The Usefulness of a Simple Classification for Bronchoscopic Findings for Diagnosis of Peripheral Pulmonary Tumour

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Keywords

Bronchoscopy · Cilia · Diagnosis · Lung cancer · Narrow-band imaging

Abstract

Background: Normal bronchial epithelium has been described in terms of transparency and smoothness. No studies have compared bronchoscopic endoscopic findings in the identification of bronchial epithelium. **Objectives:** This study aimed to classify bronchoscopic findings for peripheral pulmonary tumour (PPT) for accurate bronchoscopic diagnosis according to the presences of bronchial epithelium and bronchial stenosis using an ultrathin bronchoscope. **Methods:** We performed endocytoscopy using narrow-band imaging (NBI) on specimens immediately after lobectomy to investigate normal bronchial epithelium under the physical saline injection technique (PSIT) prior to classification of PPT. A retrospective study to classify bronchoscopic findings included 46 patients diagnosed with malignancy by bronchoscopy for PPT. **Results:** We recognized a “light blue line” (LBL) with NBI under PSIT, corresponding to strong re-

fection of short-wavelength light by cilia on the epithelial surface in an ex vivo endocytoscopic study. Bronchoscopic findings of PPT were classified morphologically into stenotic type (ST) and non-stenotic type (NonST). Tumours were also classified as exposed type (ET) and non-exposed type (Non-ET) based on the presence of epithelium. Most ST and NonET lesions (74%) were adenocarcinoma. Among squamous cell carcinoma, 55% were categorized as ST and ET. All NonST and NonET cases were adenocarcinoma. A significant difference in the presence of LBL was seen between ET and NonET. **Conclusions:** Our simple classification based on the appearance of stenosis and LBL in PPT may facilitate pathological diagnosis.

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Introduction

We have been directly observing peripheral airways using ultrathin bronchoscopy (UTB) since 2015. Before starting this study, we often encountered poor visibility in the peripheral airways using UTB (BF-XP290; outer

diameter, 3.1 mm; channel diameter, 1.2 mm; Olympus, Tokyo, Japan), despite trying to observe the lesion directly. The peripheral airways tend to collapse easily because their walls are not stiff, unlike those of central airways. Even small amount of mucous secretions in the peripheral airways tend to obscure the visual field. We recognized that airway collapse can be prevented or collapsed airways reopened by injecting a small amount of physiological saline into the airway, simultaneously eliminating obstructing mucus (shown in Fig. 1a1, a2) [1]. We desired to establish a novel method to directly observe lesions in the peripheral airway using UTB. While repeating bronchoscopic observation using UTB under a physiological saline injection technique (PSIT) [2], we noticed that a portion of the bronchial surface sometimes appeared pale and glowing on narrow-band imaging (NBI) (Olympus Medical Systems, Tokyo, Japan) (shown in Fig. 1a3) [3]. In gastrointestinal endoscopy, Uedo et al. [4] detected a fine, blue-white line on the crests of the epithelial surface/gyri with gastric intestinal metaplasia using NBI with magnifying endoscopy. They termed this finding the “light blue crest” (LBC) and demonstrated that LBC originated from the brush border on the epithelial surface with intestinal metaplasia [4]. We speculated that the presence of a light blue line (LBL) on the surface of the bronchial epithelium with NBI under PSIT might therefore indicate bronchial cilia. However, no previous studies have compared bronchoscopic and pathological findings in the identification of the bronchial epithelium, and objective observation methods remain lacking. In previous reports we identified have described observation of the bronchial epithelium with NBI under PSIT.

Tanaka et al. [5] used UTB to classify bronchoscopic findings of peripheral pulmonary tumour (PPT) into 3 types. However, bronchoscopic images using UTB without a miniature charge-coupled device were not high resolution, and no comparisons with pathological findings were presented. The aims of our study are (1) to demonstrate from pathology that the LBL on the surface of the bronchial epithelium with NBI under PSIT indicates bronchial cilia, (2) to classify bronchoscopic findings of PPT according to bronchoscopic diagnosis (Fig. 1).

Materials and Methods

Design

Pathological Demonstration of the LBL as Bronchial Cilia
Two patients were studied to observe the bronchial epithelium under endocytoscopy (EC) (CF-H290ECI; Olympus) with ultra-high magnification using NBI (NBI-EC) at JCHO Shimonoseki

Medical Center between July 2018 and August 2019. We examined which parts of the epithelium corresponded to LBL in resected specimens from patients immediately after lobectomy using NBI-EC. NBI-EC uses a gastrointestinal endoscope, which cannot be used in vivo as a bronchoscope. We therefore observed the bronchus of the resected lobe by NBI-EC under PSIT immediately after performing lobectomy with 0.05% crystal violet and 1% methylene blue staining [6]. When the LBL was observed by NBI under magnification, illumination was switched to white light. The location of the LBL on NBI was observed under maximum magnification (×520) for comparison of corresponding white light images.

Classification of Bronchoscopic Findings

Bronchoscopic Procedure

A retrospective study to classify bronchoscopic findings included 46 patients who were diagnosed as lung cancer malignancy after bronchoscopy for suspected PPT located from the fourth-generation bronchi to the subpleural area with lesion size ≤3 cm in longest diameter) and subsequently performed surgical operation at JCHO Shimonoseki Medical Center between March 2015 and June 2018. During bronchoscopy performed to obtain a diagnosis, the portion of the bronchus in contact with the peripheral pulmonary tumour was recorded as follows. Before bronchoscopy, we created a bronchial diagram corresponding to the target lesion from thin-slice computed tomography and used virtual bronchoscopic navigation (VBN) to clarify lesion location and then determined the bronchus name and generation. During bronchoscopy, the assistant followed from the central bronchus to the lesion, matching the location on the VBN and bronchial diagram with the actual bronchoscopic image. This allowed us to reach the bronchus at the exact location of the lesion, even if no findings were evident in the bronchial lumen. At this time, the display of the distance to the lesion by VBN was also helpful.

All patients with undiagnosed PPT had undergone prior observation with UTB followed by diagnosis using endobronchial ultrasonography (EBUS) using a guide sheath (EBUS-GS) [7]. EBUS-GS using a thin bronchoscope (BF-P290; outer diameter, 4.2 mm; channel diameter, 2.0 mm; Olympus) and a guide sheath (K201; Olympus) with a radial-type EBUS probe (UM-S20-17S; Olympus) under X-ray fluoroscopy was implemented, and brushing cytology and biopsy were performed approximately 5 times each [8, 9].

When the bronchoscope almost reached the target, saline was injected to wash out the mucus in the peripheral bronchus using PSIT to observe in the target lesion. Next, the target lesion was observed by white light and NBI. White light observation was carried out to observe stenosis and obstruction morphologically. NBI observation was carried out to observe the presence or absence of LBL and subepithelial blood vessels.

Cases showing a narrowed bronchial lumen due to stenosis and obstruction in bronchial lumen were defined as stenotic type (ST), and those without a narrowed bronchial lumen were defined as non-stenotic type (NonST). If the transparent and smooth epithelium regarded as normal bronchial epithelium was observed on the surface of PPT before PSIT, in addition, the presence of LBL on NBI after PSIT increased the confidence that normal epithelium was present; the tumour was judged as bronchoscopic non-exposed type (NonET). When a loss of epithelial transparency and presence of an irregular surface of PPT were seen before PSIT, and theoretically no LBL indicating normal ciliated epithelium would

Fig. 1. **a** is one of the 46 cases used in the classification study based on direct findings, and **(b, c)** are cases used in the preliminary experiment for the purpose of confirming LBC, respectively. **a1** The tip of the BF-XP290 is wedged in the left B⁸iax. **a2** Slow injection of saline from the working channel fills the airway with saline and provides an approximately 30% enlargement. **a3** In the upper wall of the left B⁸iax, the LBL is observed on the crests of bronchial epithelium under NBI. **b** The ciliated bronchial epithelium is observed in the biopsied bronchial wall with LBL on the margins with hematoxylin and eosin staining ($\times 400$ magnification). The height of cilia is measured at 4 points (mean value, $6.92 \mu\text{m}$). **c** Inset (blue box): The LBL is observed at the crest of longitudinal bronchus with EC using NBI-EC under PSIT (not shown). The bronchial wall is biopsied where the

LBL is observed. Yellow arrows show markers near the biopsy site in the inset images. **c** Inset (blue box): appearance of bronchial epithelium stained with crystal violet and methylene blue under ultra-high magnifying by EC. A ciliated tissue structure is indicated by white arrows (red box): EC is slightly separated from the airway wall under NBI, and the LBL is observed at the crest of the bronchial epithelium. Fusion of inset images to match the subepithelial vascular pattern. Cilia and LBL coincide. a and a' , b and b' indicate distances. The full-zoom photo is $500 \mu\text{m}$ long and $580 \mu\text{m}$ wide. From comparison with the full-zoom photo (not shown), $a = a'$: $667 \mu\text{m}$ and $b = b'$: $575 \mu\text{m}$. LBC, light blue crest; LBL, light blue line; NBI, narrow-band imaging; PSIT, physiological saline injection technique; EC, endocytoscopy; NBI-EC, narrow-band imaging endocytoscopy.

be observed under NBI after PSIT, the tumour was judged as bronchoscopic type (ET). We examined whether the simple classification of bronchoscopic findings for PPT into ST and Non-ST based on bronchial luminal morphology, and bronchoscopic ET and NonET for bronchial surface appearance is useful for estimating pathological features. All bronchoscopic procedures were digitally recorded.

The BF-XP290 bronchoscope was inserted into the bronchus of all the resected lobes immediately after surgical operation under

PSIT in the same manner described above. A guide wire was guided to the bronchus containing the lesion. The specimen was then stretched and fixed in formalin. By cutting along the guide wire, the specimen was split along the plane of the involved bronchus and a clear cross section of the lesion and bronchus was obtained. We performed macro- and microscopic observations of specimens and compared the findings with results from bronchoscopy. We reconfirmed ET or NonET based on the presence of bronchial epithelium (shown in Fig. 2).

Fig. 2. Study design. Directly observable findings of PPT by UTB were allocated by 2 aspects of stenosis related by tumour and tumour exposure. Pathological diagnosis was obtained by EBUS-GS using a thin bronchoscope. By cutting along the guide wire inserted into the leading bronchus to the PPT, the specimen was split along the plane of the involved bronchus and showed a clear cross section of the lesion and bronchus. Macro- and microscopic analysis of the lung was performed to compare bronchoscopic findings. EBUS-GS, endobronchial ultrasonography using a guide sheath; ET, exposed type; NBI, narrow-band imaging; NonET, non-exposed type; NonST, non-stenotic type; PPT, peripheral pulmonary tumour; PSIT, physiological saline injection technique; ST, stenotic type; UTB, ultrathin bronchoscope.

Bronchoscopic Assessment

In this retrospective study of classifications of the bronchoscopic findings, evaluations were made by 2 doctors who conducted the examination. The reason for classification into ST or NonST, and ET or NonET was based on the classification by Tanaka et al. [5] as described below. Tanaka et al. [5] reported using UTB to classify peripheral airway findings into a subepithelial obstruction type, ET, and nodular type. However, no comparisons with pathological findings were made. We therefore developed a method of taking high-resolution bronchoscopic images of PPT and compared the results with the pathological findings from the corresponding bronchus of the resected lung lobe. The results led to the identification of the new category of NonST in this study. We proposed that the subepithelial obstruction type and nodular type proposed by Tanaka et al. [5] can be grouped morphologically as the ST and added a NonST as a counterpart. Furthermore, using the ET proposed by Tanaka et al. [5] we proposed adding a NonET as a counterpart.

A bronchoscopic finding classification has been provided by the Japan Society of Respiratory Endoscopy [10]. This classification shows 3 observation items for morphological classification: (1) lesion spread (localized or non-localized); (2) surface properties (colour tone); and (3) changes in airway lumen (stenosis, dilation, ridge, flaps, depressions, etc.). Next, an endoscopic layer classification focuses on changes in the existing layer structure, such as epithelial, subepithelial blood vessels, longitudinal folds, ring-shaped folds, and collagen rings. For the main lesion, the mode of development and progression and the extent of progression are compared. These analyses can be performed on relatively central bronchial lesions, but the bronchial generation of PPT targeted in the present study involves bronchi from the fourth-generation bronchi to the subpleural area. With reference to the classification by Tanaka et al. [5] for the peripheral bronchus, peripheral airway lumen changes are classified into the presence or absence of stenosis, while we focused on the presence or absence of normal epithelium as the surface texture and, conversely, the presence or absence of tumour exposure.

The classification of NonST is determined by tracing the inner diameter of the bronchus before the lesion towards the lesion. In observation from the bronchi before the lesion, when the inner diameter of the bronchi does not change or dilate at the lesion, NonST is classified. The evaluation of ET or NonET is performed based on the presence or absence of normal epithelium with transparency and smoothness as before. In the normal epithelium with transparency and smoothness before PSIT, white light is observed and the lesion appears to shine when light enters from the front. When LBC was observed in the bronchial lumen wall under NBI after PSIT, we judged that the lesion was covered by normal epithelium.

We further examined the inter-observer variability of ST or NonST and ET or NonET classifications. Classification was performed independently by 2 of the co-authors as specialists in respiratory endoscopy (observers A and B). Observers A and B were instructed using 4 of the 46 cases in this study as representative images. Next, the 2 observers classified the remaining 42 cases as ST or NonST and ET or NonET, and inter-observer agreement was evaluated. The 42 cases were evaluated using several still images and video clips of approximately 1 min when the still images were inadequate. All still images and video clips were edited by erasing all personal information such as date, time, and name. Each evaluation was performed using the same still image and video clip, with the observer blinded to the histopathological diagnosis.

Diagnosis

Lesion confirmation was performed using radial EBUS. The positional relationship between the ultrasound probe and lesion was evaluated during bronchoscopy. The number of cases within/adjacent to/invisible was evaluated in each of the categories of ST or NonST and ET or NonET. Diagnostic yields were evaluated in relation to each of our bronchoscopic classifications.

Each histologic and cytologic specimen was interpreted separately by an experienced pathologist. Cytology included bronchial brushing, bronchial washing, and instrumental washing of the echo probe, brush, and biopsy forceps. Cytology specimens

were classified as negative, suspicious, or positive for malignancy. Suspicious cases were considered negative in our study. The final diagnoses and presence or absence of the ciliated columnar epithelium were established pathologically from surgical procedures.

Statistical Analysis

Statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [11]. Fisher's exact test was used for comparing the LBL with histological evidence of bronchial epithelium. $p < 0.05$ indicated significance.

To confirm the validity of our simple classification of the bronchoscopic findings, observer agreement was evaluated by analysing inter-observer reliabilities [12]. We used JMP Pro version 14.2.0 (SAS Institute, Cary, NC, USA) for the analysis of inter-observer reliabilities. κ values were then calculated to evaluate the degree of agreement, defined as poor, $\kappa < 0$; slight, $\kappa = 0-0.20$; fair, $\kappa = 0.21-0.40$; moderate, $\kappa = 0.41-0.60$; substantial, $\kappa = 0.61-0.80$; and almost perfect, $\kappa = 0.81-1.00$ [12].

This study was approved by the Ethics Committee at JCHO Shimonoseki Medical Center (approval Nos. 2018039, 2019002). All participants provided written informed consent to participate in this study.

Results

Pathological Demonstration that the LBL Indicates Bronchial Cilia

PSIT allowed detailed observation of the bronchial epithelium (shown in Fig. 1a2) without any adverse effects. In the first case, the bronchial wall was biopsied when LBL was observed. Ciliated bronchial epithelium was observed with haematoxylin and eosin staining. The mean height of cilia measured at 4 points in histopathological examination was $6.9 \mu\text{m}$ (range, $6.65-7.15 \mu\text{m}$) (shown in Fig. 1b).

In the second case, with observation of bronchial epithelium stained with crystal violet and methylene blue under NBI-EC, the LBL was observed at the surface of the bronchial epithelium. Under white light illumination, cilia were observed at the location of the LBL (shown in Fig. 1c inset). By superimposing these 2 images to match the subepithelial vessels, the presence of cilia and LBL were considered coincident (shown in Fig. 1c).

Classification of Bronchoscopic Findings

This study consisted of 46 patients (27 men, 19 women; mean age, 71.4 years; range, 41–91 years). Median lesion size according to longest diameter on CT was 19.6 mm (range, 8–30 mm). Eight lesions were located in the right upper lobe, 9 in the right middle lobe, 12 in the right

Table 1. Simple classification for bronchoscopic findings for diagnosis of PPT

Morphology	ET (n = 13)	NonET (n = 33)
ST (n = 40)	13 (4)	27 (20)
NonST (n = 6)	0	6 (6)

Data are presented as n (adenocarcinoma cases) / total cases. ET, exposed type; NonET, non-exposed type; ST, stenotic type; NonST, non-stenotic type; PPT, peripheral pulmonary tumour, non-stenotic type.

Table 2. Correlation of bronchoscopic findings of PPT with postoperative pathology

Postoperative pathology and comparison of bronchoscopic findings	ET (n = 9)	NonET (n = 22)	p value
NBI (n = 31)			
LBL-positive (n = 7)	0	22	<0.001*
LBL-negative (n = 9)	9	0	

ET, exposed type; LBL, light blue line; NBI, narrow-band imaging; NonET, non-exposed type; PPT, peripheral pulmonary tumour, non-stenotic type. * $p < 0.05$.

lower lobe, 6 in the left superior segment, 4 in the lingular segment, and 7 in the left lower lobe.

A pathological diagnosis was made in 46 patients (adenocarcinoma, 30 patients; squamous cell carcinoma [SCC], 11 patients; small cell lung carcinoma [SCLC], 3 patients; typical carcinoid, 1 patient; and metastatic carcinoma [renal cell carcinoma], 1 patient). Mean level of bronchial branch reached by UTB was generation 6.4 (range, generation 4–12). Mean saline volume for PSIT was 25.3 mL (range, 7–76 mL). No complications were encountered.

The simple classification for bronchoscopic findings (ST and NonST) for diagnosis of PPT in comparison with ET and NonET is shown in Table 1. NBI was performed in 31 cases. The correlation of bronchoscopic findings (NBI) of PPT with postoperative pathology is shown in Table 2. A significant difference in the presence of LBL was seen between ET and NonET.

ST and NonET

The lesion classified as ST and NonET was present in 27 cases (adenocarcinoma, 20 patients; SCC, 5 patients; SCLC, 1 patient; and typical carcinoid, 1 patient).

Fig. 3. a–d ST and NonET. **a** The tip of the BF-XP290 is wedged into the left B⁸aiiβyx. Morphological bronchoscopic findings suggest ST. The LBL is observable (yellow arrow). **b** CT shows that the bronchial lumen narrows sharply towards the periphery and is completely obstructed on the distal side. **c** Pathological specimen shows that the bronchial lumen narrows sharply towards the periphery. **d** Histology shows that the bronchial epithelium and tumour (adenocarcinoma) remains ($\times 40$ magnification) (red arrow). **e–h** ST and ET. **e** The tip of the BF-XP290 is wedged into the right B⁷aiiβxy. Morphological bronchoscopic findings suggest ST. A black arrow indicates the LBL, where the tumour is not exposed.

Red arrows indicate the areas where LBL is not observed and where the tumour is exposed. The corkscrew-like blood vessels can be observed in the ST. ST shows tumour completely obstructing the bronchus. Pathological specimen shows that the bronchus is completely obstructed on the distal side by the tumour. **h** Histology shows that the replaced bronchial epithelium and has spread to the epithelium and subepithelium ($\times 40$ magnification). The exposed cancer areas are indicated by red arrows. A black arrow indicates the edge of the normal epithelial part corresponding to the normal epithelium. LBL, light blue line; SCC, squamous cell carcinoma; ST, stenotic type; ET, exposed type; NonET, non-exposed type.

Table 3. Positional relationship between the ultrasound probe and lesion

	ST (n = 40)	NonET (n = 33)
ST (n = 40)	18/8/1	6/0/0/
NonST (n = 6)	0/0/0/	6/0/0/

Total: within/adjacent/invisible = 34 (73.9%)/11 (23.9%)/1 (2.2%). Data are presented as raw numbers. ET, exposed type; NonET, non-exposed type; NonST, non-stenotic type; ST, stenotic type.

Most lesions (74%, 20 of 27 cases) were adenocarcinoma. We present 1 representative case of adenocarcinoma of ST and NonET (shown in Fig. 3a–d). From a

comparison of bronchoscopic findings and pathological specimens, tumour with LBL was observed in adenocarcinoma with ciliated columnar epithelium. The distal bronchial lumen could not be observed for stenosis and obstruction.

ST and ET

A lesion classified as ST and ET was present in 13 cases (adenocarcinoma, 4 patients; SCC, 6 patients; SCLC, 2 patients; and renal cell carcinoma, 1 patient). Our cases (55%, 6 of 11 cases) of SCC tended to be categorized as ST and ET. We present 1 representative case of SCC of ST and ET (shown in Fig. 3e–h). From the comparison of bronchoscopic findings and pathological specimens, the absence of LBL and abnormal meandering vessels on the tumour surface indicated SCC (Fig. 3).

Fig. 4. NonST and NonET. **a** The tip of the BF-XP290 is inserted into the right B²aiay. Morphological bronchoscopic findings suggest NonST. **b** CT shows that B²aiay is bifurcated peripherally. **c** Pathological specimen shows that the bronchus is bifurcated without the tumour without obstruction. **d** Histology shows that B²aiay bifurcates peripherally and bronchial epithelium of the tumour (adenocarcinoma) remains. Tumour has collapsed alveoli ($\times 12.5$ magnification). **e** The part without smooth muscle (red arrow) is relatively depressed ($\times 100$ magnification). **f** Elastic fibres are present in the subepithelium by EVG staining ($\times 100$ magnification). NonST, non-stenotic type; NonET, non-exposed type; EVG, Elasticity van Gieson.

Table 4. Diagnostic yield in the bronchoscopic classifications

(n)	ET (n = 13)		NonET (n = 33)	
	cytological diagnosis	histological diagnosis	cytological diagnosis	histological diagnosis
ST (40)	13/13 (100)	13/13 (84.6)	26/33 (78.8)	30/30 (100)
NonST	3/6 (50)	6/6 (100)		

Data are presented as n (%). Cytology includes bronchial brushing, bronchial washing, and instrumental washing of echo probe, brush, and biopsy forceps. ET, exposed type; NonET, non-exposed type; NonST, non-stenotic type; ST, stenotic type.

NonST and NonET

All 6 cases showing NonST and NonET were adenocarcinoma. We present 1 representative case of adenocarcinoma showing NonST and NonET (shown in Fig. 4a–f). Histologically, the part between smooth muscles was relatively depressed, probably due to the shortening of elastic fibres in the tumour (Fig. 4).

Positional Relationship between the Ultrasound Probe and the Lesion

Table 3 shows the degree of reaching the lesion of radial EBUS. We used “within” when the probe was inside the lesion, “adjacent to” when the probe was located beside the lesion, and “invisible” when EBUS did not show the lesion.

Table 5. Inter-observer agreement: ST or NonST and ET or NonET

	Observer A				Total
	ST and ET	ST and NonET	NonST and ET	NonST and NonET	
Observer B					
ST and ET	7	2	1	1	11
ST and NonET	5	17	0	1	23
NonST and ET	0	0	0	0	0
NonST and NonET	0	1	0	7	8
Total	12	20	1	9	42

$\kappa = 0.58$, moderate, $\kappa = 0.41$ – 0.60 , moderate agreement. Data are presented as raw numbers. ET, exposed type; NonET, non-exposed type; NonST, non-stenotic type; ST, stenotic type.

Table 6. Inter-observer agreement: ST or NonST

	Observer A		Total
	ST	NonST	
Observer B			
ST	31	3	34
NonST	1	7	8
Total	32	10	42

$\kappa = 0.72$, substantial, $\kappa = 0.61$ – 0.80 , substantial agreement. Data are presented as raw numbers. NonST, non-stenotic type; ST, stenotic type.

Table 7. Inter-observer agreement: ET or NonET

	Observer A		Total
	ET	NonET	
Observer B			
ET	8	3	11
NonET	5	26	31
Total	13	29	42

$\kappa = 0.53$ moderate, $\kappa = 0.41$ – 0.60 , moderate agreement. Data are presented as raw numbers. ET, exposed type; NonET, non-exposed type.

Diagnostic Yield of Bronchoscopic Cytological Diagnosis and Histological Diagnosis in Relation to Each of the Bronchoscopic Classifications

The diagnostic rates for bronchoscopic cytological diagnosis and histological diagnosis in ST and ET, ST and NonET, and NonST and NonET were 100%/84.6% for ST and ET, 78.8%/100% for ST and NonET, and 50%/100% for NonST and NonET (Table 4).

Inter-Observer Agreement

Tables 5–7 show the inter-observer agreement for both morphological change and surface properties (ST or NonST and NonET), morphological change alone (ST or NonST) and surface properties alone (ET or NonET), respectively. Inter-observer agreements were moderate ($\kappa = 0.58$) for both morphological change and surface properties (ST or NonST and ET or NonET), substantial ($\kappa = 0.72$) for morphological change alone (ST or NonST) and moderate ($\kappa = 0.53$) for surface properties (ET or NonET).

Discussion/Conclusion

This study shows that (1) the LBL on the epithelial surface in the PPT is a highly accurate sign of the presence of normal ciliated bronchial epithelium and (2) pathologic findings can be indicated by classifying bronchoscopic findings of PPT into ST or NonST and ET or NonET. PSIT deepens the depth of field, eliminates halation, and provides a magnifying effect (approximately 1.33-fold, depending on the difference between refractive indexes of water and air) (shown in Fig. 1a2). The LBL under PSIT appeared as a dense reflection of light with NBI, at the surface of the bronchial epithelium. In previous reports, bronchial ciliated cells have been distinguished from other cell types by the presence of cilia (~200–300 cilia per cell) on the luminal surface, each about 0.2–0.3 μm in diameter and 6–7 μm in length in the upper airways [13]. The height of the cilia we measured was around 7 μm , not differing markedly from previous reports. On the other hand, NBI emits narrow-band light at specific wavelengths of 400–430 nm and 525–555 nm. In these images, each cilium is too thin to be resolved due to the diffraction

limit of the light. We speculated that the LBL originates from the difference in reflectance of the light at the surface of ciliated tissue structures. Supporting this speculation, the LBL was mostly observed on the surface of the bronchial epithelium and showed similar features in images and histological specimens. This LBL is presumed to be similar to the mechanism by which a “LBC” is observed with intestinal metaplasia on the pylorus side in chronic gastritis due to *Helicobacter pylori*. LBC corresponds to the brush border at the light microscope level [4]. We would thus like to call this LBL of the bronchial epithelium “LBC.”

Since this was an endoscopic classification, evaluation was made from 2 perspectives: morphological changes to the bronchial lumen; and surface properties of the bronchus considered to be a lesion from the viewpoint of the lumen and simply applied to the 4-cell classification. Regarding surface properties, we found that more accurate differentiation between exposure and non-exposure was achieved by adding the presence or absence of LBC, as indicative of the presence or absence of cilia. From the κ values obtained in the examination of inter-observer variability, the classification of bronchoscopic findings of PPT into ST or NonST and ET or NonET was considered to be at least moderately reproducible.

Lesions classified as NonET were mostly adenocarcinoma (79%, 26 of 33 cases). Three-quarters of adenocarcinoma originating from peripheral lung parenchyma show replacement growth of alveolar structures [1]. Peripheral lung adenocarcinoma is thus rarely observed at bronchoscopy when the tumour itself is not exposed in the bronchial lumen. This is consistent with the notion that bronchial epithelium and cilia are expected to remain in peripheral lung adenocarcinoma.

When a bronchoscope entered NonST in all 6 adenocarcinomas, the colour of the bronchial surface was often bright, and ring-shaped folds of bronchus were clearly evident. Pathologically, the part without smooth muscle seemed to be compressed by the shortening of elastic fibres of the tumour, and the bronchial lumen was dilated. Contraction with smooth muscle may have contracted the ring-shaped folds presumably resulting in marked ring-shaped folds of the bronchial lumen. The lesion could not be seen through the thinned airway wall, as shown in Figure 4. This phenomenon was attributed to the thin airway wall in NonST and the white colouration of the cancer lesion. The reason for no cases of NonST and ET might be attributed to the fact that NonST often involves adenocarcinoma which rarely destroys the epithelium.

Generally, SCC often advances in the bronchial epithelium and tends to spread along the bronchi while replacing the epithelium [15, 16]. In this study, the epithelium was replaced by peripheral SCC in the same way as central SCC. Furthermore, the following types of peripheral SCC in this study were confirmed: one that narrowed and closed the lumen by growing under the bronchial epithelium and the other that involved a nodule or polyp on the bronchial surface by developing in the respiratory tract.

Our classification may be beneficial in deciding whether the target lesion is present in the bronchus endoscopically and in differentiating epithelial from non-epithelial invasion of peripheral lung lesions. Therefore, in malignant lesions, this method may be potentially useful for differentiating SCC from adenocarcinoma/small cell carcinoma.

PSIT can prevent peripheral airway collapse or can reopen collapsed peripheral airways, simultaneously eliminating obstructive mucus from the area of interest for the bronchoscope. Performing PSIT allows direct observation of the endoluminal characteristics of peripheral pulmonary lesions, that the exact location of the lesion can be determined and a biopsy performed from the lesion site. Similar to the water immersion technique using gastrointestinal endoscopy, we suspect that PSIT was able to improve the depth of the field and allow clearer observation of endo-luminal findings of peripheral lesions. Bronchoscopic cytological diagnostic yield may indicate the difference of location of tumour cells in the lesion (Table 4). ST and ET are more likely to have tumour cells on the surface of the bronchial lumen compared to NonET, and bronchial brushing and bronchial washing cytology are thus expected to be more likely to be positive. This classification may be useful to some extent for selecting effective diagnostic procedures and will be an issue for future study.

Some limitations in this study need to be considered. This study showed that the pathological findings could be classified into 4 quadrants, but no significant differences were identified. The number of cases may have been too small to allow the detection of significant differences.

What is important in bronchoscopic findings of PPT is whether abnormal findings are truly captured. Obtaining a high-quality image of PPT is of primary importance. Active observation of the lesion should be performed with NBI under PSIT. We believe that this approach will help clarify the lesions, localization in the bronchial wall, and diagnosis of PPT.

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Statement of Ethics

This study was approved by the Ethics Committee at JCHO Shimonoseki Medical Center (approval Nos. 2018039 and 2019002). All participants provided written informed consent to participate in this study.

Conflict of Interest Statement

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Author Contributions

Dr. Morita contributed to the design, acquisition, analysis, and interpretation of data for the work, revised it critically, and gave final approval of the version to be published. Dr. Kanda contributed to the design, acquisition, analysis, and interpretation of data for the work and wrote and revised the manuscript. Dr. Kurimoto contributed to the design, acquisition, analysis, and interpretation of data for the work and revised it critically. Dr. Setoguchi and Dr. Nosaka contributed to acquisition, analysis, and interpretation of data for the work and revised it critically. Dr. Masumata contributed to the design and revised it critically. All authors gave final approval of the version to be published, agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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