Peer Review File

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Reviewer A

Nakajima and colleagues analyzed the concordance of Ki67-LI in core needle biopsies and related surgical excisions, and found that adding information on the PET/CT SUVmax of the tumor tissue in situ could be helpful in estimating proliferative activity in core needle biopsies of breast cancers.

The paper is well written and deals with an interesting topic. In my view, there are just several minor issues that should be considered by the authors.

1. Different assessment methods were used for CNB (digital image analysis) and SRS (visual assessment of 500 cells by one pathologist). Although there was no difference in achieved concordance between CNB and SRS compared to other studies, this point should at least be discussed as a potential cause of discordance.

Reply 1:

We appreciate the reviewer's helpful comment. As stated in the penultimate paragraph of the "Discussion," recent studies reported that the accordance between Ki67LI obtained by digital image analysis and that by visual assessment; however, we cannot exclude the possibility that the difference in analysis method affected the Ki67LI in the present study. We added some descriptions and edited the penultimate paragraph of the "Discussion" as follows.

Changes in the text:

In addition, two methods were used to calculate the Ki67LI in CNBS and SRS, i.e., image analysis software, and visual evaluation, respectively. Although recent studies reported an almost perfect correlation between the two methods [29, 30], we cannot exclude that some of the observed discrepancies might be due to different measurement methods. Hence, larger numbers of patients will be needed to confirm our results. (see Page 18, line 2-6)

2. Maybe the authors could have an eye on the wording in the statistical analysis in the Methods section? I'm not a statistician, but to my knowledge, the Mann Whitney test

compares medians and is not a measure of true correlation, and the Chi² test analyzes associations between variables. And I couldn't find the description for the (r) correlation between CNB and SRS here which is reported in the first section of the Results.

Reply 2:

We appreciate the reviewer's helpful comment. As suggested, we did not describe the statistical methods used in the data demonstrated in Figure 1. We used a Spearman's rank correlation coefficient for the analysis. We have added the following description about the statistical methods in "Statistical analysis" in "Materials and methods." A statistically significance was observed by the analysis, we edited the description in "Comparison of Ki67LI between CNBS and SRS" in the "Results."

Changes in the text:

Materials and methods

A correlation between Ki67LI evaluated in CNB and SRS was analyzed by Spearman's rank correlation coefficient. (see Page 10, line 4-6)

Thus, there was no difference in Ki67LI distribution between the two types of specimens ($p = 0.13$), and a significantly positive correlation was observed between the two indexes (Fig. 1, coefficient: $|r| = 0.76$, $p < 0.01$). (see Page 12, line 5-6)

3. The authors report that in the discordant groups, the SUVmax differed significantly from those in concordant groups. Can the authors imagine and discuss a potential underlying cause (e.g. tumor heterogeneity)?

Reply 3:

We thank the reviewer for the insightful comment. As the reviewer suggested, we thought a possibility that SUVmax reflected the heterogeneity of the tumor. We speculated that the heterogeneity in the distribution of proliferating cells in the tumor could be low both in highly proliferating tumors with high Ki67LI and high SUVmax and slowly proliferating tumors with low Ki67LI and SUVmax. We added a discussion on this in paragraph 3 of the "Discussion" as follows.

Changes in the text:

These findings suggested a possibility that the heterogeneity in the distribution of proliferating cells in the tumor could be low in highly proliferating tumors with high Ki67LI and high SUVmax and slowly proliferating tumors with low Ki67LI and

SUVmax. (see Page 16, line 17-18, Page 17, line 1-2)

4. Discussion: It would be helpful for the reader to understand the main findings, if the authors could go a bit deeper into detail on how the addition of the SUVmax could be used to refine proliferation estimation in CNB.

Reply 4:

We appreciate the reviewer for the helpful comment. In the first paragraph of the "Discussion," we added advantage of evaluation by combining Ki67LI in CNBS and SUVmax as follows.

Changes in the text:

In the present study, we demonstrated that Ki67LI obtained by CNBS could be more reliable when combined with the SUVmax value of tumors obtained by FDG PET/CT. (see Page 15, line 10-12)

5. Table 1: I recommend using the WHO classification of breast tumors for the histological tumor types as this makes the data more comprehensive to a broader readership.

Reply 5:

We appreciate the reviewer for constructive comments. We have reclassified histological tumor types based on WHO classification. We described this in the paragraph on "Patients and tissues" in the "Materials and methods" and revised Table 1.

Changes in the text:

Histological tumor types of breast cancer were classified according to the WHO classification of tumors (5^{th} edition)[24]. (see Page 7, line 13-14)

Reviewer B

This study proposed the use of SUVmax obtained from pretreatment PET/CT in combination with Ki67LI to reduce discordance in Ki67 assesment in CNBSs and SRSs.

Major observations

First of all, considering the aim of the study, title should be modified, including the use of PET/CT.

Reply: We appreciate the reviewer for an insightful comment. We have reconsidered the title of the manuscript and have edited the Background in the “Abstract” as follows.
New title of the manuscript:
Concomitant use of 18F-FDG PET-CT SUVmax is useful in the assessment of Ki 67 labeling index in core-needle biopsy specimens of breast cancer (see Page 2, line 2-4)

Here, we aimed to compare the Ki67 labeling index (Ki67LI) between core-needle biopsy specimens (CNBSs) and surgically resected specimens (SRSs) of invasive breast cancer, and verify whether the discordance in Ki67LI can be reduced by analyzing the SUVmax obtained from pretreatment PET/CT in combination with Ki67LI. (see Page 2, line 6-8)

Second: these results, even if interesting, imply the use of PET/CT in all cases, however its indication is not mandatory in early breast cancers. In addition PET/CT is not reliable in some cases such as lobular carcinomas, with a number of false negative results.
Reply: We appreciate the reviewer for insightful questions. As the reviewer pointed out, PET/CT is not necessary in preoperative diagnosis of early breast cancer. However, we think that PET/CT-mammography (mammo-PET/CT) is going to spread in the near future, and it is anticipated that comparative analysis with the histopathological diagnosis will become necessary. Thus, concomitant evaluation of PET/CT-mammography and Ki67LI could provide more useful information. We have added these considerations in the "Discussion" as follows.
Changes in the text:
Although PET/CT is not necessary in preoperative diagnosis of early breast cancer at present, PET/CT-mammography has been introduced in the diagnosis of breast cancer and concomitant evaluation of PET/CT-mammography and Ki67LI could provide more useful information. Therefore, it is clinically significant to add the information obtained by PET/CT to the preoperative evaluation. (see Page 17, line 2-7)

Third: PET/CT is useful in low and high Ki67 index groups, however in these groups generally the concordance of Ki67 between pathologists is higher than in intermediate group. Please add a comment in the discussion regarding this topic. Moreover, it is necessary to add some considerations regarding molecular assays, recently proposed in

AJCC 2018 edition, that can overcome the problem of Ki67 assessment in luminal breast cases.

Reply: We thank the reviewer for the insightful comment. As the reviewer suggested, we think that intratumor heterogeneity may be one of the causes of the Ki67LI discordance between CNBS and SRS. We speculate that SUVmax reflects the heterogeneity of the tumor and that the heterogeneity in the distribution of proliferating cells in the tumor could be low both in highly proliferating tumors with high Ki67LI and high SUVmax and slowly proliferating tumors with low KI67LI and SUVmax. We added some descriptions of this in paragraph 3 of the "Discussion" as follows.

We heartily appreciate the reviewer for another insightful comment. As the reviewer suggested, molecular profiling is crucial for risk stratification for ER-positive/HER2-negative breast cancer. We have added the following sentences in the "Discussion" and have referred to two references.

Changes in the text:

These findings suggested a possibility that the heterogeneity in the distribution of proliferating cells in the tumor could be low in highly proliferating tumors with high Ki67LI and high SUVmax and slowly proliferating tumors with low Ki67LI and SUVmax. (see Page 16, line 1-2, Page 17, line 1-2)

To optimize patient care and to allow appropriate treatment de-escalation, the eighth edition of the American Joint Committee on Cancer (AJCC) staging system has recommended molecular profiling in T1/T2 tumors without lymph node metastases and ER-positive/HER2-negative status and the following four tools are recommended: Oncotype DX®, Mammaprint®, Endopredict®, and Breast Cancer Index® [27]. However, none of these molecular tests is reimbursed by the national health insurance system in many European countries and Japan. Hence, the attempts to use Ki67LI as partly substitute information obtained by molecular profiling have been reported, since most of the genes evaluated by these molecular profiling assays are related to cell proliferation [28]. From this point of view, we think that it is significant to utilize Ki67LI together with the information obtained from diagnostic imaging. (Page 17, line 8-18)

Minor observations

Abstract

“allowing a tolerance margin of 5%.

This sentence lacks the closed quotation marks.

Reply: We added the closed quotation mark. (see Page 1, line 11)

In the abstract it is not clear the method used: tumors are classified in three groups using Ki67 value, however even SUV was used. Please can you better specify what is the combination?

Reply:

As the reviewer pointed out, we did not describe the SUVmax classification method in the “Materials and methods” as well as the “Abstract,” and we apologize for making it difficult to understand the analysis method due to the insufficient description. As described in the Results, we divided the patients into three groups according to the SUVmax; SUVmax ≤ 4, 4 <SUVmax <8, and SUVmax ≥ 8. We have added the classification by SUVmax in the “Materials and methods” and added some description in the “Abstract” and Results as follows.

Changes in the text:

We divided the Ki67LI values into three groups (Low: 0 ≤ Ki67LI ≤ 10, Intermediate: 10 < Ki67LI < 30, and High: 30 ≤ Ki67LI) and the maximum standardized uptake value (SUVmax) into three groups (SUVmax ≤ 4, 4 <SUVmax <8, and 8 ≤ SUVmax). We then verified the concordance rate between CNBS and SRS in each group in combination with the SUVmax obtained by whole-body PET/CT. (see Page 2, line 11-16)

Introduction

Sentence: However, there have been paradoxical results in the assessment of prognosis and response to neoadjuvant chemotherapy

Comment: this is incorrect. There are not paradoxical results, but KI67 has a different impact considering it as a prognostic or a predictive factor. Please modify this sentence.

Reply:

We appreciate the reviewer for careful reading of our manuscript. We corrected the sentence as follows.

Changes in the text:

On the other hand, Ki67LI shows a different significance in predicting response to neoadjuvant chemotherapy. (see Page 4, line 5-6)

Materials and Methods

Table 1: authors should be classify histotypes even according to new edition of WHO 2020.

Reply:

We appreciate the reviewer for very helpful comment. We have reclassified histological tumor types based on WHO classification. We described this in the paragraph on Patients and tissues in Materials and methods and revised Table 1.

Changes in the text:

Histological tumor types of breast cancer were classified according to the WHO classification of tumors (5^{th} edition)[24]. (see Page 7, line 13-14)

It is not clear why CNBS Ki67LI was calculated using digital image analysis, while SRS Ki67LI was calculated using visual assessment. Doesn't it influence the reproducibility?

Reply:

We appreciate the reviewer's helpful comment. As stated in the penultimate paragraph of the Discussion, recent studies reported that the accordance of Ki67LI between obtained by digital image analysis and that by visual assessment; however, we cannot exclude the possibility that the difference in analysis method affected the Ki67LI in the present study. We added some descriptions and edited the penultimate paragraph of the Discussion as follows.

Changes in the text:

In addition, two methods were used to calculate the Ki67LI in CNBS and SRS, i.e., image analysis software, and visual evaluation, respectively. Although recent studies reported an almost perfect correlation between the two methods [29, 30], we cannot exclude that some of the observed discrepancies might be due to different measurement methods. Hence, larger numbers of patients will be needed to confirm our results. (see Page 16, line 2-6)