pCR) rate. However, in the study, patient who received AC-THP regimen harbored more HR-positive breast cancer than those with TCbHP. And authors identified that the pCR rate was higher with TCbHP than AC-THP regimen. The difference of the pCR rate would be biased by the difference of HR status between the two group?

Reply 1: Thank you for this suggestion. Your suggestion is very helpful to the improvement of our article. In the subgroup analysis of our study, the results of the univariate analysis shown that the HR status had an impact on the overall pCR rate of the early stage HER2-positive breast cancer patients receiving dual HER2 blockade containing NACT (Table 5). The HR-negative breast cancer had a higher pCR rate in both the chemotherapy regimens with and without anthracyclines in the presence of dual HER2 blockade. The subgroup analysis of the TRYPHAENA and TRAIN-2 trials had the same conclusions (PMID: 30413379, PMID: 23704196). Therefore, the difference of the pCR rate in AC-THP regimen and TCbHP regimen in our study might be biased by the difference of HR status between the two regimens. But in the studies of TRYPHAENA, the neoCARH trial and Xiangmin Ma et al. (PMID: 30413379, PMID: 33959195, PMID: 36221359), the pCR rate of TCbHP regimen was higher than that of AC-THP regimen, which was the same as our conclusion. We have added the possible influence of HR status difference on PCR rate to the **DISCUSSION** section.

Changes in the text:

Page10 line21-25

The baseline of HR status in the AC-THP group and TCbHP group was not balanced, therefore, the difference of the pCR rate in AC-THP regimen and TCbHP regimen in our study might be biased by the difference of HR status between the two groups. But in the studies of TRYPHAENA and neoCARH trial, the pCR rate of TCbHP regimen was higher than that of AC-THP regimen, which was the same as our conclusion[13,14].

Page12 line13-19

Secondly, patients who received AC-THP regimen harbored more HR-positive breast cancer than those with TCbHP regimen in our study, and the difference of the pCR rate would be biased by the difference of HR status between the two regimens. However, as mentioned above, several results in previous studies support our

conclusion. We need to add more cases in the future before we draw the final conclusion.

Comment 2: Given the nature of retrospective study, choice of chemotherapy regimen should also be biased. Authors need to imply and address these possibilities.
Reply 2: Thank you for this suggestion. In the present study, clinicians chose NACT regimens for patients according to the NCCN guidelines of each year (https://nccn.medlive.cn/index). From 2019 to 2020, the AC-THP and TCbHP regimens were recommended by the NCCN guidelines with the same priority, both of which were recommended as the preferred regimen. From 2021, as the cardiotoxicity of AC-THP regimen was reported more and more, the priority of the AC-THP regimen was degraded from the preferred regimen to the "useful in certain circumstance" regimen.

However, according to the baseline analysis in **Figure 1** in the manuscript, breast cancer patients in the two chemotherapy regiments were relatively balanced at baseline except for differences in HR status. But as shown above, in the NCCN guideline of breast cancer, hormone receptor status is not an indicator for the clinicians to choose the NACT regimen for the Her2+ breast cancer.
Changes in the text:
Page12 line18-20
In addition, as the nature of a retrospective study, the choice of chemotherapy regimen might be biased, the RCT needs to be conducted in the future to exclude this issue.

Comment 3: Also, multivariate analysis in Table 5 should also include the choice of regimen (TCbHP vs. AC-THP) to demonstrate that the TCbHP regiment independently associated with better pCR rate.
Reply 3: Thank you for this suggestion. We have added " regimen " comparison to the multivariate analysis in **Table 5**. And we added the description of "regimen" in **Results** section.
Changes in the text:
Page8 line7-14
The univariate analysis of the relationship between these characteristics and pCR rate showed that ER status (P = 0.011, OR: 0.437, 95% CI: 0.231-0.829), PR status (P = 0.001, OR: 0.309, 95% CI: 0.157-0.608), and IHC HER2 status (P = 0.001, OR: 7.167, 95% CI: 1.970-26.076) and regimen (P = 0.002, OR: 0.338, 95% CI: 0.168-0.681) were significantly correlated with pCR rate. In the multivariate analysis, PR status (P = 0.016, OR: 0.297, 95% CI: 0.110-0.799), IHC HER2 status (P = 0.011, OR: 6.130, 95% CI: 1.516-24.793), and T classification (P = 0.027, OR: 0.316, 95% CI: 0.114-0.877) and regimen (P = 0.037, OR: 2.293, 95% CI: 1.053-4.994) were

independent predictors of pCR rate.
Page18 line3-4
Table 5

Comment 4: The pCR rate with AC-THP regimen was relatively lower than that previously reported in other real-world data (Spring L et al. Breast Cancer Res Treat. 2018 Dec;172(3):733-740.; González-Santiago S et al. Breast Cancer Res Treat. 2020 Nov;184(2):469-479.; Ma X et al. Medicine. 2022 Oct 7;101(40):e30892.). Authors are required to clarify possibilities explaining why the pCR was relatively lower with AC-THP regimen in the present study.

Reply 4: Thank you for this suggestion. We have reviewed the three articles you mentioned, and tried our best to reply to your question based on the comparison of research contents.

In the study of Spring L et al. (Spring L et al. Breast Cancer Res Treat. 2018 Dec;172(3):733-740.), the clinical stage of patient in AC - THP group was: I: 4.4%, II: 82.2%, III: 13.3%. In our study, the clinical stages of AC-THP group were: I: 2.1%, II: 47.9%, III: 50.0%. Considering that clinical stage is an important correlation factor for pCR rate, therefore, the earlier clinical stage in the first article might be one of the reasons for the higher pCR rate compared to our study. In the second paper (González-Santiago S et al. Breast Cancer Res Treat. 2020 Nov), the authors divided the treatment into anthracycline/taxane-based chemotherapy regimen, single-agent taxane regimen and platinum-based combinations regimen，and the pCR rates of the three groups of patients were also given. AC-THP accounted for only 32.1% of anthracycline/taxane-based chemotherapy regimen. Therefore, the exact pCR rate of the AC-THP protocol was not given in this paper. The third article has a relatively balanced baseline (Ma X et al. Medicine. 2022 Oct 7;101(40):e30892.). However, the rate of ER-negative breast cancer in AC-THP group in this study was 50.8%, which was higher than that in our study (41.7%)，and ER-negative patients were more likely to achieve pCR. Therefore, the differences in ER status at baseline may result in higher pCR rates compared to our study. The pCR rates of platinum-based regimens and anthracycline regimens in these three papers were 63% and 60%, 48.6% and 71%, 73.1% and 65.1%, respectively. The PCR rates of the platinum-based regimen in the first and third articles were higher than those of the anthracycline regimen, similar to our conclusions, while the conclusions in the second article were opposite. Considering that these three articles are both retrospective studies and our study, they tend to be descriptive, and the number of patients receiving different protocols is not balanced, so there may be differences in data due to sample size and other possible

reasons.

Comment 5: Also, authors need to describe novelties compared with these previous reports.

Reply 5: Thank you for this suggestion. Cardiotoxicity is an important indicator to evaluate the safety of HER2+ NACT. Electrocardiogram (ECG), which reflects whether the electrophysiological activity of the heart muscle is normal, is a routine item for chemotherapy patients every cycle. Therefore, one of the innovations of our paper is to include abnormal ECG events occurring during chemotherapy cycles in the comparison of cardiotoxicity. The previous studies generally focused on the PCR rate and adverse reactions, our article also focused on and analyzed the factors affecting the pCR rate, especially the imaging aspects. We summarized and sorted out the MRI image information of breast cancer patients to study the correlation between post-NACT MRI image features and pCR under the condition that the differences in chemotherapy regimens were minimized.

Comment 6: Authors evaluated MRI imaging characteristics which might potentially predict pCR. Is it clinically imperative? Even if MRI imaging indicates potential pCR, primary tumor still needs to be surgically resected for actual pathological evaluation as well as to achieve cure, correct? Authors need to clarify why the prediction of pCR by MRI imaging is clinically important.

Reply 6: Thank you for this suggestion. MRI is required for initial diagnosed and preoperative breast cancer patients, especially for patients who need breast preservation. In the clinical practice, primary tumor needs to be surgically resected together with other surgical performance such as axillary lymph nodes evaluation. And pathological evaluation was performed for the resected primary tumor and the axillary lymph nodes to decide whether pCR was reached, which is essential for deciding the postoperative treatment strategy. However, it normally takes about two weeks or longer for the pathological evaluation before the postoperative treatment regimen can be decided. Therefore, if a reliable pCR prediction model can be established to know whether patients can achieve pCR before we get the pathological results, patients can be given appropriate treatment in time. Therefore, we attempted to conduct a preliminary exploration by exploring the relationship between some imaging features on MRI images and pCR.

Changes in the text:

Page4 line16-25

In the clinical practice, primary tumor needs to be surgically resected together with other surgical performance such as axillary lymph nodes evaluation. And pathological evaluation was performed for the resected primary tumor and the axillary lymph nodes to decide whether pCR was reached, which is essential for deciding the postoperative treatment strategy. However, it normally takes about two weeks or longer for the pathological evaluation before the postoperative treatment regimen can be decided. Therefore, if a reliable pCR prediction model can be established to know whether patients can achieve pCR before we get the pathological results, patients can be given appropriate treatment in time. Therefore, we attempted to conduct a preliminary exploration by exploring the relationship between some imaging features on MRI images and pCR.

Comment 7: -Page 5, line 9: the unit "mg/m2": "2" (square) should be superscript through the manuscript.

Reply 7: Thank you for this suggestion. We have revised the English grammar/ expressions in the manuscript.

Changes in the text:

Page5 line16-21

Patients in the AC-THP group were given four cycles of doxorubicin (60 mg/ m^2) and cyclophosphamide (600 mg/m^2) intravenously, followed by four cycles of docetaxel (100 mg/ m^2) or nab-paclitaxel (260 mg/ m^2), and trastuzumab plus pertuzumab every 3 weeks. Patients in the TCbHP group were treated with docetaxel (75 mg/ m^2) or nab-paclitaxel (260 mg/ m^2), carboplatin (area under the concentration-time curve = 6 mg/mL/min) given every 3 weeks for six cycles concurrently with trastuzumab and pertuzumab.

Comment 8: -Page 6, line 7: Please describe details about how to evaluate ECG abnormalities: assessed manually by a trained cardiologist among co-authors? Evaluation automatically by ECG machine? If so, please describe what ECG machine was used (e.g. company, product identification number etc...).

Reply 8: Thank you for this suggestion. The ECG abnormalities were manually evaluated by a professional cardiologist. This doctor is also one of co-authors of our article（Han Yan，Department of Cardiac Function, Renmin Hospital of Wuhan University, Wuhan, Hubei, China. We have included the description of how to evaluate ECG abnormalities.

Changes in the text:

Page6 line17-18
The ECG abnormalities were manually evaluated by a professional cardiologist.

Comment 9: -Page 6, line 10: Authors need to clarify how to evaluate characteristics of MRI imagings: Evaluated by a trained radiologist among co-authors? Authors also need to clarify reference(s) describing the methods authors used to characterize MRI imaging.
Reply 9: Thank you for this suggestion. MRI imaging features were evaluated by two experienced breast radiologists. References describing MRI image features have been added to the article (PMID: 33754824).
Changes in the text:
Page6 line21-22
MRI imaging features were evaluated by two experienced breast radiologists. The description of MRI features referred to the study of Soo-Yeon et al[20].

Comment 10: -Page 8, line 3: What do these percentage indicate? LVEF? The sentence needs to be organized.
Reply 10: Thank you for this suggestion. The mean LVEF of patients at baseline, 6 months, and 12 months were 63.4%, 61.2%, 61.0% in the AC-THP group and 62.9%, 60.6%, 61.2% in the TCbHP group. We have made revision in the original text.
Changes in the text:
Page8 line17-19
The mean LVEF of patients at baseline, 6 months, and 12 months were 63.4%, 61.2%, 61.0% in the AC-THP group and 62.9%, 60.6%, 61.2% in the TCbHP group.

Comment 11: -Page 8 line 14: “Deviation” and “Wave”: do not have to use capitals.
Reply 11: Thank you for this suggestion. We have revised the original text.
Changes in the text:
Page8 line27, Page9 line1
Sinus tachycardia (33.0%), T-wave inversion (38.3%) and ST deviation with T-wave change (20.0%) were the most common ECG abnormalities observed in the two groups.

Comment 12: -Page 8 line 18: the last “cycle” of chemotherapy, not “stage”?
Reply 12: Thank you for this suggestion. We have revised the original text.
Changes in the text:
Page9 line4-6

A total of 88 patients with post-NACT MRI image data after the last stage circle of chemotherapy and before surgery were collected, including 36 patients of AC-THP group and 52 patients of TCbHP group.

Comment 13: -Page 8 line 21: This sentence needs to be revised/organized.
Reply 13: Thank you for this suggestion. We have revised the original text.
Changes in the text:
Page9 line6-10
The relationship between pathological results and clinical characteristics, post-NACT MRI images characteristics was explored in Table 7. The results showed that, in addition to ER status, PR status, IHC HER2 status and NACT regimens, mass features and enhancement type at post-NACT MRI were also independent predictors of pCR.

Comment 14: -Figure 1: Left bottom square: Is this "88 women with baseline and post-NACT MRI"? The description seems to be same as middle square.
Reply 14: Thank you for this suggestion. We apologize for the error in the **Figure 1**. We have changed the description "88 women with clinical baseline collection" to "88 women with baseline and post-NACT MRI". Please see the newly uploaded **Figure 1**.

Comment 15: -Figure 2: Were these P values examined by paired test such as Wilcoxon signed rank test? If so please indicate so either in methods or figure legend.
Reply 15: Thank you for this suggestion. The P values in Figure 2 were obtained by comparing the baseline LVEF with the 6 - and 12-month LVEF using Welch's t test. We have added this to the legend of Figure 2.
Changes in the text:
Page22 line9-11
Figure 2: Mean left ventricular ejection fraction per month for one year over time from initiation of HER2 blockade drugs. AC-THP group **(a)**, TCbHP group **(b)**. The LVEF at baseline was compared with LVEF at 6- and 12- month using Welch's t test.

Comment 16: -Figure 2: Probably authors do not have to show LVEFs other time points than baseline, 6 months, and 12 months since less numbers of patients evaluated LVEFs in interval time points. Figure 2 would be changed to dots and line figures like below.
Reply 16: Thank you for this suggestion. We assume that you were intended to send us a dots and lines figure example, but we did not receive the appendix dots and lines figure example. We consider that the line graph can make comprehensive use of the

collected data and can show the change of LVEF over time. If you have more information for us in the future, we will be glad to refer to your comments.

Comment 17: -Table 3: BERNICE study (Swain SM et al. Ann Oncol. 2018 Mar 1;29(3):646-653.) also evaluated efficacies of NACT with anthracycline containing regimen, would be better to include in the table.

Reply 17: Thank you for this suggestion. We have added the regimens used in BERNICE study to **Table 3**.

Changes in the text:

Page8 line1-3

Besides, the pCR rates and the regimen of each group of the clinical trials TRYPHAENA, GeparSepto, and TRAIN-2 and BERNICE were summarized in Table 3[12, 13, 21, 22].

Table 3.

Comment 18:

Some suggestions / comments:

P2 Line 15: maybe it is better to keep as “pCR”

P3 Line 25: “is another option”? (please review)

P4 Line 3: add “s” to “guidelines”

P4 Line 14: “they might have ignored”?

P4 Line 24: Add a space before “Inclusion”

P6 Line 5: “LVEF (left ventricular ejection fraction)” => “left ventricular ejection fraction (LVEF)”

P6 Line 17: “to finished” => “to finish”

P8 Line 21-24: Please review again as there are some repetitions

Reply 17: Thank you for these suggestions. We have revised the problems you pointed out in the original text using the revision mode, please review our newly submitted manuscript.