Author’s response to reviews

Title: A Retrospective Analysis on Metastatic Rate of the Internal Mammary Lymph Node and Its Clinical Significance in Adjuvant Radiotherapy of Breast Cancer Patients

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Author’s response to reviews:

ZhaoZhi Yang (Reviewer 1):
General comments:
Q: Although from anatomy, the anterior mediastinal region is close to sternum, the authors need to prove that anterior mediastinal node metastasis is not part of extensive multisite metastasis which could come from the blood circulation.
A: The anterior mediastinum is a stenotic region between the sternum, pericardium and the mediastinal pleura. Anatomically, the anterior mediastinal lymph nodes include the parasternal and anterior mediastinum group, while the parasternal group corresponds to the IMNs in breast cancer, so we think that the internal mammary lymphatic chain and the anterior mediastinal lymph node region may be seen as an entirety. The anterior mediastinum, which receives lymphatic drainage from the adjacent pleura, is not a region of high-risk lymph node metastasis even in lung cancer, except when there is pleural involvement or reflux of the enlarged mediastinal lymph node is formed. In this study, we excluded patients with widely lung or pleural metastases, so we think that the anterior mediastinal node maybe connected with the IMNs, but not come from the blood circulation.

Specific revision:
Title:
Q: The title needs to be reorganized because it doesn't accurately depict the content in this paper.
A: The reorganized title may be: A Retrospective Analysis on Metastatic Rate of the Internal Mammary Lymph Node and Its Clinical Significance in Adjuvant Radiotherapy of Breast Cancer Patients

Abstract:
Q: the highlight of this paper is to redefine the IMN metastasis, but in the methods, we couldn't find it.
A: The diagnosis of the internal mammary/anterior mediastinal lymph node metastasis was according to clinical symptoms, signs and imaging modalities (mainly based on contrast-enhanced CT of chest).
The definition of lymph node metastasis include that the internal mammary lymph nodes whose short diameter is more than 6mm, or the lymph node is tightly connected to the internal mammary blood vessels regardless of the short diameter, lymph node who meet one of the above conditions can be seen as metastatic lymph node; and the hypermetabolism in PET-CT also be diagnosed as metastases even the short diameter was less than 6mm or 10mm. Anatomically, the anterior mediastinal lymph nodes include the parasternal and anterior mediastinum group, while the parasternal group corresponds to the IMNs in breast cancer, so we infer that the internal mammary region and the anterior mediastinal region can be seen as an entire region, which we called the extensive internal mammary region in this study.

Method

P 2. Line 32 "first" should be "firstly"
A: P 2. Line 32 "first" had been instead of "firstly".

P 3. Line 16 French trial should be cited
A: P 3. Line 16 the French trial had been cited.

P 3. Line 26 "to explain this contradiction", your study is just the composition rate of the IMN Mets in 114 patients, not the incidence of IMN mets in BC patients.
A: The metastatic rate of the internal mammary lymph node is very low according to some reports, but in our clinical work, we found that the metastasis is not uncommon probably. Based on the phenomenon that we observed, we just try to investigate the natural metastatic rate of the internal mammary lymph node in patients that didn’t undergo adjuvant radiotherapy. In this study, the interval estimation of the population rate was based on approximate normal distribution method when \( nP \) and \( n(1-P) \) were both greater than five, otherwise look-up table method was used, so based on the results, we think that it may can present the incidence of IMNs metastases partly, but this is a retrospective study, we just have found a clinical phenomenon, further prospective work should be done to verify this phenomenon.

P3. Method

When these patients were treated in your center. Whether this study was approved by the ethic committee.
A: These patients were collected from January 2015 to January 2019, but were treated before January 2015, some of them were treated in other hospitals, the others were treated in our hospital. This retrospective study was approved by the ethical approval for Scientific Research Projects under medical ethics committee, Zhongnan Hospital of Wuhan University (approval no. 2017025). Due to the retrospective nature of the study, informed consent was waived.

Results:

P 4. Line 44 Fig (1E-F) this figure demonstrated medial supraclavicular node metastasis. Maybe it is not related to IMN metastasis.
A: P 4. Line 44 Fig 1(E-F) illustrates that the metastatic lymph node that above the upper edge of the first rib, we can also call it medial supraclavicular node, not refer to that it is the internal mammary lymph node. But in our opinion, the internal mammary lymph node is consistent with the medial supraclavicular lymph node, once the metastases of the IMNs happened, it is easy to metastases to the medial supraclavicular region, so this is why we suggested that the upper bound of the internal mammary lymphatic chain should be up to the subclavian vein with 5mm margin, which is connected to the caudal board of the supraclavicular CTV in breast cancer patients with high risk of recurrence.

San Gang Wu (Reviewer 2):

1. Methods: More details should be included in the Methods including enrolled and exclusion criteria; the definition of hormone receptor positive and HER2; the definition of TMN stage;
A: Eligibility criteria included unilateral histologically confirmed invasive breast carcinoma of stage I,
II, or III with any quadrant located primary tumor, irrespective of axillary involvement, eligible patients had undergone mastectomy or breast-conserving surgery and axillary dissection; Exclusion criteria included patients that male breast cancer, bilateral breast cancer, and noninvasive breast carcinoma, patients with widely lung or pleural metastases were also excluded; the definition of hormone receptor-positive is estrogen and/or progesterone receptor positive (the positive rate is equal or greater than 1%), and the definition of HER2 positive is immunohistochemistry [IHC] 3+ or IHC2+/ in situ hybridization-positive. The definition of TNM stage is according to the 7th edition of AJCC cancer staging.

2. Results: It is appreciated to assess the risk factors related to IMN recurrence; The stage IIIA is 71.9%, However, only 14.9% of patients were stage IIIA in Table 1.
A: In our manuscript, we mean that the rate of tumor staging under stage IIIA (include stage IIIA) is 64.9%, but the percentage of stage IIIA was only 14.9%. (I am so sorry to explain that patients of stage IIB is twenty-eight and fifteen for stage IIIC owing to my cursoriness, so the rate of tumor staging under stage IIIA (include stage IIIA)).

3. Discussion: The incidence of IMN recurrence was significantly higher than previous studies. More discussion should be performed the association and difference to the previous studies.
A: In our study, we found that the incidence of the internal mammary lymph node was 37.7%, which is in accord with the metastatic rate after the extended mastectomy of breast cancer in the 1970s. But according to some reports, the metastatic rate of IMNs is about 2%-5%. The reason is perhaps that IMNs are small, whose diameter are usually between 2mm to 5mm, the routine imaging examinations easily miss IMN for it is usually 6mm in diameter even if it metastasis, unless the huge mass is formed in the very late stage. In addition, the recurrence of IMNs rarely exists isolatedly, often combining with systemic metastases such as liver, lung and so on. And there are no obvious clinical symptoms when the recurrence of IMNs occurs, so the physicians usually concentrate their attentions on other recurrence/metastases. In this study, the patients enrolled didn’t undergo postoperative adjuvant radiotherapy owing to various reasons such as lack of indications of radiation therapy, poor economic conditions and so on, but most of the previous studies enrolled patients that underwent postoperative radiotherapy which may reduce the metastatic rate; secondly, the reason is perhaps that nearly 30% of the patients studied underwent PET-CT examination which could improve the detection rate of metastatic lymph nodes probably; and thirdly, in our opinion, the internal mammary lymph node region and the anterior mediastinal lymph node region can be seen as an entirety, so when the metastasis of the anterior mediastinal lymph node occurs, we think that the internal mammary lymph node has a very high probability of metastasis even if the short diameter is less than 6mm.

4. Limitation: Limitation should be added in this study.
A: Firstly, this is a retrospective study, so there may exist selection bias; secondly, most of the patients did not undergo adjuvant radiotherapy owing to that most of them were treated in county hospitals before recurrence, and the others had no indications of radiotherapy according to guidelines at that time; thirdly, our study were mainly based on enhanced-CT, further studies should be done based on more accurate imageological examinations such as PET-CT and so on.

5. Abbreviations: Lack of partial abbreviation such as HR, et al.
A: HR: Hormone Receptor; CT: Computed Tomography; IHC: Immunohistochemistry

Toshiaki Iwase, M.D., Ph.D. (Reviewer 3): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be
The authors retrospectively analyzed 114 patients with breast cancer to investigate the metastatic rate of IMNs and also to provide recommendation target delineation of IMNs for radiotherapy. The indication of radiotherapy to IMNs needs special consideration especially for the patients with high risk of cardiac function. Therefore, to determine the group which can be de-escalate the therapy is highly demanded.

**Major revision**

1. The definition of adjuvant radiotherapy in the article is unclear. Does it mean radiotherapy to remnant breast or regional LN?
   - A: Postoperative adjuvant radiotherapy of breast cancer in this article refer to irradiation to the chest wall plus regional lymph node (including the internal mammary, supraclavicular and axillary lymph nodes) for mastectomy and to whole-breast plus regional lymph nodes for breast-conserving surgery, which mainly refers to patients that with T3, T4 stage and/or lymph node positive.

2. The authors should describe a reason why the patients didn't receive radiotherapy. 35.1% of the patients is TNBC and most of cases was advanced. Is it because poor PS? Age? or any other reason?
   - All clinical factors which possibly contributed to the decision should described in background table.
   - A: All of the patients enrolled did not undergo adjuvant radiotherapy owing to lack of indications of radiotherapy according to guidelines at that time, and other reasons such as poor economic conditions, patient’s willness, the condition of local hospital and so on, but this is the problem in China. There were many advanced patients with the molecular subtype of TNBC, on one hand, TNBC possess worser biological behavior, on the other hand, it may be because the output tube of IMN is located in the supraclavicular and anterior mediastinum region, entering the superior vena cava through the left and right jugular veins, thus increasing the risk of widely systemic metastases. More clinical factor such as Age was described in Table 1.

3. The authors should describe detail of imaging analysis and clear definition of LN metastasis in method section and statistical considerations.
   - A: The definition of lymph node metastasis include that the internal mammary lymph nodes whose short diameter is more than 6mm, or the lymph node is tightly connected to the internal mammary blood vessels regardless of the short diameter, lymph node who meet one of the above conditions can be seen as metastatic lymph nodes; the anterior mediastinal lymph nodes whose short diameter were more than 10mm, or the lymph node is tightly connected to the blood vessels regardless of the short diameter, or lymph node with ring-enhancement in contrast-enhanced CT images also can be seen as metastatic lymph node; and the hypermetabolism in PET-CT also be diagnosed as metastases even the short diameter was less than 6mm or 10mm. In this study, the interval estimation of the population rate was based on approximate normal distribution method when nP and n(1-P) were both greater than five, otherwise look-up table method was used. We just have found a clinical phenomenon from this retrospective study, and further prospective work should be done to verify this phenomenon.

4. The authors should show flow chart how you selected the patients.
   - A: This is the selection process of patients included in this study: 126 patients were initially identified as having chest-wall recurrence and regional lymph node metastases. 12 patients were subsequently excluded for the following reasons: 1 patient was excluded with the reason that he was male breast cancer patient; 3 patients were excluded with the reason that the pathology was invasive lobular carcinoma; 8 patients had widely lung or pleural metastases, and therefore not included. The remaining 114 patients were included in this study. And the flowchart is uploaded in the additional file.
Minor revision
1. Authors should show the anatomical location of LNs in figure because most of the readers are not radiologist.
A: The anatomical location of LNs were shown in figure 2, which is uploaded.
2. Language editing service is needed throughout the paper.
A: The language of this paper was edited by professional English and academic editing services (editage, https://www.editage.com/).