TRANSLATIONAL LUNG CANCER RESEARCH

Peer Review File

Article Information: Available at http://dx.doi.org/10.21037/tlcr-20-828

Reviewer A

This is an interesting study that explores the pitfall involved in intraoperative decision making based on pathological examination by frozen section in small sized lung adenocarcinoma. I think there are some issues to modify.

Major concerns:

Comment 1:

P3, L65; Authors concluded complementary treatment was associated with better prognosis based on recurrence-free survival. But was there statistically significant difference between 2 recurrent cases of 50 patients without complementary treatment and no recurrent cases of 13 patients with treatment (96.1% vs 100%)? Could it be explained 1 patient with VPI and another with STAS out of 2 with VPI and 1 with STAS were included in no complementary treatment group accidentally?

Reply 1:

We thank the reviewer for pointing out this issue. In this study, a total of 63 cases upstaged to invasive adenocarcinoma in final pathology (FP) diagnosis. Of the 63 invasive adenocarcinoma cases, 57 exhibited lepidic-predominant, two exhibited papillary-predominant, and four exhibited acinar-predominant subtypes. 13 patients received complementary treatment, and 50 patients not received complementary treatment. Two invasive adenocarcinoma cases without complementary treatment experienced a local recurrence after surgery. Because the number of recurrent events is relatively small, it is difficult to achieve statistical differences between the two groups. But our results provided important clues that invasive adenocarcinoma cases with underestimation by frozen section (FS) who do not receive supplementary treatment are

potentially at risk of recurrence. Future studies with larger sample size will verify our results.

To be cautious, we deleted the sentence "Regarding the 63 invasive adenocarcinomas, complementary treatment was associated with better prognosis (5-year recurrence-free survival: 96.1% versus 100%)".

Changes in the text:

We have deleted the sentence "Regarding the 63 invasive adenocarcinomas, complementary treatment was associated with better prognosis (5-year recurrence-free survival: 96.1% versus 100%)" in our revised manuscript.

Comment 2:

P11, L277; authors think most of the upstaged cases was attributed by sampling error and AIS and MIA ≥1cm by FS were more likely upstaged to invasive adenocarcinoma. Furthermore, pathologists should be more cautious about AIS and MIA ≥1cm by FS. Then, what do you think for pathologist to improve FS? Please discuss. I think comparison of clinicopathological features may be needed between cases of invasive adenocarcinoma who enrolled to this intentional sublobar resection protocol, which was correctly diagnosed by FS and converted to lobectomy and 63 cases with invasive adenocarcinoma underdiagnosed.

Reply 2:

We appreciate the reviewer's professional suggestions. The protocols of FS and FP diagnoses are shown in **Figure 1** in this letter. Specimens were immediately sliced into block 1 and block 2 after being removed by a thoracic surgeon. To avoid the sampling errors, at least two or three levels of tissue section were taken for diagnosis at the largest diameter interface. It is worth noting that timeliness is one of the most important features of FS diagnosis, which is used to guide the surgical decision-making. In general, the diagnosis of FS was based on only one block for a quick diagnosis. Although several

measures were developed to minimize the sampling errors, including sliced the tumors along the largest diameter and two or three levels of tissue section were taken for diagnosis, sometimes sampling error is still inevitable. (1,2)

In this study, we found that adenocarcinoma in situ (AIS) and minimally invasive adenocarcinoma (MIA) ≥ 1 cm by FS were more likely to be invasive adenocarcinoma due to sampling errors. Because of the large tumor volume, the range of invasion component in paraffin-embedded tissues that may exceed that observed in the FS due to the limitations of FS sampling. For pathologists, FS results and tumor size measured in fresh specimens should be considered jointly to predict the final diagnosis; for thoracic surgeons, FS diagnosis of AIS and MIA should be considered cautiously for tumor ≥ 1 cm to avoid insufficient resection.

During the study period, a total of 2153 patients with early-stage lung adenocarcinoma who enrolled to this intentional sublobar resection protocol, which were correctly diagnosed as invasive adenocarcinoma by FS and converted to lobectomy. The comparison of clinicopathological features were performed between invasive adenocarcinomas with correct diagnosis by FS and 63 invasive adenocarcinomas with underestimation by FS (**Table 1** in this letter). Between the two groups, the characteristics of age, gender, smoking history, preoperative carcinoembryonic antigen (CEA), tumor location, visceral pleural invasion (VPI), lymph node (LN) positive, tumor spread through air space (STAS) and video-assisted thoracic surgery (VATS) and postoperative chemotherapy did not differ. However, invasive adenocarcinomas with correct diagnosis by FS had a larger whole tumor size (1.58 ± 0.35 versus 1.47 ± 0.45 cm; $P = 0.044$), larger solid component size (0.83 ± 0.34 cm versus 0.71 ± 0.46; $P = 0.036$) on the CT scan, and more total LN removed (4.34 ± 2.46 versus 3.69 ± 4.85; $P = 0.044$) than the invasive adenocarcinomas with underestimation by FS.

Changes in the text:

We have added the discussion about how the pathologist to improve FS according to our findings that AIS and MIA ≥ 1 cm by FS were more likely to be invasive adenocarcinoma in the discussion section (page 17 in red font in the revised manuscript). Moreover, the comparison of clinicopathological features between invasive adenocarcinomas with correct diagnosis by FS and 63 invasive adenocarcinomas with underestimation by FS were added in the revised supplemental Table 1 and the results section (page 11 in red font in the revised manuscript).

Minor concerns:

Comment 3:

The most important factors in predicting the prognosis and determining the extent of resection in the surgical practice of early-stage small lung adenocarcinoma are solid component size and CTR in preoperative images, especially thin-section CT. Therefore, solid component size and should be entered in the multivariate logistic regression (Table 3). In multivariate analysis, the prognostic factors that are extracted differ depending on the factors that are input. It may be better to exclude sex, smoking history, lobes of tumor location, etc.

Reply 3:

We appreciate the reviewer's professional and detailed suggestions, and agree with the reviewer. We added the solid component size, and excluded sex, smoking and tumor location in the multivariate logistic regression model in the revised Table 3. We found pathological tumor size was the only independent predictive factor of upstage (< 1cm: reference; 1-1.4 cm: odds ratio [OR] 3.71, 95% confidence interval [CI] 1.94–11.36, $P < 0.001$; > 1.5 cm: OR 3.19, 95% CI 1.52–14.25; $P = 0.008$).

Changes in the text:

We added the solid component size, and excluded sex, smoking and tumor location in

the multivariate logistic regression model in the revised Table 3, the result has been changed in the revised manuscript (page 12 in red font in the revised manuscript).

Comment 4:

P3, L66: please correct "5-year recurrence survival" to "5-year recurrence-free survival".

Reply 4:

Sorry for this misspelling. It has been corrected.

Changes in the text:

"5-year recurrence survival" has been corrected to "5-year recurrence-free survival" in the revised manuscript.

Reviewer B:

This is an interesting study that involves a substantial sample size. Below are several comments and recommendations.

Comment 1:

Upgrade should be changed to upstage throughout (title, text, tables, figure legends, supplementary materials)

Reply 1:

We appreciate the reviewer's professional and detailed suggestions. Upgrade has been changed to upstage throughout the revised manuscript, including the title, text, tables, figure legends and supplementary materials.

Changes in the text:

Upgrade has been changed to upstage throughout the revised manuscript.

Comment 2:

The entire paper (abstract, text, figure legends, tables) requires word for word editing

by a native English speaker to correct spelling, grammar, tense, and syntax.

Reply 2:

Thanks for the reviewer's kind reminding. We had asked a native English speaker to help us polish the language of our manuscript.

Changes in the text:

The language of the revised manuscript has been polished by a native English speaker.

Comment 3:

Image quality is poor in Figures 2 and 3; at minimum, the images of permanent sections should be replaced with better quality images.

Reply 3:

Thanks for the reviewer’s detailed suggestion. We have replaced the original pictures 2 and 3 with high-quality images to strengthen our study.

Changes in the text:

Figures 2 and 3 have been replaced by high-quality images.

Comment 4:

Supplementary Figure 2 and Supplementary Figure 3 should be deleted. The information is and/or can be presented in the text.

Reply 4:

Thanks for your helpful suggestion. We have deleted Supplementary Figure 2 and Supplementary Figure 3. The information of these two figures was described in the text.

Changes in the text:

Supplementary Figure 2 and Supplementary Figure 3 have been deleted. The information of these two figures was described in the result section.

Comment 5:

line 274: What do the authors mean by "tissue cells"? Alveolar macrophages? other? A clear alternative term should be used instead.

Reply5:

Sorry for our irregular expression. We have replaced the tissue cells with intra-alveolar macrophages.

Changes in the text:

We have replaced the tissue cells with intra-alveolar macrophages.

Comment 6:

The data in Supplementary Table 1 and in Table 2 would be better presented in a single informative table.

Reply 6:

Thanks for your helpful suggestion. We have merged Supplementary Table 1 and Table 2 into a single table (**Table 2 in this letter**).

Changes in the text:

Supplemental Table 1 and Table 2 were merged into a single Table 2 in the revised Tables.

Comment 7:

Given that the vast majority of pulmonary adenocarcinomas show heterogeneous histological growth patterns, a skeptical reader may question designating "the predominant" growth pattern on only 2 sections from each tumor even though the tumors studied were = or < 3 cm. Also, is the percentage of a given growth pattern in the frozen section or in the entire tumor more closely predictive of frozen section error rate?

Reply 7:

We thank the reviewer for pointing out this important issue. The protocols of frozen

section (FS) and postoperative final pathological (FP) diagnoses are shown in **Figure 1** in this letter. Specimens were immediately sliced into block 1 and block 2 along the largest diameter interface of the tumor after being removed by a thoracic surgeon. FS diagnosis was based on only one block for a quick diagnosis in the operation (two or three levels of tissue section were taken for the diagnosis at the largest diameter interface). Remaining tissues from block 1 and block 2 were collected and fixed in 10% formaldehyde, paraffin-embedded, and prepared for final pathological examination. FP diagnosis was based on the entire tumor, including block 1 and block 2.

Timeliness is one of the most important features of intraoperative FS diagnosis, which is used to guide the surgical decision-making. To ensure the maximal benefit for patients from FS diagnosis and reduce the waiting time for FS diagnosis in the operation, the diagnosis of FS was based on only one block for a quick diagnosis. Although several measures were developed to minimize the sampling errors, including sliced the tumors along the largest diameter and two or three levels of tissue section were taken for diagnosis, sometimes sampling error is still inevitable. (1,2)

Reference in response letter:

1. He P, Yao G, Guan Y, et al. Diagnosis of lung adenocarcinoma in situ and minimally invasive adenocarcinoma from intraoperative frozen sections: an analysis of 136 cases. J Clin Pathol 2016;69:1076-80.

2. Walts AE, Marchevsky AM. Root cause analysis of problems in the frozen section diagnosis of in situ, minimally invasive, and invasive adenocarcinoma of the lung. Arch Pathol Lab Med 2012;136:1515-21.

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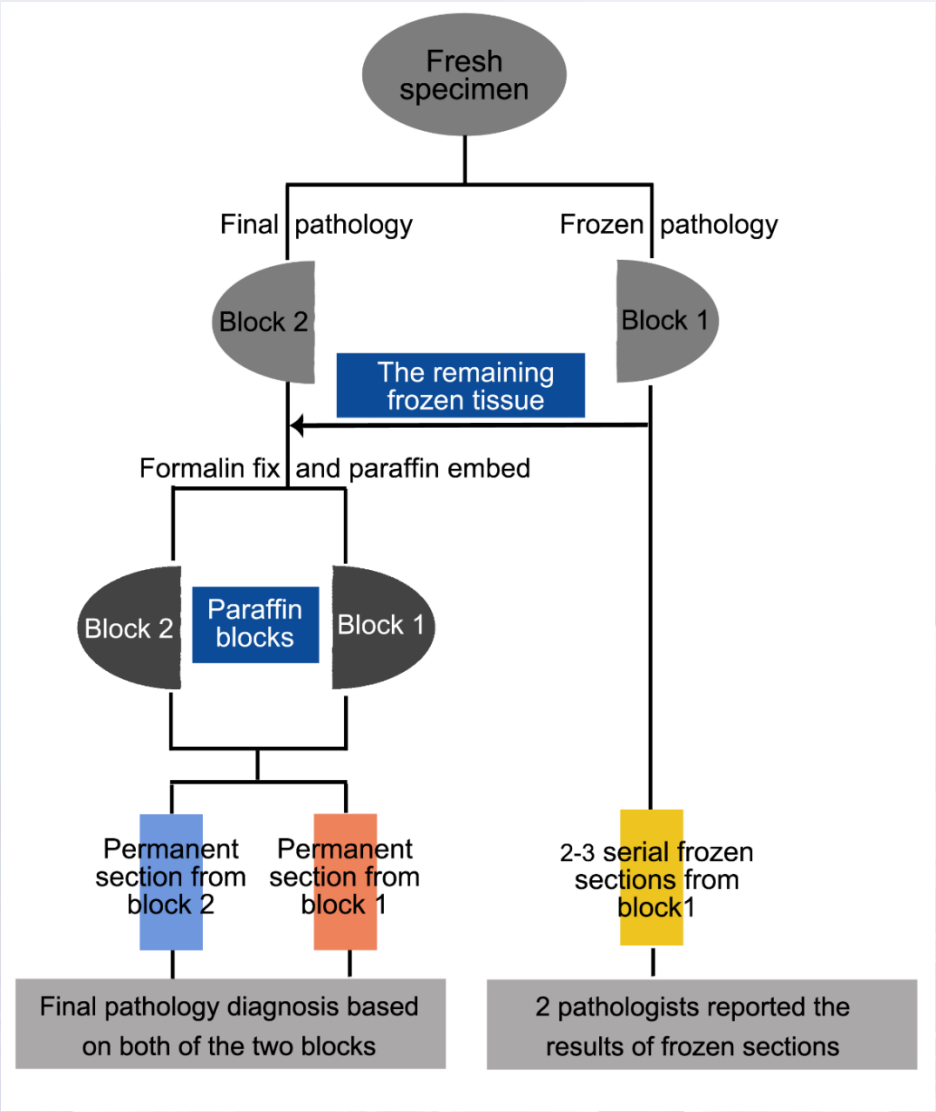

Figure 1:

The protocols of FS and postoperative FP diagnoses: FS diagnosis based on only one block for a quick diagnosis, and FP diagnosis based on both two blocks from entire tumor. (FS, frozen section; FP, final pathology).

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Table 1. The comparison of clinicopathological features between invasive adenocarcinomas with correct diagnosis by FS and 63 invasive adenocarcinomas with underestimation by FS.

Characteristics	IA underestimated by FS (n = 63)	IA correctly diagnosed by FS (n = 2153)	*P*
Age, years, No. (%)			0.538
< 60	36 (57.1)	1313 (60.9)	
≥ 60	27 (42.9)	840 (39.1)	
Gender, No. (%)			0.957
Male	27 (42.9)	930 (43.2)	
Female	36 (57.1)	1223 (56.8)	
Smoking history, No. (%)			0.785
Ever/current	10 (15.9)	370 (17.2)	
Never	53 (84.1)	1783 (82.8)	
Preoperative CEA			0.719
≤ 5 ng/ml	55 (87.3)	1845 (85.7)	
> 5 ng/ml	8 (12.7)	308 (14.3)	
Radiologic measurements (on CT)			
Whole tumor size, cm	1.47 ± 0.45	1.58 ± 0.35	0.044
Solid component size, cm	0.71 ± 0.46	0.83 ± 0.34	0.036
CTR	0.48 ± 0.26	0.53 ± 0.17	0.063
Primary tumor location, No. (%)			0.486
Upper and Middle lobe	37 (58.7)	1169 (54.3)	
Lower lobe	26 (41.3)	984 (45.7)	
Pathological tumor size, cm	1.17 ± 0.45	1.25 ± 0.33	0.103
Total LN removed	3.69 ± 4.85	4.34 ± 2.46	0.044
VATS, No. (%)			0.617
Yes	57 (90.5)	1985 (92.2)	

No	6 (9.5)	168 (7.8)	
VPI, No. (%)			0.068
Yes	2 (3.2)	219 (10.2)	
No	61 (96.8)	1934 (89.8)	
STAS, No. (%)			0.177
Yes	1 (1.6)	118 (5.5)	
No	62 (98.4)	2035 (94.5)	
LN positive, No. (%)			0.143
Yes	0 (0)	71 (3.3)	
No	63(100)	2082 (96.7)	
Postoperative chemotherapy, No. (%)			0.575
Yes	7 (11.1)	292 (13.6)	
No	56 (88.9)	1861 (86.4)	

IA, invasive adenocarcinoma; CT, computed tomography; LN, lymph node; CTR, consolidation-to-tumor ratio; VATS, video-assisted thoracic surgery; VPI, visceral pleural invasion; STAS, tumor spread through air space.

Table 2. Accuracy of the diagnosis of frozen section.

Frozen section	Final pathology								Total (N = 2006)
	AAH		AIS		MIA		Invasive adenocarcinoma		
	No.	%	No.	%	No.	%	No.	%	
AAH	77	100	82	7.9	0	0	0	0	159
AIS	0	0	957	92.1	127	15.4	9	14.3	1093
MIA	0	0	0	0	700	84.6	54	85.7	754
Accuracy %	95.9		89.1		91		/		
Sensitivity%	100		92.1		84.6		/		
Specificity%	95.7		85.9		95.4		/		
PPV%	48.4		87.7		92.8		/		
NPV%	100		91		89.9		/		

AAH: Atypical adenomatous hyperplasia; AIS: Adenocarcinoma in situ; MIA: Minimally invasive adenocarcinoma; NPV: negative predictive value; PPV: positive predictive value.

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