Peer review file
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Reviewer A

Comment 1: "The total concordance rate between the diagnoses of AIS by intraoperative frozen sections and postoperative paraffin-embedded sections was 82.7%". I wonder whether the experience of pathologists would have impact on the accuracy of the frozen section? What did the authors do in this study to avoid this situation?

Reply 1: Thank you for your comments. Two or more pathologists agreed upon all cases, adding to a senior pulmonary pathologist（T.H.), to formulate the diagnosis. We believe that the frozen section diagnosis by some pathologists including one specialist in thoracic oncology would contribute to improvement of diagnostic accuracy. We reconstructed the chapter on pathological evaluation in methods on comments by you and reviewer B.

Changes in text:

Pathological evaluation

Regarding frozen-section diagnosis, after sectioning the frozen samples, some slides of tumor center were stained with hematoxylin-eosin (H.E.). For final diagnosis, after fixing the specimens with 10% formalin and embedding them in paraffin, staining was performed with H.E. and elastica Van Gieson to assess the degree of pleural and vascular invasions.

According to the fourth edition of the World Health Organization histological classification, the cases were reviewed and staged according to the eighth edition of the TNM classification of the Union for International Cancer Control (8). Briefly, AIS was defined as a localized, small (≤3 cm) adenocarcinoma with growth restricted to neoplastic cells along with pre-existing alveolar structures (lepidic growth) that lacks stromal, vascular, or pleural invasion; MIA was defined as a small, solitary adenocarcinoma (≤3 cm) with a predominantly lepidic growth pattern and ≤5 mm invasion in the greatest dimension of anyone foci. AIS or MIA was not diagnosed if any of the following conditions were present: (1) histological subtypes other than a lepidic growth pattern (i.e., alveolar, papillary, micropapillary, and/or solid) or (2) if the tumor invaded lymphatics, blood vessels or pleura, or contained necrosis.

Three or more pathologists (T.H. and his colleagues) performed the histological evaluation without knowing preoperative clinical data. Disagreements among observers on the tumor invasion were discussed using a multiheaded microscope until agreement. These disagreements were mainly due to discrepancies on whether the interstitial fibrotic hypertrophy accompanied the destruction of pre-existing alveolar structures.

When an intraoperatively diagnosed AIS tumor was confirmed as invasive adenocarcinoma

postoperatively, we strongly recommended that the patients undergo additional lung resection after the operation. (Pages 7-8, Lines 98-114)

Comment 2: More information should be described on patients demographics in the Table 1, such as pulmonary function，tumor location，comorbidities, etc.
Comment 3: Whether precise positioning technique (for example, hookwire) was preoperatively performed to definitely locate the nodules?
Comment 4: Please give more information about surgical technique: VATS or open surgery？ single port or three ports?
Comment 5: Surgical outcomes are necessary to described, such as operation duration, blood loss, morbidity, and postoperative hospital stay.

Reply2-5: Thank you for your suggestion. We have restructured Table 1 based on recommendation of you and reviewer B. We have added the description of precise positioning technique and surgical technique to the chapter on extent of surgical resection.

Changes in text:
Extent of surgical resection
The type of lung resection determined the surgical technique. In all cases, wedge resections were performed by 3-port video-assisted thoracic surgery (VATS), and segmentectomy and lobectomy were performed by open thoracotomy. We found the targeted nodule by palpation intraoperatively and performed partial resection with a surgically safe margin. When the nodule was not palpable or identified, we performed segmentectomy or lobectomy depending on preoperative CT findings including the tumor size and tumor location. On the other hand, we performed CT-guided lipiodol marking before partial resection for some non-palpable nodules to identify their position during surgery. The non-palpable nodule was defined as a small diameter (<1 cm), deep (>1 cm from visceral pleura), or pure-ground glass density on preoperative CT findings. (Page 6, Lines 74-82)

Comment 6: What were the sub-types of 5 cases which were finally diagnosed as invasive adenocarcinoma? Papillary? Micropapillary? Or other types?
Comment 7: The following sentences are hard to understanding: "Because of the tumor location, 3 patients underwent lobectomy and another patient underwent segmentectomy. As aforementioned, we strongly recommended that the other patient undergo additional lobectomy, and we performed the operation." (in Line 142-144). Please give a clearer explanation.

Reply 6,7: T Thank you for your comment on this point. We have added a table (new Table 2) on sub-types of 5 cases, finally diagnosed as invasive adenocarcinoma. We have reformulated these sentences you pointed out to explain more clearly as below.

Changes in text:

The concordance of diagnosis between frozen-section and FFPE slides

Among 151 iAIS cases, 125 nodules were confirmed as AIS by FFPE-based diagnosis (accuracy rate, 82.7%). The other cases consisted of 21 MIAs and 5 invasive adenocarcinomas. For patients with proven MIA nodules, we decided to follow up with them closely in our outpatient clinic because the need for additional resection in such cases is not generally confirmed. However, 5 patients were finally diagnosed as having invasive adenocarcinoma (Table 2). Case 1,2 and 3 underwent lobectomy due to a location close to the hilum. Case 4 underwent segmentectomy due to a keep safety margin. Case 5 underwent partial resection. In these cases, as aforementioned, we strongly recommended additional lobectomy, and we performed a second surgical resection. The HRCT and pathology slides of 2 cases among such 5 cases are shown in Figure 3. (Pages 10-11, Lines 147-150)

Reviewer B

The authors have tried to prove the usefulness of intraoperative frozen section diagnosis in the surgical resection of pulmonary nodules. Although this is a topic requiring further efforts to improve, this manuscript actually adds scarce significant value to the topic. The intraoperative frozen section diagnosis is a well-established and routinely performed procedure during pulmonary surgery in many institutes, if not all of them. Therefore, the current research focus should direct to how to improve its accuracy and efficiency, instead of proving its significance. This manuscript would be more attractive if it could be re-designed according to what mentioned above. The authors should also address several other issues in the manuscript before its acceptance for publication:

Reply: Thank you for your comments on our manuscript. As you told us, our manuscript seems not to provide a strong impact on the current clinical works regarding frozen section diagnosis of undiagnosed small lung nodule. We know some papers have already reported that the concordance rate between frozen section diagnosis and final pathology of peripheral lung adenocarcinoma. However, there are few papers which focus only on the cases with iAIS during operation. Many iAIS tumors look like invasive adenocarcinomas radiologically. We completely understand our weak points in our study cohort. But, as mentioned in discussion, we believe our data would motivate surgeons to offer frozen section diagnosis of undiagnosed small lung nodules during operation regardless of radiological findings.

Comment 1: Selection bias: The authors did not provide a diagram of inclusion and exclusion process, where the number of patients who were diagnosed as MIA or invasive adenocarcinoma intraoperatively, and their corresponding concordance with final pathology should also be provided for reference.

Reply 1: Thank you for this comment. We have added the diagram of the inclusion and exclusion process (Figure 1) and reconstructed the chapter on patients in methods on your comment. We added the breakdown list of intraoperative and final diagnosis of intraoperatively-diagnosed adenocarcinoma (≤3cm) (Table 3), and reconstructed the chapter on the concordance of diagnosis between frozen-section and FFPE slides.

Changes in text:

Patients

Of the 1253 patients who underwent resection for lung cancer at our institute between January 2012 and December 2019, we retrospectively reviewed 143 patients diagnosed as having AIS intraoperatively (iAIS) by comparing to HRCT findings and referring to the final diagnosis using formalin-fixed and paraffin-embedded (FFPE) specimens. Exclusion criteria are listed in Figure 1. Because limited resection for MIA and IA is not established as a feasible operation yet, cases with MIA and invasive adenocarcinoma diagnosed intraoperatively were excluded. (Pages 6, Lines 68-73)

Comment 2: Pathological evaluation: The definitions of AIS and MIA should be further clarified in this section. Besides, what was the number of pathologists participating in the reviewing process, whether they worked independently, how did they achieve consensus regarding the disconcordant results, are not known yet in this part.

Reply 2: Thank you for your comments. We reconstructed the chapter on pathological evaluation in methods on comments by you and reviewer A. Definitions of AIS and MIA have been added. Two or more pathologists agreed upon all cases, adding to a senior pulmonary pathologist（T.H.), to formulate the diagnosis. The frozen section diagnosis by some pathologists including one specialist in thoracic oncology would contribute to improvement of diagnostic accuracy.

Changes in text:

Pathological evaluation

Regarding frozen-section diagnosis, after sectioning the frozen samples, some slides of tumor center were stained with hematoxylin-eosin (H.E.). For final diagnosis, after fixing the specimens with 10% formalin and embedding them in paraffin, staining was performed with H.E. and elastica Van Gieson to assess the degree of pleural and vascular invasions.

According to the fourth edition of the World Health Organization histological classification, the cases were reviewed and staged according to the eighth edition of the TNM classification of the Union for International Cancer Control (8). Briefly, AIS was defined as a localized, small (≤3 cm) adenocarcinoma with growth restricted to neoplastic cells along with pre-existing alveolar structures (lepidic growth) that lacks stromal, vascular, or pleural invasion; MIA was defined as a small, solitary

adenocarcinoma (≤3 cm) with a predominantly lepidic growth pattern and ≤5 mm invasion in the greatest dimension of anyone foci. AIS or MIA was not diagnosed if any of the following conditions were present: (1) histological subtypes other than a lepidic growth pattern (i.e., alveolar, papillary, micropapillary, and/or solid) or (2) if the tumor invaded lymphatics, blood vessels or pleura, or contained necrosis.

Three or more pathologists (T.H. and his colleagues) performed the histological evaluation without knowing preoperative clinical data. Disagreements among observers on the invasion size were discussed using a multiheaded microscope until agreement. The disagreements were mainly due to discrepancies on whether the interstitial fibrotic hypertrophy accompanied the destruction of pre-existing alveolar structures.

When an intraoperatively diagnosed AIS tumor was confirmed as invasive adenocarcinoma postoperatively, we strongly recommended that the patients undergo additional lung resection after the operation. (Pages 7-8, Lines 98-114)

Comment 3: Survival analysis: The authors have to further describe their follow-up plans and the disease surveillance workup for patients. What's more, the definition of recurrence should be clear. It was said that "Survival curves were plotted according to the Kaplan-Meier method and compared using the log-rank test", however, no related results were provided in this manuscript.

Reply 3: Thank you for your comment. We added a description of the follow-up details. When the data were recalculated to create the latest 5-year OS rates and overall survival curves, the number of deaths during the observation period went from 1 to 5, and the 5-year survival rate changed accordingly, so the description was corrected. We added survival curves as new Figure 4.

Changes in text:

Survival and statistical analyses

All patients received every 3 months follow-ups at our outpatient department for the first 2 years post-resection, and every 6 months for the third year, and once per year for the subsequent year, consisting of physical examination, blood analysis, chest radiography, and routine CT scans of the chest and upper abdomen. Brain magnetic resonance imaging and radionuclide bone scan were performed every year. Positron emission tomography of the neck, chest, and abdomen was performed when patients with suspicious symptoms or recurrence signs. Tumor recurrence was determined according to the results of these evaluations. (Pages 8-9, Lines 118-123)

Postoperative survival

Among 143 patients, 5 patients died of another disease during the observation period. No patient had tumor recurrence postoperatively. The respective 5-year OS rates were 94.3% in AIS cases, 94.4% in MIA, and 100% in invasive adenocarcinoma (Figure 4). RFS rates were the same as OS rates because of no recurrence. There was no statistical difference in postoperative survival between AIS cases, MIA,

and invasive adenocarcinoma. (Pages 11, Lines 162-164)

Comment 4: Data presentation and statistical analyses: I suggest the authors to reformat the table 1 for easier reading. Percentage could be calculated for each datum as it is more intuitive. Besides, I have doubt on the saying of "representative slide" in the legend of figure 1. It should be noted that the extreme case cannot be presented as representative result but the most common ones, as it might be misleading.

Reply 4: Thank you for your comment. We reformatted the Table 1 on the comment by you and reviewer A. We revised the chapter on representative H.E. stained frozen-section slides to avoid misleading.

Changes in text:

A typical case in which we could avoid excessive lobectomy

Images of a typical case that we could avoid excessive lobectomy, including HRCT and staining slides, are shown in Figure 2. Although this nodule has no ground-glass component radiologically, the frozen-section slide shows that all the tumor cells are well-differentiated without the destruction of the original fibrotic texture, consistent with AIS's typical findings. In this case, as aforementioned, we finished the operation without further resection. The final pathology diagnosis was AIS. (Pages 10, Lines 136-141)

Comment 5: Study design: I do believe that this study could be better designed if it had included a group of control. For example, the patients who did not have intraoperative frozen section diagnosis, received the pulmonary resection only under the guidance of radiological tests. This would generate stronger evidence for us.

Reply 5: Thank you for your comment. As you pointed, this study would have stronger impact on the evidence of importance regarding intraoperative diagnosis for undiagnosed lung nodule if our study had control cases. Unfortunately, this study was performed at only one institute. As shown in Table 1, 54 patients underwent resection for undiagnosed lung nodule based on radiological findings without an intraoperatively frozen diagnosis. Among them, only 14 had small tumors less than 3 cm in diameter. Due to its small numbers, we were not able to provide them as a control group. However, some articles demonstrated the concordance between preoperative radiological evaluation and pathological findings regarding tumor invasiveness (Suzuki et al. A prospective radiological study of thin-section computed tomography to predict pathological noninvasiveness in peripheral clinical IA lung cancer, JTO 2011). What we emphasize in this manuscript is not only the accuracy of intraoperative frozen section diagnosis for AIS but also high proportion of AIS cases “mimicking” like invasive adenocarcinoma based on radiological findings.

Comment 6: The style and deepness of discussion: The discussion of this manuscript provide no further knowledge about the research front of intraoperative frozen section diagnosis. The comparison between the current study and others was also not adequate, which give no prominence to the strength of this study. I would suggest the authors to go deeper in discussion, and to help the readers to discover the valuable research directions in the future.

Reply 6: Thank you for your comment. As you pointed out, our manuscript seems not to provide a novel knowledge regarding frozen section diagnosis of undiagnosed small lung nodule. As mentioned in discussion, some papers have already reported that the concordance rate between frozen section diagnosis and final pathology of peripheral lung adenocarcinoma. Additionally, as mentioned above, a couple of articles have already revealed the radiological characteristics of the concordance between preoperative radiological evaluation and pathological findings regarding tumor invasiveness. However, there are few papers which focus on the cases with iAIS. Surprisingly, a lot of iAIS cases “pretend” like invasive adenocarcinoma radiologically. As mentioned in discussion, our “take home message” is that surgeons should offer frozen section diagnosis of undiagnosed small lung nodules during operation regardless of radiological findings.