Original Article

An Accurate Diagnosis of Noguchi Classification Is Possible after the Modification of Preoperative Biopsy-Induced Fibrosis

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Purpose: After computed-tomography–guided needle biopsy (CTNB), the lung may undergo iatrogenic fibrosis (post-CTNB fibrosis), which can be misdiagnosed as tumor-induced fibrosis. The purpose of the study was to examine if an accurate evaluation of pulmonary adenocarcinoma can be made using the Noguchi classification (type A or B vs. type C), even after CTNB.
Materials and Methods: The subjects were 71 patients with primary pulmonary adenocarcinoma of 20 mm or less that had been resected surgically after CTNB. Twenty-four patients who did not undergo a preoperative biopsy served as controls. Resected specimens were stained with hematoxylin-eosin (HE) and elastic-fiber staining for a precise observation of fibrosis.
Results: The period from CTNB to surgery ranged from 12 to 153 days. Post-CTNB fibrosis consisted primarily of collagen fibers with a few thin elastic fibers observed only with high magnification, which was able to distinguish post-CTNB fibrosis in 39 of 48 patients (81.3%) with bronchioloalveolar carcinoma (BAC) lesions (types A/B/C) and in 6 of 23 patients (26.1%) without BAC lesions (types D/E/F/E + F), showing a significant difference ($p < 0.0001$). In the control group, no lesions that resembled post-CTNB fibrosis were observed.
Conclusion: An evaluation of pulmonary adenocarcinoma by Noguchi classification can be accurately performed even after CTNB. (Ann Thorac Cardiovasc Surg 2009; 15: 221–226)

Key words: bronchioloalveolar carcinoma, computed-tomography–guided needle biopsy, fibrosis, lung cancer, Noguchi classification

Introduction

Precise screening with computed tomography (CT) can identify small suspected lesions in the chest. In these cases it is often difficult to collect tissues and cells by transbronchial lung biopsy (TBLB), since the lesion cannot be confirmed fluoroscopically or reached with forceps. Consequently, CT-guided needle biopsy (CTNB) is often performed, but pricking of the lung with a biopsy needle may cause fibrosis in the wound-healing process.[1)] It is difficult to distinguish post-CTNB fibrosis from other types of fibrosis, especially tumor-induced desmoplasia, by hematoxylin-eosin (HE) staining.

In 1995, Noguchi et al. reported a 5-year survival rate of 100% for patients with bronchioloalveolar carcinoma (BAC) of 20 mm in diameter or less, but only 74.8% for BAC patients having active fibroblastic proliferation with Noguchi type C. Thereafter, pulmonary adenocarcinoma of 20 mm or less has been diagnosed using a Noguchi

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classification.[2] However, post-CTNB fibrosis leads to confusion between the Noguchi types A and B without active fibroblastic proliferation and type C with it. Consequently, many institutions do not perform preoperative diagnosis, but depend on thoracoscopic lung biopsy in patients with a suspected malignant ground-glass opacity (GGO).[3] In such cases, intraoperative frozen section diagnosis is required to determine the treatment approach; however, this is inappropriate for patients with atypical adenomatous hyperplasia (AAH) and pure GGO of possible types A and B for the following reasons. First, frozen sections used in intraoperative diagnosis are of inferior quality compared to normal formalin-fixed specimens, resulting in difficulty discriminating between benign and malignant lesions and establishing the Noguchi classification.[4] Second, accurate pathological evaluation is important not only for the patient's benefit, but also in research for an improvement of therapy. However, if part of a tumor of 20 mm or less is removed, the remaining lesion is too small to perform formalin fixation and accurate Noguchi classification.[5,6] CTNB provides preoperative diagnosis. This means that an intraoperative frozen section merely for the diagnosis of a lesion is avoidable. In this context, if the discrimination between tumor-induced fibrosis and CTNB-induced fibrosis is possible, a Noguchi classification is more accurately done by the CTNB followed by operation procedure than by intraoperative frozen section.

At the Nippon Medical School Hospital, patients with GGO and a surgical specimen requiring Noguchi classification undergo CTNB.[7] Surgically resected specimens after CTNB are analyzed with elastic fiber staining to discriminate post-CTNB fibrosis from tumor-induced active fibroblastic proliferation. In this paper, we show that CTNB is a useful modality and that a Noguchi classification can be evaluated even in the presence of post-CTNB fibrosis.

Materials and Methods

The subjects were 71 consecutive patients with pulmonary adenocarcinomas 20 mm in diameter or less that were resected surgically after CTNB in the Nippon Medical School Hospital from January 2003 to November 2007. The controls were 24 consecutive patients with pulmonary adenocarcinomas 20 mm in diameter or less who did not undergo preoperative CTNB or TBLB during the same period. CTNB is performed using an 18-gauge needle 9 or 15 cm long (SuperCore™ Biopsy Instrument, Sieman, Osaka). In all control cases, a lung biopsy was performed by partial resection of lesions using video-assisted thoracic surgery (VATS). Each specimen was diagnosed histologically, using World Health Organization (WHO) criteria and the Noguchi classification.[1] Pulmonary adenocarcinomas 20 mm in diameter or less are defined in the Noguchi classification as follows: type A, BAC; type B, BAC with macroscopic foci because of the collapse of the alveolar structure; type C, BAC with active fibroblastic proliferation or mixed BAC and invasive adenocarcinoma; type D, poorly differentiated adenocarcinoma without a BAC component; type E, acinar adenocarcinoma without a BAC component; type F, papillary adenocarcinoma without a BAC component. Formalin-fixed and paraffin-embedded tissue sections containing carcinoma were stained with elastic fiber staining (Elastica Masson Goldner [EMG]) to observe the fibrotic area of the lesion; in EMG staining, elastic fibers turn black and collagen fibers are green. In the lung, elastic fibers are present in the alveoli and in the walls of the blood vessels and bronchi, and their structure is clearly visible (Fig. 1). Using these characteristics, a fibrotic lesion with elastic fibers in structures including alveoli, blood vessels, and bronchioli that are interrupted (as if cut off) can be diagnosed as artificial post-CTNB fibrosis and discriminated from tumor-induced desmoplasia, especially called the active fibroblastic proliferation, which is the hallmark of Noguchi type C (Figs. 2 and 3). The subjects were classified into two groups based on the presence or absence of post-CTNB fibrosis, and correlations among the Noguchi classification, the period from CTNB to surgery, and the presence of elastic fibers in fibrosis were examined.

The unpaired Student's *t*-test was used for statistical analysis between two groups. A probability value P of <0.05 was considered significant.

Results

The 71 subjects comprised 42 males and 29 females from 40 to 85 years old (mean: 67.1). Tumor maximum diameters ranged from 4 to 20 mm (mean 15.0 mm) and the period from CTNB to surgery from 12 to 153 days (mean 57.1). The controls comprised 10 males and 14 females from 34 to 80 years old (mean 65.3), and the diameters of the tumors ranged from 5 to 20 mm (mean 13.8). There was no significant difference in male-female ratio, age, or diameter of carcinoma between the subjects and controls.

Post-CTNB fibrosis was confirmed in 45 patients

Table 1. Comparison between groups with and without identifiable apparent post-CTNB fibrosis

	Total	Post-CTNB fibrosis		P value
		Apparent	Not apparent	
Gender (male/female)	42/29	27/18	15/11	>0.999
Age (years)	40–85 (mean: 67.1)	68.6 ± 9.19*	64.5 ± 8.60*	0.063
Period from CTNB to surgery (days)	12–153 (mean: 57.1)	58.3 ± 19.4*	55.0 ± 16.5*	0.548

*Data are expressed as mean ± standard deviation (SD).
There was no significant differences in gender, age, or period from CTNB to surgery between the two groups.
CTNB, computed-tomography–guided needle biopsy.

(63.4%) (Table 1). The shapes were in ellipsoidal or rectangle form, and they consisted mainly of collagen fibers associated with blood vessels and lymph vessels and with short, thin elastic fibers. No patients had post-CTNB fibrosis containing tumor cells, and no hemorrhages surrounding regions of post-CTNB fibrosis or hemosiderosis were observed. The thicknesses of post-CTNB fibrosis ranged from 0.22 mm to 0.65 mm (mean 0.39). The longer axis of post-CTNB fibrosis ranged from 0.51 mm to 4.3 mm (mean 2.84). The diameter of a needle to use for CTNB was 0.94 mm, and the outer sheath diameter of the needle was 2 mm. In the control group, no lesions indicating post-CTNB fibrosis were observed, except that the interlobular septum had characteristics resembling post-CTNB fibrosis resulting from the similar thickness of these regions and the similar content of mainly collagen fibers. The thickness of interlobular septum ranged from thin as alveolar septum (approximately 20 μm) to thick as post-CTNB fibrosis (approximately 0.6 mm).

The discrimination rates of post-CTNB fibrosis by Noguchi classification are shown in Table 1. Post-CTNB fibrosis was found in 39 of 48 patients (81.3%) with BAC lesions (types A/B/C) and in 6 of 23 patients (26.1%) without BAC lesions (types D/E/F/E + F), showing a significant difference ($P < 0.0001$). In particular, the discrimination rate in 8 patients with a type A tumor was 100% (Table 2, Fig. 4). The mean period from CTNB to surgery in patients with and without post-CTNB fibrosis was 58 and 55 days, respectively, showing no significant difference ($p = 0.55$) (Table 1).

The mean periods from CTNB to surgery in patients having post-CTNB fibrosis with and without thin elastic fibers in the post-CTNB fibrosis area were 65 and 48 days, showing a tendency for an increase in elastic fibers with time ($p = 0.02$) (Table 3). However, the elastic fibers observed in post-CTNB fibrosis were thin and observable only by high-power magnification; they were clearly different from preexisting elastic fibers (Fig. 5).

Discussion

In our hospital, the first choice modality approaching small pulmonary lesion is transbronchial biopsy (TBBx), generally. CTNB is selected for cases in which lesions are not visible under conventional X-ray, are too peripherally located to reach with fiber optic biopsy forceps, or have not reached diagnosis by TBBx. In this context, CTNB is often selected for lesions less than 20 mm in diameter and radiologically showing ground glass opacity. The period from CTNB to operation depends on each case including reasons that are sometimes private. In our hospital, the accuracy of CTNB is 98%.[7]

Primary lung cancer is often associated with elastosis.[8] BACs usually have abundant alveolar elastosis, which causes the CTNB wound to be identifiable, because post-CTNB fibrosis within our observatory period (within 5 months) does not evolve elastosis as abundantly as BAC-associated elastosis does (Fig. 4). Of 71 patients who underwent surgery after CTNB, 45 (63.4%) had post-CTNB fibrosis. In the other 26, post-CTNB fibrosis may not have been detected because the lesions in the tissue through which the needle passed were not cut off, even though multiple sections of each tumor lesion were prepared for diagnosis. However, post-CTNB fibrosis was found in approximately 80% of patients of Noguchi types A, B, and C (and in 100% of type A patients), but only in 26% of patients of types D, E, F, and E + F. This suggests that it might be difficult to detect post-CTNB fibrosis in a non-BAC region, perhaps because of abundant cancer

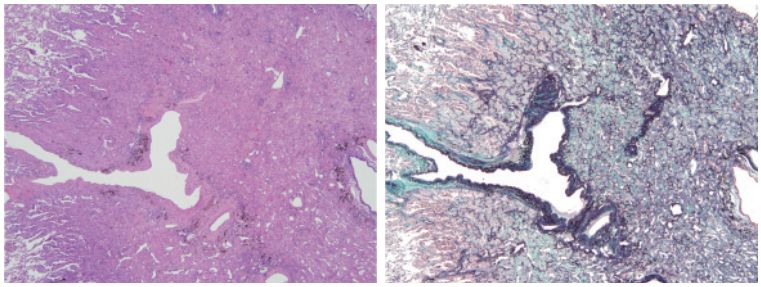

Fig. 1. Low magnification of small adenocarcinoma of the lung, Noguchi type C.
a: Elastic and collagen fibers are both stained pink by HE staining.
b: In Elastica Masson Goldner (EMG) staining, collagen fibers are green and elastic fibers turn black, showing the network of abundant elastic fibers.

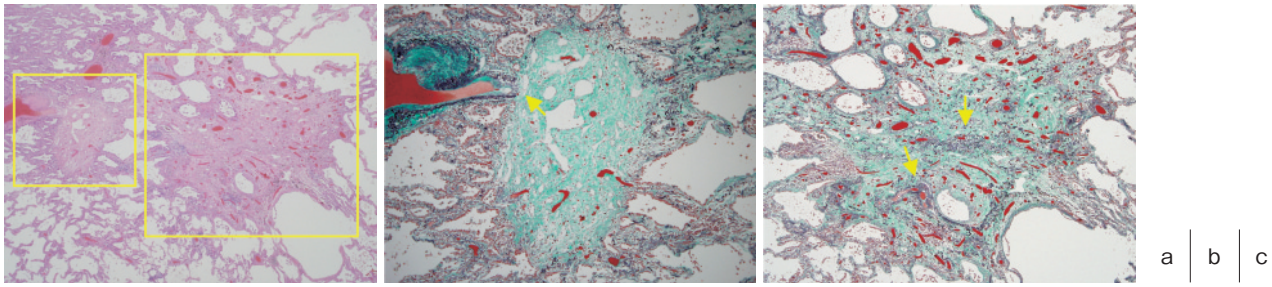

Fig. 2.
a: Two fibrosis lesions in adenocarcinoma (square).
b: High magnification of EMG-stained tissues in **(a)** showing fibrosis in the left section. The surrounding mesh work of elastic fibers has disappeared as if cut off. Elastic fibers in the blood vessel wall are cut off (arrow). These findings indicate post-CTNB fibrosis.
c: High magnification of EMG-stained tissues in **(a)** showing fibrosis in the right square. No cut off of elastic fibers is observed in the lesion. A distribution of thick elastic fibers (arrows) is also noted. These findings suggest tumor-induced fibrosis.

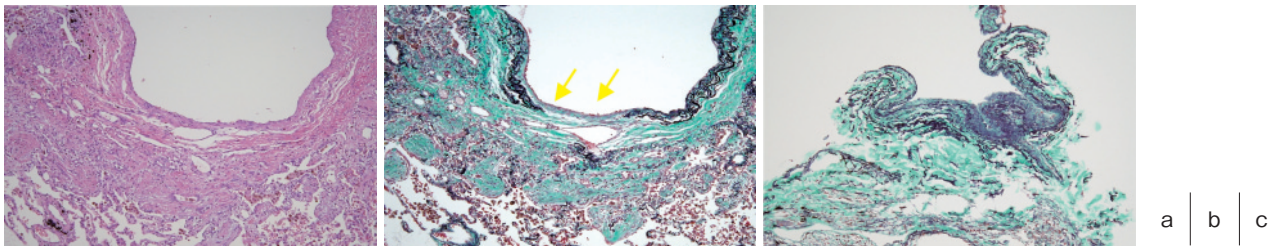

Fig. 3. Comparison between surgical and CTNB specimens.
a,b: Surgical specimen status 41 days post-CTNB with HE and EMG staining. Elastic fibers in the blood vessel are partially lost (cutoff appearance), which indicates post-CTNB fibrosis.
c: CTNB specimens from the same patient, with confirmation of a cut off of the blood vessel in **(b)**.

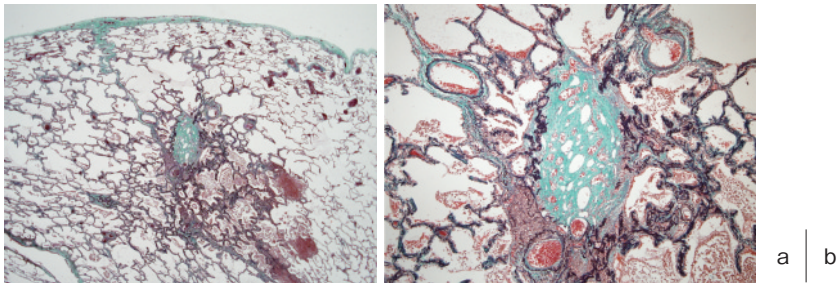

Fig. 4. Post-CTNB fibrosis in BAC (Noguchi type A).
Video-assisted thoracic surgery was performed 153 days after CTNB.
a: EMG staining at low magnification.
b: EMG staining at high magnification. The BAC lesion shows elastosis, and the elastic fibers surrounding the post-CTNB fibrosis are cut off.

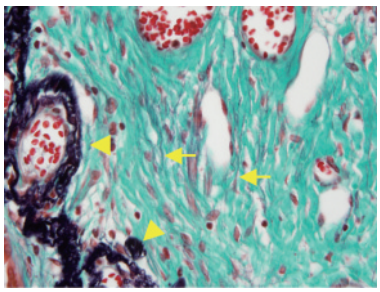

Fig. 5. High-powered view of thin elastic fibers in post-CTNB fibrosis shown in Fig. 4. The thin elastic fibers (arrows) are clearly different from preexisting thick elastic fibers (arrowheads).

Table 2. Discrimination of post-CTNB fibrosis in types A, B, and C lesions with BAC in tumors compared to types D, E, and F lesions without BAC

Noguchi classification	Post-CTNB fibrosis (cases)		Discrimination rate (%)*
	Apparent	Not apparent	
A (BAC without alveolar collapse)	8	0	100
B (BAC with alveolar collapse)	3	1	75
C (mixed BAC and non-BAC adenocarcinomas)	28	8	77.8
D (poorly differentiated adenocarcinoma)	1	3	25
E (acinar adenocarcinoma)	0	4	0
F (papillary adenocarcinoma)	1	2	33.3
E + F (mixed acinar and papillary adenocarcinoma)	4	8	33.3

*P<0.0001 for discrimination of post-CTNB in types A, B, and C lesions with BAC vs. types D, E, and F lesions without it.
CTNB, computed-tomography–guided needle biopsy; BAC, bronchioloalveolar carcinoma.

Table 3. Comparison between groups with and without thin elastosis in post-CTNB fibrosis

	Total	Thin elastosis		P value
		Apparent	Not apparent	
Gender (male/female)	27/18	11/13	16/5	0.076
Age (years)	42–85 (mean: 68.6)	68.4 ± 10.1*	67.8 ± 8.86*	0.831
Period from CTNB to surgery (days)	12–153 (mean: 58.5)	65.7 ± 21.1*	48.8 ± 16.6*	0.021

*Data are expressed as mean ± standard deviation (SD).
There is no significant difference in gender or age between the two groups, but we observed a tendency for the amount of the elastic fibers to increase over time.
CTNB, computed-tomography–guided needle biopsy.

cell proliferation eliminating elastic fibers destroying the surrounding tissues and mixing with tumor-induced desmoplasia. However, this issue is not a serious problem, since the main concern is the distinction of fibrosis in patients of Noguchi types A or B vs. type C after CTNB. Other suggested reasons why post-CTNB fibrosis after surgery was not detected is the thickness of the needle used for CTNB and the wound-healing process. Shown in the results, the longest length of post-CTNB fibrosis was 4.3 mm, even though the needle itself traveled through the lung at least 1 cm at the time of CTNB procedure. This discrepancy may be due to the angle between the direction of needle passage and that of the paraffin-embedded specimen. Moreover, the results showed the discrepancy between the thickness of post-CTNB fibrosis and the thickness of the needle for CTNB. The wound-healing process and the preparation of the specimen may lead to the shrinkage of post-CTNB fibrosis.[9–11)]

There were no significant differences in time from CTNB to surgery between patients with and without identifiable apparent post-CTNB fibrosis. The process of wound healing is known to involve fibrin deposition and inflammatory cell migration immediately after wound formation, and also capillary angiogenesis and fibroblast proliferation with collagen fiber hyperplasia 3 to 4 days later.[9,12)] In this study, the shortest period from CTNB to surgery was 12 days. If a period of approximately one week from surgery to suture removal is allowed, post-CTNB fibrosis can be detected around 7 days post-

operatively. The period from CTNB to surgery is generally 1 to 2 months, and in this study post-CTNB fibrosis was detected 12 to 158 days after CTNB, suggesting that in a regular treatment process it will always be possible to discriminate post-CTNB fibrosis from other fibroses over time.

The difference of the period occurred between groups with apparent and nonapparent thin elastosis in Table 3. This may correspond to the wound-healing process of lung. That is, the period from CTNB to surgery is longer, and the synthesis of elastic fiber is more in this study period. Experimental observations showed that the microscopic appearances of elastic fibers are fewer than collagen fibers during the wound-healing process in lung.[13] In this study, newly synthesized elastic fiber deposition was observed in post-CTNB fibrosis over time, but the fibers were usually thin and observable only with high magnification; i.e., they could be distinguished from elastic fibers existing in the pulmonary tissue, especially in cases with carcinoma-associated elastosis, which we showed in Fig. 2.

Patients with pulmonary adenocarcinomas of 20 mm or less who did not undergo preoperative biopsies were examined as controls, and no fibrosis with abrupt interruption of preexisting elastic fibers was found in them. However, adenocarcinomas that proliferate by destroying elastic fibers show fibrosis without elastic fibers, making these cases difficult to distinguish from post-CTNB fibrosis, which is also characterized by fibrosis with a sudden interruption of preexisting elastic fibers. The interlobular septum consists mainly of collagen fibers, including small blood vessels and lymph vessels, and its thickness is similar to that observed in post-CTNB fibrosis; however, the continuous nature of the interlobular septum allows discrimination from post-CTNB fibrosis.

Based on the above, we conclude that fibrosis after CTNB can be discriminated from tumor-induced active fibroblastic proliferation by elastic fiber staining, and that types A and B BAC in the Noguchi classification can be discriminated from type C BAC with fibroblastic proliferation, even after CTNB.

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