Original Article

Concordance between cryobiopsy and forceps biopsy specimens in assessment of immunohistochemistry staining for non-small cell lung carcinoma

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Background: Cryobiopsy is recently being promoted for biopsy of tumors in the lung periphery in precision medicine for lung cancer; the obtained tissue samples have been reported to be more useful compared to those obtained using forceps, because of the larger volume and higher quality. However, the influence of freezing and thawing of tissues when performing cryobiopsy on the results of immunohistochemistry (IHC) has not been completely understood.

Methods: In this study, consecutive patients who underwent diagnostic bronchoscopy with cryobiopsy for peripheral pulmonary lesions (PPLs) at our institution between June 2017 and November 2021 were reviewed retrospectively. Specimens of diagnosed cases of unresectable or recurrent non-small cell lung carcinoma (NSCLC) were selected. We compared the results of IHC assessment for programmed death-ligand 1 (PD-L1), human epidermal growth factor receptor 2 (HER2), and human epidermal growth factor receptor 3 (HER3) in cryobiopsy specimens versus conventional forceps biopsy specimens from the same site in the same procedure.

Results: Twenty-four of 40 patients were male (60%). The most frequent histologic type of cancer was adenocarcinoma (n=31, 77.5%), followed by NSCLC (n=4, 10%), squamous cell carcinoma (n=3, 7.5%), and others (n=2, 5%). The concordance rates of the tumor proportion scores (TPSs) for PD-L1, IHC score for HER2 and, IHC scores for HER3 were 85%, 72.5%, and 75%, respectively; the weighted kappa were 0.835, 0.637, and 0.697, respectively.

Conclusions: Freezing and thawing associated with cryobiopsy had virtually no effect on the results of IHC. We suggest that cryobiopsy specimens would therefore be ideal for precision medicine and translational research.

Keywords: Cryobiopsy; immunohistochemistry (IHC); human epidermal growth factor receptor 2 (HER2); human epidermal growth factor receptor 3 (HER3); lung cancer

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Introduction

In the past, cytotoxic chemotherapy was the only treatment for advanced non-small cell lung carcinoma (NSCLC). Now, various kinds of molecular targeted drugs are used in the presence of driver mutations such as epidermal growth factor receptor (*EGFR*) (1-4), anaplastic lymphoma kinase (*ALK*) (5-7), ROS proto-oncogene 1 (*ROS1*) (8), b-raf proto-oncogene (*BRAF*) (9), mesenchymal epithelial transition factor (*MET*) (10), ret proto-oncogene (*RET*) (11), Kirsten rat sarcoma viral oncogene homologue (*KRAS*) (12), and tumor-agnostic neurotrophic tyrosine receptor kinase (*NTRK*) (13). In the absence of driver mutations, the first choice of treatment is a single agent immune checkpoint inhibitor (ICI) or its combination of chemotherapy. The expression of programmed death-ligand 1 (PD-L1) in tumors, as assessed by tumor proportion score (TPS), is important as a predictor of the effect of ICIs (14-16). Thus, there is a need for the comprehensive genetic mutation analysis of tumors and assessments of the immune profile to determine the indications for appropriate treatments.

Tissue is essential not only for definitive diagnosis but also for determining the treatment strategy for advanced NSCLC, and bronchoscopy plays an important role in obtaining tissues. Although forceps is the most frequently used biopsy device in bronchoscopy, it has the following problems: (I) only anteriorly located components can be obtained, (II) tissue destruction is likely to occur during pinching, and (III) sample volume is small.

In contrast, recently introduced cryobiopsy has the potential to facilitate the collection of larger volume and higher quality of tissue samples (17). Cryobiopsy requires a cryoprobe, which uses compressed gas that is decompressed within its metallic tip, causing a cooling (Joule-Thomson) effect. As a result, tissue surrounding the tip is frozen and can be extracted. In addition, cryobiopsy allows bronchoscopists to perform an entirely circumferential biopsy of areas in contact with its tip. It has already been reported to be useful for diagnosis of endobronchial tumors and interstitial lung diseases (ILDs) (18,19). Cryobiopsy is also being actively promoted for biopsy of tumors in the lung periphery, and the obtained tissue samples have been reported to be more useful compared to those obtained using forceps in precision medicine for lung cancer (20).

The development and introduction of antibody-drug conjugates (ADCs) have been focused on as a new drug therapy. ADCs consist of three elements: an antibody, a cytotoxic payload, and a linker that connects them. ADCs form complexes with antigens on the cell membrane and are then internalized and translocated to lysosomes, where the ADCs are digested, thereby releasing their payload. The payload is usually a cytotoxic compound such as emtansine, which inhibits tubulin polymerization. In the early stages of their development, chimeric antibodies were used, which were unstable in the blood, resulting in toxicity and low antitumor effects. Thereafter, the use of human monoclonal antibodies has allowed the drug to reach tumor cells more safely and effectively by extending its half-life in the blood and increasing its stability. The anti-human epidermal growth factor receptor 2 (HER2) antibodies, CD30 and CD22 are examples of such antibodies that have been used (21).

Alterations in *HER2* gene, including mutation, amplification, and HER2 overexpression, are known to be associated with poor prognoses in lung cancer along with other cancers. In breast and gastrointestinal cancers, *HER2*-targeted therapies, such as tyrosine kinase inhibitors (TKIs) that bind to intracellular domains and ADCs that bind to extracellular domains, are in clinical use (22). In NSCLC, there have been clinical trials of molecularly targeted drugs for *HER2* amplification/mutation, but all of them ended in negative studies (23). Meanwhile, the development of HER2-targeted ADCs is now underway. Phase II clinical trials of trastuzumab deruxtecan, one of the ADCs, showed high efficacy in patients with *HER2* mutants and poor efficacy in those with its overexpression evaluated by immunohistochemistry (IHC) 2+/3+ (24,25).

In addition, another target for ADCs is human epidermal growth factor receptor 3 (HER3). HER3 is aberrantly

Highlight box

Key findings

- Freezing and thawing associated with cryobiopsy had little influence on immunohistochemistry (IHC) results.

What is known and what is new?

- Cryobiopsy allows for the collection of larger volume and higher quality of tissue samples than conventional forceps biopsy, but knowledge of the influence on IHC due to its freezing and thawing process is limited. In the present study, we focused particularly on HER2 and HER3, which are the major targets of antibody-drug conjugates (ADCs), and revealed that the influence on these IHC was little.

What is the implication, and what should change now?

- Cryobiopsy specimens would be potentially ideal for precision medicine and translational research.

expressed in many cancers, including NSCLC. Accumulated evidences indicate that HER3 plays a crucial role in survival of cancer cells and drug resistances. HER3 expression has been reported to contribute to the TKI resistance in *EGFR*-mutated NSCLC by maintaining anti-apoptotic HER3/PI3K/AKT signaling (21,26). A phase I clinical trial of patritumab deruxtecan, one of the HER3-targeted ADCs, in patients with or without *EGFR*-mutated NSCLC demonstrated a good response rate and a disease control rate (27,28).

As aforementioned, to select appropriate drug therapies for NSCLC, it is necessary to investigate various tumor characteristics for each case; there are high expectations for cryobiopsy specimens, which are larger in volume and have minimal tissue destruction. However, the influence of freezing and thawing of tissues when performing cryobiopsy on IHC is still not well understood. Accordingly, cryobiopsy specimens are not currently recognized as a standard for translational research. We therefore aimed to compare the results of IHC assessment in samples obtained by cryobiopsy versus those obtained by conventional forceps biopsy from the same site and in the same procedure. We selected PD-L1 and HER2, which are well-established in NSCLC, as well as HER3 as an exploratory target, for IHC assessment in this study. All procedures were performed by or under the supervision of bronchoscopist experts. We present this article in accordance with the STARD reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-621/rc).

Methods

Patients

This study was approved by the National Cancer Center Institutional Review Board (No. 2019-123). Written informed consent for the clinical procedure and for comprehensive researches using residual specimens were obtained from all eligible patients. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Consecutive patients who underwent diagnostic bronchoscopy with cryobiopsy for peripheral pulmonary lesions (PPLs) at our institution between June 2017 and November 2021 were reviewed retrospectively. Among them, cases of diagnosed unresectable or recurrent NSCLC were selected for the study. Finally, cases were included in the analysis if a sufficient amount of tumor cells, i.e., more than 100 cells, without crush artifacts, were seen in slides prepared from both cryobiopsy and forceps biopsy specimens.

Tissue sampling

The diagnostic bronchoscopy with cryobiopsy was performed as previously described by us (17). All patients underwent bronchoscopy under moderate to deep sedation with a combination of opioids and sedatives compliant with general anesthesia.

One of the following bronchoscopes (P260F, P290, 1T260, or 1TQ290; Olympus, Tokyo, Japan) was inserted through the intubated tracheal tube (Portex® Uncuffed Ivory PVC, Oral/Nasal Tracheal Tube; Smiths Medical, Minneapolis, MN, USA) and deeply wedged near the relevant bronchus, navigated by virtual bronchoscopy. The location of the target lesion was estimated by a radial endobronchial ultrasound under X-ray fluoroscopic guidance, and biopsies were performed 4–6 times using a forceps (1.8-mm FB-15C-1 or 1.9-mm FB-231D; Olympus, Tokyo, Japan). Next, a 1.9-mm flexible cryoprobe was inserted into the same position in combination with ERBECRYO® 2 (Erbe Elektromedizin GmbH, Tübingen, Germany). The sampling position was confirmed by matching the X-ray fluoroscopic image with the virtual fluoroscopic image in up to three directions.

The tip of the cryoprobe was then frozen to approximately –42 ℃ for 3–6 seconds, and the frozen tissue was pulled out along with the bronchoscope. The tip was then thawed in saline solution, and the tissue was detached and fixed in individual formalin bottles. If the bleeding was well controlled, cryobiopsy was performed up to two times.

Tissue staining

Tissue samples were fixed in 10% neutral buffered formalin, and after 24–48 hours, paraffin-embedded blocks were prepared in an ISO15189-certified laboratory. After hematoxylin and eosin (HE) staining, IHC staining was performed on a new set of slides containing 4-μm thick sections using primary antibodies targeting the following proteins: PD-L1 (PD-L1 IHC 22C3 pharmDx, Dako/Agilent, Tokyo, Japan), HER2 [Ventana I-VEW PATHWAY anti-HER-2/new (4B5), Roche, Basel, Switzerland], and HER3 (HER3/ErbB3 (D22C5) XP Rabbit mAb, Cell Signaling Technology, Danvers, the United States). The PD-L1 and HER3 IHC staining were conducted on the

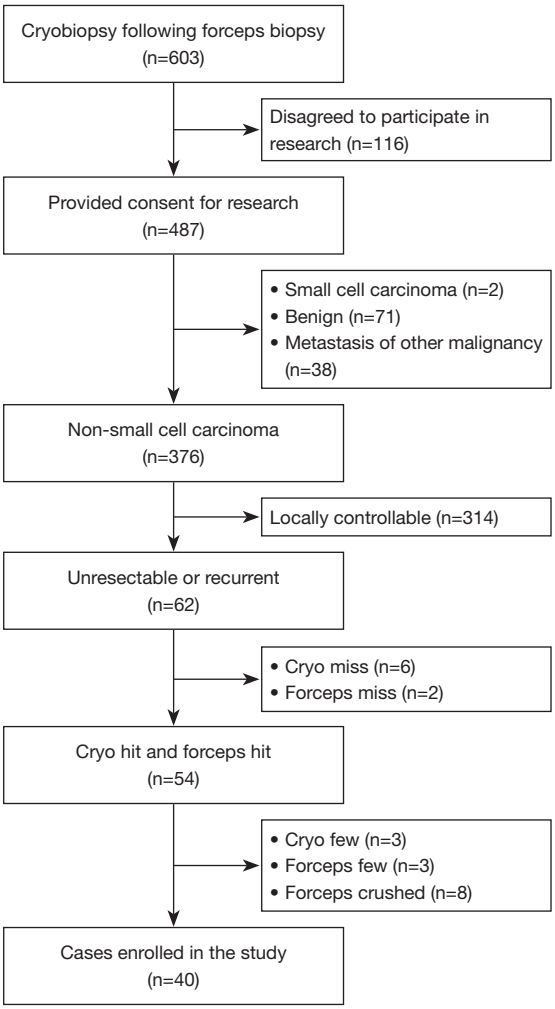

Figure 1 Patients flow diagram.

Autostainer Link 48 platform (Dako/Agilent, Tokyo, Japan) according to manufacturer's protocol. The HER2 IHC staining was conducted on VENTANA BENCHMARK XT (Roche, Basel, Switzerland).

Tissue assessment

The following clinicopathological features of the patients were collected from clinical records and pathological reports: age, sex, histology, and TNM stage. The histology of lung cancer was classified according to the 2021 WHO classification for lung tumors.

The protein expression of PD-L1 was measured using a TPS based on the percentage of viable tumor cells exhibiting partial or complete membrane IHC staining on the slide, following the manufacturer's protocol. The TPS was evaluated in 5% increments and scored at three levels: namely, TPS <1%, TPS 1% to 49%, and TPS ≥50%.

The IHC score for HER2 was determined according to the scoring guidelines for breast cancer. Although there is no established method for assessing HER3, we followed the scoring of HER2 in breast cancer. The expression levels were categorized as 0, 1+, 2+, and 3+, respectively, according to the degree of IHC staining on the membranes of tumor cells.

Tumor cells that were no longer present after thin sectioning were considered for evaluation, in which case the score was set to 0.

The TPS (%) for PD-L1 and the IHC scores (0–3+) for HER2 and HER3 were assessed by a medical doctor (KN) and the results were confirmed by a pathologist (YY). Clinical information was blinded during the assessments.

Statistical analysis

The Pearson's correlation coefficients were calculated to evaluate the agreement and inconsistency between the TPS results of each biopsy specimen. The closer the value of coefficient of determination (R^2) is to 1, the more it is interpreted as a match. The κ coefficient and weighted κ coefficient were used to evaluate the agreement and inconsistency between the IHC results of each biopsy specimen. The κ coefficient of 0.75 or less indicates poor to fair agreement, and the value of greater than 0.75 indicates almost perfect agreement. Statistical significances were set at P values <0.05. Statistical analyses were performed using an SPSS® 28.0 software (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

During the study period, there were 603 patients who underwent cryobiopsy following forceps biopsy for PPLs (*Figure 1*). Of the 487 patients who consented to comprehensive research using residual specimens, 62 were diagnosed with unresectable or recurrent NSCLC. Of these, we excluded cases in which no or few tumor cells were detected, as well as cases in which tumor cells were crushed either in cryobiopsy or forceps biopsy specimens. Three cases were excluded due to few tumor cells in forceps specimens and three cases in cryobiopsy specimens, respectively. Eight cases were excluded due to crush for forceps specimens and no case for cryobiopsy specimens.

Table 1 Patient characteristics

Variable	Value
Age in years, median [range]	64 [39–85]
Sex, n (%)	
Male	24 (60.0%)
Female	16 (40.0%)
Histology, n (%)	
Adenocarcinoma	29 (72.5%)
Squamous cell carcinoma	3 (7.5%)
Non-small cell carcinoma, not otherwise specified	4 (10.0%)
Others	4 (10.0%)
TNM stage, n (%)	
III	6 (15%)
IV	33 (82.5%)
Recurrence	1 (2.5%)

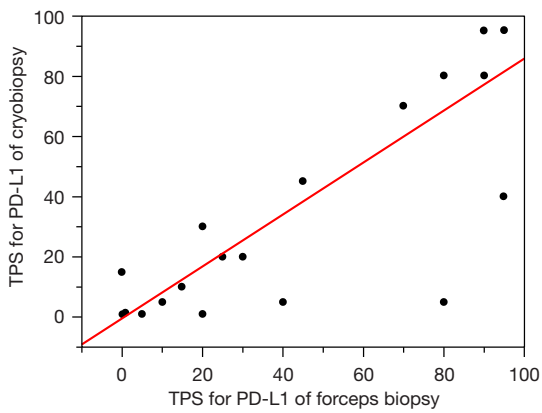

Figure 2 Pearson's correlation coefficient for PD-L1. The horizontal and vertical axes indicate the tumor proportion scores of the forceps biopsy and cryobiopsy, respectively. There is a strong correlation between the two specimens (R^2=0.831). TPS, tumor proportion score; PD-L1, programmed death-ligand 1.

Finally, 40 patients were eligible for the study.

The patient characteristics and histology are summarized in *Table 1*. Twenty-four of 40 patients were male (60%). Six patients had stage III disease, 33 had stage IV, and one had recurrent disease. The most frequent histologic type was adenocarcinoma (n=31, 77.5%), followed by NSCLC, not otherwise specified (n=4, 10%), squamous cell carcinoma (n=3, 7.5%), and others (n=2, 5%).

Table 2 Tumor proportion scores for PD-L1

PD-L1	Cryobiopsy				Weighted kappa value (P value)
	<1%	1–49%	50–100%	Total	
Forceps biopsy					0.835 (<0.001)
<1%	13	4	0	17	
1–49%	0	13	0	13	
50–100%	0	2	8	10	
Total	13	19	8	40	

PD-L1, programmed death-ligand 1.

Table 3 Immunohistochemistry scores for human epidermal growth factor receptor 2

HER2	Cryobiopsy					Weighted kappa value (P value)
	0	1+	2+	3+	Total	
Forceps biopsy						0.637 (<0.001)
0	17	7	0	0	24	
1+	2	12	1	0	15	
2+	0	0	0	1	1	
3+	0	0	0	0	0	
Total	19	19	1	1	40	

HER2, human epidermal growth factor receptor 2.

IHC assessment

The TPS for PD-L1 showed strong correlations between the cryobiopsy and forceps biopsy specimens (R^2=0.831, *Figure 2*). Thirty-four of 40 samples (85%) produced the same results between the two sampling methods (weighted kappa value: 0.835, P<0.001, *Table 2*). Only six samples produced different results, four scored higher with cryobiopsy and two scored higher with forceps biopsy.

For HER2, 29 samples (72.5%) produced the same results between the cryobiopsy and forceps biopsy specimens (weighted kappa value: 0.637, P<0.001) (*Table 3*). Nine samples scored higher with cryobiopsy and two scored higher with forceps biopsy. When the IHC scores were binarized to 0/1+ and 2+/3+, 39 samples (97.5%) showed the same results (weighted kappa value: 0.850, P<0.001), almost a perfect match (*Table 4*).

For HER3, 30 samples (75%) produced the same results

Table 4 Binarized immunohistochemistry scores for human epidermal growth factor receptor 2

HER2	Cryobiopsy			Weighted kappa value (P value)
	0/1+	2+/3+	Total	
Forceps biopsy				0.850 (<0.001)
0/1+	38	1	39	
2+/3+	0	1	1	
Total	38	2	40	

HER2, human epidermal growth factor receptor 2.

Table 5 Immunohistochemistry scores for human epidermal growth factor receptor 3

HER3	Cryobiopsy					Weighted kappa value (P value)
	0	1+	2+	3+	Total	
Forceps biopsy						0.697 (<0.001)
0	2	0	1	0	3	
1+	1	6	4	0	11	
2+	0	3	17	1	21	
3+	0	0	0	5	5	
Total	3	9	22	6	40	

HER3, human epidermal growth factor receptor 3.

Table 6 Binarized immunohistochemistry scores for human epidermal growth factor receptor 3

HER3	Cryobiopsy			Weighted kappa value (P value)
	0/1+	2+/3+	Total	
Forceps biopsy				0.545 (<0.001)
0/1+	9	5	14	
2+/3+	3	23	26	
Total	12	28	40	

HER3, human epidermal growth factor receptor 3.

between the cryobiopsy and forceps biopsy specimens (weighted kappa value: 0.697, P<0.001) (*Table 5*). Six samples scored higher with cryobiopsy and four scored higher with forceps biopsy. When binarized as in HER3, 32 samples (80%) showed the same results (weighted kappa value: 0.545, P<0.001) (*Table 6*). A representative case with completely concordant results for each IHC is shown in *Figure 3*.

Discussion

In the present study, we compared the IHC expressions of PD-L1, HER2, and HER3 in cryobiopsy specimens with those in conventional forceps biopsy specimens. We observed fine agreements for PD-L1 and HER2, and a reasonable agreement for HER3, suggesting that the effect of freezing and thawing during cryobiopsy is minimal. Tumors are collections of tumor tissues with heterogeneous characteristics, and they can change over time. While it is easier to use surgical specimens from the same case for comparison, we considered that it was important to particularly compare specimens taken from the same location at the same time in order to derive correct results. Therefore, comparisons between biopsy specimens obtained during the same procedure and from the same site are valuable; although limited number of cases (n=40) were analyzed herein, we were able to draw valuable comparisons. Some previous reports have shown that the IHC expressions of TTF-1, p40, and PD-L1 in cryobiopsy specimens were approximately the same as those in forceps biopsy specimens (20,29). In this study, the results of the TPS for PD-L1 were similar in line these previous studies. These aforementioned results and our findings combined suggest that the effect of freezing during cryobiopsy on the IHC results of PD-L1 is insignificant. However, no study till date has evaluated the IHC results for the expression of HER2 and HER3 in cryobiopsy specimens.

In breast cancer, *HER2* gene amplification or HER2 protein overexpression is observed in 15–25% of invasive breast cancers and is evaluated by IHC and/or *in situ* hybridization (ISH) methods. Among the four expression levels of IHC (i.e., 0, 1+, 2+, and 3+), 0/1+ is considered HER2-negative, 2+ is equivocal, and 3+ is HER2-positive (30). The results of IHC 0/1+ and 3+ are reported to be in good agreement with those of ISH (31). In contrast, for cases of IHC 2+, the frequency of *HER2* gene amplification by ISH ranges from 17% to 81%, and trastuzumab is less effective in cases without *HER2* gene amplification (31,32). Therefore, ISH is recommended to be performed on the same specimen in cases of IHC 2+. In gastric cancer, IHC 0/1+ is considered HER2-negative, 2+ is equivocal, and 3+ is HER2-positive, as in breast cancer. In cases of IHC 2+, further ISH is performed, and the cases are considered positive if there is *HER2* gene amplification (*HER2*/CEP17 ≥2.0) (33). Meanwhile, it has been reported that there is no correlation between HER2 protein overexpression and *HER2* gene mutation/amplification in NSCLC (34). Therefore, the

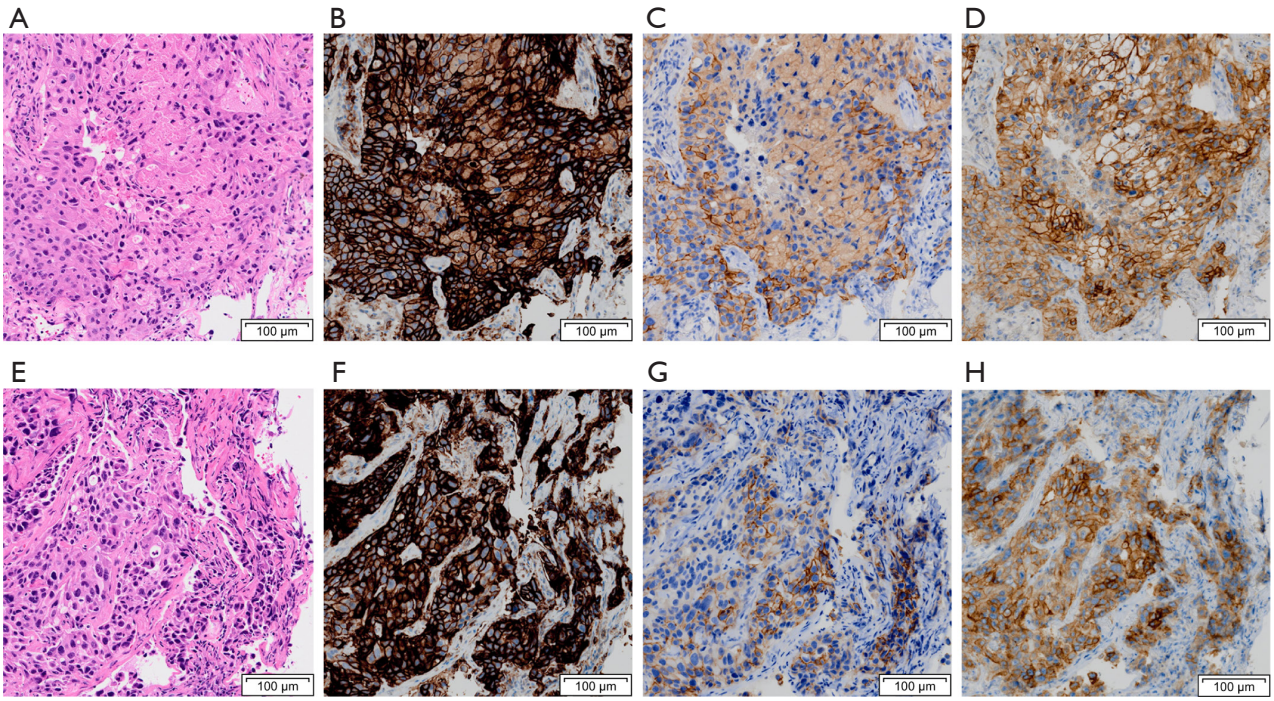

Figure 3 A representative case of favor adenocarcinoma showing complete concordances in the results of the respective IHC. The upper images show serial sections of cryobiopsy specimens (A-D ×10 magnification) whereas the lower ones show those of forceps specimens (E-H ×10 magnification). Hematoxylin and eosin-stained sections demonstrate that the tumor cells proliferate in a solid pattern (A,E). The tumor proportion scores for programmed death-ligand 1 are both 95% (B,F). Incomplete and faintly perceptible membrane staining of >10% of the tumor cells is observed; thus, the IHC scores for anti-HER 2 are both 1+ (C,G). Moderate complete membrane staining is observed in >10% of tumor cells; hence, IHC scores for HER3 are both 2+ (D,H). IHC, immunohistochemistry; HER, human epidermal growth factor receptor.

overexpression needs to be assessed separately from the gene alterations, and consequently IHC is expected to play an important role in precision medicine also.

In addition, as aforementioned, HER3 is attracting attention as an acquired resistance mechanism for EGFR-TKIs, and the development of drugs targeting HER3 is underway (35,36). Since there are no clear criteria for assessing the IHC score for HER3 in NSCLC and other carcinomas, we conducted an exploratory evaluation and found concordant results between the cryobiopsy and forceps biopsy specimens. HER3 has been reported to be overexpressed in a wide range of NSCLC, ranging from 7% to 86% (37,38). The HER3 protein overexpression in NSCLC, as well as HER2, is not necessarily caused by *HER3* gene amplification (39). Regardless of gene alterations, the expression of HER3 is one of the key targets for ADCs. In the phase I study of patritumab deruxtecan (HER3-DXd, anti-HER3 ADC), patients with locally advanced or metastatic *EGFR*-mutated NSCLC with prior EGFR-TKI therapy were eligible. Responses were observed in patients with known and unknown EGFR-TKI resistance mechanisms, and it suggested that HER3-DXd could be an approach to a broad range of drug-resistant cancers (40). Therefore, IHC for HER3 may play a key role in screening for those targeted drugs.

One common feature of IHC for PD-L1, HER2, and HER3 is that it assesses the staining on the cell membranes. Of course, accurate assessment requires preservation of cellular structure, and the presence of crush artifacts in tumor cells can have a significant impact on IHC results. In this study, cases that did not contain more than 100 evaluable tumor cells in each specimen were excluded beforehand, and forceps biopsy specimens had to be excluded more often than cryobiopsy specimens, mainly due to crush artifacts. In particular, HER3 can be stained not only in the cell membrane but also in the cytoplasm, but the presence of crush artifacts obscures the boundary between the two, making the determination of IHC staining

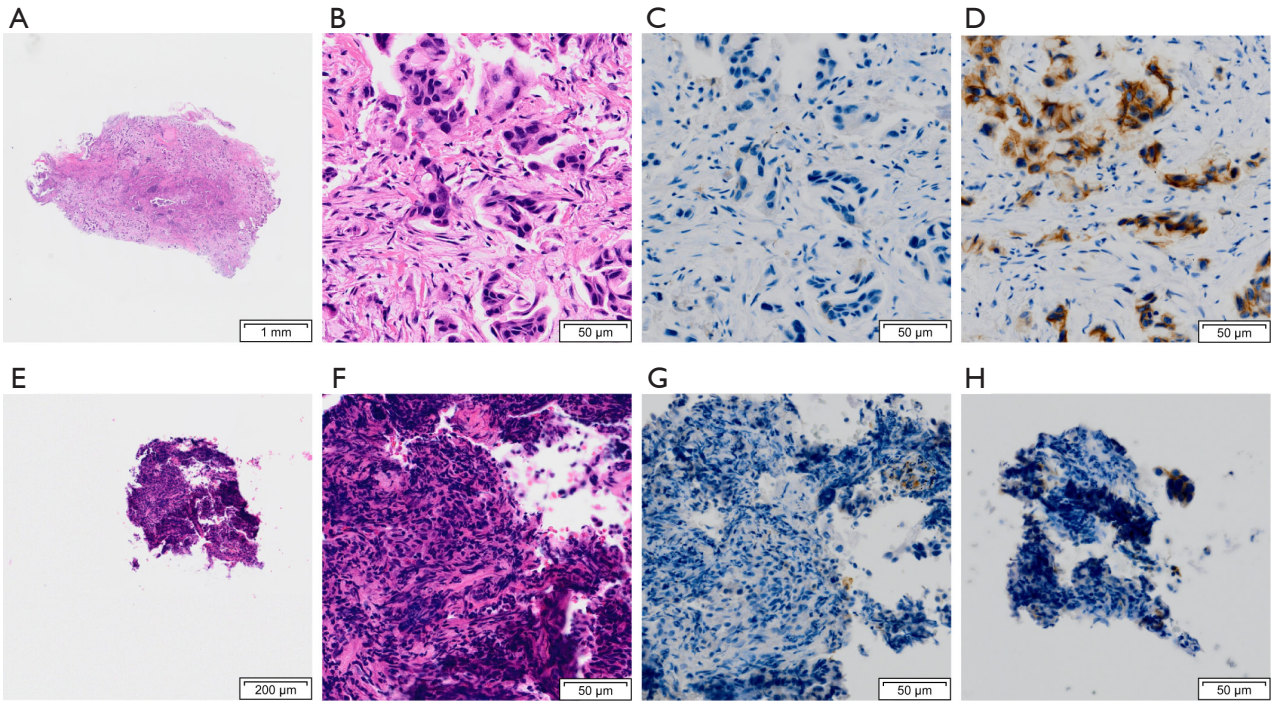

Figure 4 A representative case of adenocarcinoma showing discordance in the results of the IHC scores for anti-HER3. The upper images show serial sections of cryobiopsy specimens (A ×1 magnification, B-D ×20 magnification) whereas the lower ones show those of forceps specimens (E ×2 magnification, F-H ×20 magnification). The sizes of the cryobiopsy and forceps biopsy specimens are 3.7 mm × 5.4 mm (A) and 0.8 mm × 0.6 mm (E), respectively. The cryobiopsy specimen retains a well-defined architecture (B) whereas the forceps biopsy specimen is somewhat crushed (F). The tumor proportion scores for programmed death-ligand 1 are both <1% (C,G). Although the IHC score for HER3 can be determined as 2+ for the cryobiopsy specimen (D), it is difficult to evaluate the IHC score for the forceps biopsy specimen due to loss of tumor cells during thin sectioning and is determined as 0 (H). IHC, immunohistochemistry; HER, human epidermal growth factor receptor.

susceptible. In fact, in the present study, there was a case in which the HER3 results were incorrect owing to a decrease in the number of tumor cells in the forceps biopsy specimen during additional thin sectioning, combined with crush artifacts (*Figure 4*). Therefore, the results of cryobiopsy specimens might more accurately reflect the characteristics of the tumor in each case.

Furthermore, cryobiopsy, when performed using a 1.9-mm cryoprobe, results in difficulty in maneuverability owing to the thickness and rigidity of the probe. Specifically, there were some cases that had to be excluded because the cryobiopsy probe did not reach the lesion, and the consequent specimen did not contain any tumor cells. Recently, new cryoprobes with smaller diameters of 1.1 and 1.7 mm have been clinically introduced to improve the maneuverability. Despite the smaller diameters, it has been reported that the sample size collected was comparable to that with conventional cryoprobes (41). The improved induction is especially important for PPLs, and the chances of diagnosis of NSCLC made using cryobiopsy specimens is expected to increase (42).

There are several limitations to our study. First, this is a single-center retrospective study with a relatively small number of cases. However, for an accurate comparison of IHC, this small number of cases is still considered to be worthwhile because only a limited number of cases can be obtained from the same location at the same time with a sufficient amount of tumor cells by both cryobiopsy and forceps biopsy. Second, we investigated staining comparability for cryobiopsy versus conventional biopsy specimens against only three different antibodies. The more the types of antibodies to be examined, the greater the volume of tissue samples are needed for the study, but it is often difficult to secure larger samples, especially with conventional forceps biopsy. Meanwhile, the effects of freezing and thawing are expected to remain basically the

same even if the type of antibody is changed, and thus the results could be extrapolated to other antibodies.

Conclusions

We found that freezing and thawing associated with cryobiopsy had little influence on IHC results. Cryobiopsy specimens would be potentially ideal for precision medicine and translational research.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-621/rc

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Ethical Statement: The authors are accountable for all aspects of work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by The National Cancer Center's Institutional Review Board (No. 2019-123). Written informed consent for the clinical procedure and for comprehensive researches using residual specimens were obtained from all eligible patients.

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