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Characterization of pulmonary cysts in Birt-Hogg-Dubé syndrome: histopathologic and morphometric analysis of 229 pulmonary cysts from 50 unrelated patients

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A short running title: Pathologic features of lung cysts in BHDS

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Abstract

Aims: To characterize pathologic features of pulmonary cysts and to elucidate the possible mechanism of cyst formation in lungs of patients with Birt-Hogg-Dubé syndrome (BHDS), a tumor suppressor gene syndrome, we used histologic and morphometric analyses.

Methods and results: We evaluated 229 lung cysts from 50 patients with BHDS and 117 from 34 patients with primary spontaneous pneumothorax (PSP) for the number, size,

location, and absence or presence of inflammation. These BHDS-cysts abutted interlobular septa (88.2%) and had intracystic septa (13.6%) or protruding venules (39.5%) without cell proliferation or inflammation. Frequencies of these histologic characteristics differed significantly from those of patients with PSP ($P<0.05$). Although the intrapulmonary BHDS-cysts were smaller than the subpleural BHDS-cysts ($P<0.001$), there was no difference in size between them when there was no inflammation. The number of cysts diminished logarithmically and the proportion of cysts with inflammation increased as their individual sizes enlarged ($P<0.05$).

Conclusions: These results imply that the BHDS-cysts are likely to develop at the periacinar region, an anatomically weak site in a primary lobule, where alveoli attach to connective tissue septa. We hypothesize that the BHDS cysts possibly expand in size as the alveolar walls disappear at the alveolo-septal junction and grow even larger when several cysts fuse.

Introduction

Birt-Hogg-Dubé syndrome (BHDS) is an autosomal dominant disorder characterized by hamartomas of the hair follicle, renal tumors, and multiple lung cysts accompanying spontaneous recurrent pneumothorax[1]. The *FLCN* gene responsible for BHDS was cloned in 2002[2], but the function of folliculin, a protein encoded by *FLCN*, is not completely clear. At present, several reports have documented that folliculin-binding proteins (FNIP1 and FNIP2)

involve 5'AMP-activated protein kinase (AMPK) and a mammalian target of rapamycin (mTOR) pathway, and that a complete loss of folliculin function leads to BHDS-associated tumorigenesis through dysregulation of AMPK and the mTOR pathway[3-8].

Clinically, 84-85% of BHDS patients have fibrofolliculoma diagnosed by histologic testing of skin or lung cysts detected by CT imaging of the chest; additionally, 29-34% of these patients have renal tumors visible by CT imaging[1, 9]. In molecular analysis, BHDS-associated renal tumors have a somatic mutation of a second copy of *FLCN*[10], whereas fibrofolliculomas of BHDS patients do not necessarily have a *FLCN* loss of heterozygosity (LOH), indicating that a haploinsufficiency of *FLCN* leads to tumor-like lesions of the hair follicle[11]. In contrast to kidney and skin lesions, pulmonary manifestations appear to be unique, since neither tumor formation nor proliferation of abnormal cells has ever been reported. Instead, multiple cysts are the sole abnormality reported from radiological as well as pathological studies. In addition, high penetrance of lung cysts like fibrofolliculomas[11, 12] may indicate that cysts occur through haploinsufficiency of *FLCN* and that LOH analysis of cysts is not as useful as for renal tumors and fibrofolliculomas.

The mechanism of cyst formation in BHDS is not well understood. Therefore, we believe it crucial to define histopathological findings for these cysts and underlying parenchyma from a large number of BHDS patients. Previously, in lung specimens from such patients, bullae or blebs were found underlying emphysematous changes[13-15]; thin-walled

cysts were surrounded by normal parenchyma[16-18], or the cysts manifested a predominance of type II pneumocyte-like cuboidal cells[4]. However, the number of BHDS patients examined in those studies was small, and the focus might have been on pleura or subpleural cysts for which pathological findings would be profoundly influenced by pneumothorax and pneumothorax-associated inflammation.

Here we report the histologic and morphometric characteristics of 229 lung cysts from 50 patients with BHDS, the largest cohort of BHDS patients ever included in a determination of lung pathology.

Materials and methods

Lung specimens were obtained from 50 Asian patients (49 Japanese and 1 Chinese) with BHDS from the archives or consultation files in the Pneumothorax Center, Tamagawa Hospital, and Division of Respiratory Medicine, Juntendo University Faculty of Medicine and Graduate School of Medicine (Table 1). BHDS was diagnosed by applying *FLCN* genetic tests as described previously[19, 20]. The age (median) at operation in 50 patients was 38.5 years ranging from 24 to 66 (38 ranging from 27 to 50 in 19 men; 41 ranging from 24 to 66 in 31 women) (Table 1). Thirteen patients were smokers, 4 were ex-smokers, 30 had never smoked, and 3 lacked any documented smoking history. A total of 229 lung cysts (79 in men, 150 in women) were identified in the 350 tissue sections we examined: ranging from 1 to 15 tissue

sections per patient, 4 cysts ranging from 1 to 15 per patient, and 0.6 ranging from 0.1 to 3 of a cyst per tissue section.

Lung tissues were obtained with video-assisted thoracic surgery (VATS) for the treatment of pneumothorax or for the diagnosis of cystic lung diseases, appropriately inflated, and fixed with 10%-buffered formaldehyde. After routine preparation, the formalin-fixed paraffin-embedded tissues were sectioned and stained with Hematoxyline-eosin and Elastica-Masson trichrome (EM) or Elastica-van Gieson (EVG) stains. We evaluated chronic inflammation in each cyst on low power field (4x objective lens attached to BX51, Olympus Corp., Tokyo, Japan): the presence of cellular inflammation defined by accumulation of lymphocytes or plasma cells; the presence of fibrous inflammation defined by dense (sub)pleural scars and/or lung tissue fibrosed with replacement of architecture [21]. We measured the maximum diameter of each cyst on the tissues stained with EM or EVG by using the ocular micrometer on a microscope (U-OCM10/100, Olympus Co. Ltd., Tokyo, Japan) or the micrometer calipers on a glass slide (Shinwa Co. Ltd., Nagoya, Japan). As a control for the analysis of pulmonary cysts, lung tissues from 34 Japanese patients with primary spontaneous pneumothorax (PSP) in the Japanese Red Cross Medical Center were used. Median age of 34 patients with PSP including 33 men and a woman (25 year old) was 24 ranging from 18 to 30. All of the 117 cysts associated with PSP were diagnosed as bullae

and/or blebs. This study was approved by the ethical committee in Juntendo University School of Medicine (No.17053) and by the ethical committee for clinical studies in the Japanese Red Cross Medical Center (No.429).

Statistical analysis was performed by using the Mann-Whitney U test and χ^2 test (StatMate III for Windows, ATMS Co. Ltd., Tokyo, Japan), or Kruskal Wallis test (IBM SPSS Statistics, IBM Japan, Tokyo, Japan). A P value less than 0.05 was considered to be statistically significant.

Results

Histological characteristics

The lung tissues obtained from 45 of 50 patients with BHDS had normal parenchyma, whereas those from the other 5 had centrilobular emphysema (2 patients), granuloma (2), and fibrosis (1) in the parenchyma (Table 1, and data not shown). Macroscopic findings demonstrated that the lung cysts, which occasionally contained intracystic septa composed of interlibular septa, had very thin and translucent walls and were surrounded by normal lung parenchyma in all patients (Figs.1A and 1B). The intracystic septa seen in 13.6% of BHDS cysts were composed of interlobular septa and the venules protruding into the cyst (seen in 39.5% of BHDS cysts) sometimes had the surrounding connective tissue being regressed (Fig.1C). The anatomical and histological findings were

characterized by the following features (Table 2): half of the lung cysts were located in the subpleural area (Fig. 1D) and the remaining in the intrapulmonary area (Fig. 1E); the cysts abutted interlobular septa but rarely bronchioles. The cysts of BHDS, especially those located in lung parenchyma, were not at all or little affected with inflammation including fibrosis (Fig. 1F), although approximately one-third of subpleural cysts showed mild inflammation including fibroblast proliferation (Fig. 1G) and lymphocyte infiltration (Fig. 1H). When compared with the pathological findings of pulmonary cysts (bullae or bleb) of PSP patients, the BHDS groups' pulmonary cysts exhibited far more clearly defined and, in fact, statistically significant pathological features (Table 2). In BHDS, 1) cysts were distributed at subpleural as well as intrapulmonary areas, 2) cysts frequently abutted interlobular septa, often had venules protruding into the cyst, and occasionally accompanied intracystic septa, suggesting the periacinar development of cysts in a primary lobule; however, 3) cysts usually had no sign of inflammation, especially those at intrapulmonary areas.

Next, BHDS and PSP patients were compared for the pathological features (inflammatory site and type) of subpleural cysts with inflammation (Fig.2). In fact, most cysts from PSP patients were located at subpleural areas and had inflammatory infiltrates. Subpleural BHDS cysts that were inflamed were less likely to have such inflammation (especially fibrous type) at a basal site (i.e., proximal part of subpleural cyst) (Table 3). The

most prominent feature of subpleural BHDS cysts that distinguished them from PSP cysts was the former's virtual absence of fibrous inflammation at the basal area with 94.8% of sensitivity and 92.2% of specificity.

Morphometric analysis

We examined the histologic features of cysts in terms of its size and location in the lung parenchyma. The maximum diameter of cysts associated with BHDS ranged from 1.0 to 15.7 (median, 3.8) millimeters (mm) and two-thirds of them were equal to or less than 5 mm. A histogram depicting our analysis of size showed that the number of the cysts logarithmically diminished as maximum cyst size increased (correlation coefficient for the fitted curve, $y = -23.3\ln(x) + 63.0$, $R^2 = 0.925$) (Fig. 3). In addition, the proportion of cysts positive for inflammation increased as the maximum cyst size increased. However, no significant difference was noted in maximal cyst size between men and women (median, 4.0 mm ranging from 1 to 15.7 vs. 3.5 mm ranging from 1.0 to 13.2, $P = 0.6908$) or between patients with or without a history of smoking (median, 4.0 mm ranging from 1.0 to 12.6 vs. 3.4 mm ranging from 1.0 to 15.7, $P = 0.1508$). Meanwhile, statistical significance was evident for the larger size of subpleural cysts compared to intrapulmonary cysts (median, 5.0 mm ranging from 1.0 to 15.7 vs. 3.0 mm ranging from 1.0 to 9.8, $P < 0.0001$) and for the larger size of cysts with inflammation than those without inflammation (median, 4.7 mm ranging

from 1.1 to 15.7 mm vs. 3.3 mm ranging 1.0 to 9.8, $P < 0.0001$). When we evaluated the influence of location or inflammation on maximum cyst size, the results demonstrated that subpleural cysts with inflammation were significantly larger than those without inflammation. However, the size of intrapulmonary cysts was not affected by the presence or absence of inflammation, and the size of subpleural cysts without inflammation resembled that of non-inflamed intrapulmonary cysts (Fig. 4).

Discussion

We have demonstrated the unique histologic characteristics of pulmonary cysts from 50 unrelated patients with BHDS, the largest cohort ever included in a study of lung pathology focused on BDHS. Our results document that pulmonary BHDS cysts are 1) surrounded by normal alveolar walls; 2) abut interlobular septa, and 3) may have intracystic septa and/or protrusion of venules into the cystic space, indicating disappearance of the surrounding alveolar wall and/or regression of connective tissue of interlobular septa. These histologic characteristics can differentiate BHDS from other cystic lung diseases. For example, tuberous sclerosis complex (TSC)-associated lymphangioleiomyomatosis (LAM) always shows LAM cell proliferation on the cyst walls. In other hereditary cystic lung diseases such as cystic fibrosis, Ehlers-Danlos syndrome, and Malfan syndrome, patients have nonspecific cystic lesions with cellular or fibrous inflammation[22-24]. In the non-

hereditary lung cystic diseases including Langerhans cell histiocytosis, amyloidosis, Sjögren syndrome, and lymphocytic interstitial pneumonia, the infiltration of inflammatory cells and/or matrix deposition always occur[25].

The present study clearly establishes that neither inflammation nor cell proliferation contributes to cyst formation in patients with BHDS, because most of their cysts, especially intrapulmonary BHDS cysts that do not suffer from the secondary effects of pneumothorax, show neither inflammation nor abnormal cell proliferation. Since the majority of BHDS cysts are located far from bronchioles, the mechanism for cyst formation in BHDS is less likely to be associated with a check-valve mechanism, which is supposedly operative on cyst formation in PSP, smoking-related diseases, Sjögren syndrome, or other non-hereditary cystic lung diseases. We demonstrated in the present study that the most of the intrapulmonary BHDS cysts (88/113, 77.9%) lacks inflammation whereas only approximately one-third of the subpleural cysts (37/116, 31.9%) have no inflammation (Table 2). Accordingly we think that most if not all the inflammatory changes observed would be associated with pneumothorax. It has already been described well that pneumothorax caused pleural inflammation especially at the pleural side of cyst not at the basal side of cyst[26]. In addition, there is an animal experiment clearly showing that repeated injection of air into pleural space caused inflammation and formed “neomembranes” composed of fibroblast and collagens that

was variably covered by proliferation of mesothelial cells[27]. In addition, the findings that the BHDS cysts without inflammation had no significant difference in their size, irrespective of their locations whether subpleural or intrapulmpnary (Fig. 4), suggesting that inflammation secondary to pneumothorax is likely to contribute to the subsequent growth of subpleural cysts in BHDS. Interestingly, we have demonstrated a logarithmical decline in the number of cysts examined as the size of individual cysts increased. Possibly the fusion of small cysts resulted in the enlargement we noted. That process may also explain how intracystic septa develop in BHDS cysts, since abutted cysts could fuse with intervening interlobular septa. In this context, venules protruding into about 40% of the BHDS cysts we found may be caused by not only the disappearance of alveolar walls adjoining interlobular septa but also regression of their surrounding connective tissue in the septa.

The mechanism for development of pulmonary cysts in BHDS has been discussed in several reports. Graham et al[14]. speculated that a genetic abnormality was responsible for postnatal alveolar proliferation of the peripheral lung based on pathologic examination of three BHDS nonsmokers, since they found cysts predominantly in the subpleural area. Warren et al.[28] found *FLCN* expressed in type I pneumocytes and stromal cells including fibroblasts and macrophages in the lungs, hence proposed a possible role for functional abnormalities of these folliculin-expressing cells in cyst formation. Recently, Furuya et al.[4]

reported that dysregulation of the mTOR pathway through haploinsufficiency of the *FLCN* gene may induce cyst formation by proliferation of type II pneumocytes. However, the foregoing mechanisms were deduced from an examination of limited numbers of lung specimen, therefore, are unlikely to fit the histopathologic features of BHDS cysts and lungs defined after detailed analysis of the much larger specimen sampling presented here. For example, cysts are distributed not only at subpleural areas but also in parenchyma, and most cysts have neither cellular proliferation nor inflammation, especially cysts of a parenchymal area where secondary changes due to pneumothorax would not affect pathologic findings in contrast to cysts at subpleural sites. In addition, when bullae/blebs are affected with pneumothorax, they will usually have reactive proliferation of type II pneumocytes. If proliferation of type II pneumocytes were actively involved in cyst formation, those cysts would show no predilection for the location and distribution recorded here; instead, they should be detectable at not only the area surrounded by interlobular septa but also the centrilobular area. Furthermore, one would expect cyst formation to proceed by proteolysis as in smoke-related inflammatory diseases, collagen diseases, or neoplastic diseases like LAM[29]. Otherwise, the proliferation of type II pneumocytes might form a lung tumor like multifocal micronodular pneumocyte hyperplasia occurring via dysregulation of the mTOR pathway in patients with TSC[18]. However, in the present study, lung specimens from BHDS patients showed no destruction of lung architecture by either proliferating type II pneumocytes or

inflammation, indicating that BHDS cysts may not develop through proliferation of type II pneumocytes or proteolysis mediated by proliferating type II pneumocytes.

Presumably, considering the unique histologic characteristics of BHDS cysts defined by the present study, almost all cysts abutting interlobular septa without significant inflammation should ensue naturally from the inherent mechanism of cyst formation. In this context, we postulate that a *FLCN* mutation causes the abnormal alveolo-septal junction in a parenchymal structure of the lungs. Several reports describe folliculin, a protein encoded by the *FLCN* gene, as a regulator of TGF-β signaling, especially TGF-β_2[30], or cell-cell adhesion through the interaction with adherence junction protein[31]. TGF-β_2 is involved in epithelial-mesenchymal interactions, cell growth, production of extracellular matrix proteins, and tissue remodeling during development or normal subepithelial matrix homeostasis[32, 33]. Warren et al.[28] demonstrated that *FLCN* mRNA was strongly expressed in stromal cells within the connective tissue and weakly in type I pneumocytes in the lung. This topographic expression pattern indicates that folliculin may participate in maintaining the homeostasis of alveolar walls composed of stromal cells and/or type I pneumocytes. Actually, we recently revealed that lung fibroblasts isolated from patients with BHDS showed diminished migration as well as decreased expression of TGFβ and extracellular matrix proteins (Manuscript in preparation). These findings may imply that the alveolo-septal junction becomes vulnerable

to mechanical forces throughout the entire alveolar wall meshwork during respiratory cycles of inflation and deflation, leading to cyst formation due to the disruption of alveolar homeostasis. Supporting this notion is that the periacinar regions where alveoli attach to connective tissue septa are weak from an anatomical as well as physiological viewpoint. Capillaries in alveolar walls abutting connective tissue septa or visceral pleura are less numerous than elsewhere and then the periacinar regions have less vascularity and greater compliance[34]. Furthermore, our hypothesis generated by histopathological study seems well-formulated to explain the results of our previous radiological study that BHDS cysts are irregularly-shaped rather than oval and predominantly located in the lower medial lung zone[35]: 1) a cyst's periacinar development would contribute to its irregular shape caused by imbalanced elastic recoil forces of remaining alveolar tissues within an unit structure of secondary lobule[36], and 2) greater mechanical force would be loaded onto the lower lobes than upper lobes during respiratory cycles. However, further studies are needed to confirm this hypothesis.

In conclusion, we have defined the unique histological features of pulmonary cysts in patients with BHDS, almost all cysts abutting interlobular septa without significant inflammation. The 229 BHDS cysts derived from lung specimens of 50 unrelated patients surveyed here constitute the most extensive of such studies.

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Figure legends

Figure 1. Representative pathological findings of pulmonary cysts from patients with BHDS. The cyst at the subpleural area has, macroscopically, a very thin, translucent wall with an intracystic septum indicated by a white arrow (scale span: 5 mm) (A). The cyst pictured in Fig. 1A was located at the area adjacent to an interlobular septum including pulmonary veins and has a very thin intracystic septum (indicated by black arrow) (B, Elastica-Masson trichrome stain). Vessels in the interlobular septa frequently protrude into the cyst. Note that the surrounding connective tissue of the vessel is decreased (indicated by small black arrow) (C, Elastica-Masson trichrome stain). The two subpleural cysts abut interlobular septum (small arrowheads) and the opposite side of each cyst wall is composed of thin pleural wall (D, CL indicates a centrilobular area). The intrapulmonary cyst abuts interlobular septum (large arrowheads) and the other side of cyst wall is composed of thin alveolar wall (E, CL indicates a centrilobular area.). Approximately half of all cysts we examined in this study were composed of normal alveolar walls with neither cell proliferation nor inflammatory cell

infiltrates (F, * indicates intracystic area). However, some cysts from BHDS have inflammations and the representative photomicrographs of subpleural cysts are presented in parts (G and H). The basal side of a subpleural cyst abuts interlobular septum without inflammation whereas its pleural side showed thickened visceral pleura with fibroblast proliferation (G). Very thin wall of a subpleural cyst showed no fibrous thickening but lymphocyte infiltration (H).

Figure 2. The representative photomicrographs showing cyst-associated cellular and/or fibrous inflammation: no inflammation (A from an intrapulmonary cyst in BHDS: note the cyst abuts an interlobular septum in the upper area); cellular inflammation (B from a subpleural cyst in BHDS); fibrous inflammation (C from bullae in PSP); and cellular and fibrous inflammation (D from bullae in PSP) (x4 objective lens).

Figure 3. Distribution of the maximum diameter of pulmonary cysts in patients with BHDS. Black or gray columns indicate the number of pulmonary cysts with or without inflammation, respectively.

Figure 4. Comparison of the maximum diameter between intrapulmonary and subpleural cysts in patients with BHDS.

Table 1. Case summary

No.	Age	Sex	Smoking history	Location	*FLCN* mutation	Number of tissue sections	Number of cysts	Other findings
1	38	M	S	exon 4	c.119delG	7	2	
2	38	F	S	exon 5	c.328C>T	1	1	
3	38	F	S	intron 5	c.396+1G>A	3	3	
4	29	M	N	intron 5	c.397-2A>C	8	1	

5	36	F	U	exon 6	c.397-13_397-4del GGCCCTCCAG	1	3	
6	37	F	U	exon 6	c.402delC	2	3	
7	39	F	S	exon 7	c.769_771delTCC	7	8	
8	40	F	N	exon 7	c.769_771delTCC	9	8	
9	47	F	N	exon 8	c.853C>T	7	1	Fibrosis
10	38	F	N	exon 9	c.889_890delGA	7	5	
12	48	F	N	exon 9	c.932_933delCT	6	3	
11	44	F	N	exon 9	c.991_992dupTC	10	11	
13	48	M	N	exon 9	c.997_998delTC	8	4	
14	34	M	S	intron 9	c.1063-2A>G	8	8	Emphysema
15	53	F	N	exon 10	c.1063-10_1065del TCTTGTTTAGGTC	5	6	
16	24	F	S	exon 11	c.1285dupC	6	1	
17	29	M	S	exon 11	c.1285dupC	4	2	
18	33	F	N	exon 11	c.1285dupC	7	1	Granuloma
19	35	F	S	exon 11	c.1285dupC	7	13	
20	35	M	N	exon 11	c.1285dupC	3	1	
21	38	M	S	exon 11	c.1285dupC	5	3	
22	39	M	S	exon 11	c.1285dupC	12	6	
23	41	F	N	exon 11	c.1285dupC	20	9	
24	43	F	N	exon 11	c.1285dupC	4	8	
25	47	F	N	exon 11	c.1285dupC	6	1	
26	50	M	N	exon 11	c.1285dupC	10	6	
27	62	F	N	exon 11	c.1285dupC	4	1	
28	64	F	S	exon 11	c.1285dupC	24	15	Granuloma
29	31	F	N	exon 12	c.1347_1353dupCCACCCT	5	4	
30	32	M	U	exon 12	c.1347_1353dupCCACCCT	3	7	
31	38	F	N	exon 12	c.1347_1353dupCCACCCT	15	5	
32	42	F	S	exon 12	c.1347_1353dupCCACCCT	7	8	
33	43	F	S	exon 12	c.1347_1353dupCCACCCT	7	5	
34	43	F	N	exon 12	c.1347_1353dupCCACCCT	5	6	
35	45	F	N	exon 12	c.1347_1353dupCCACCCT	8	4	
36	48	M	S	exon 12	c.1347_1353dupCCACCCT	6	4	
37	57	F	N	exon 12	c.1347_1353dupCCACCCT	5	4	
38	66	F	N	exon 12	c.1347_1353dupCCACCCT	4	2	Emphysema
39	46	M	N	exon 12	c.1429C>T	5	2	
40	32	F	N	intron 12	c.1433-1G>T	8	7	
46	43	M	N	exon 13	c.1489_1490delGT	4	1	
41	26	F	S	exon 13	c.1533_1536delGATG	1	1	
42	27	M	S	exon 13	c.1533_1536delGATG	5	1	
43	34	M	N	exon 13	c.1533_1536delGATA	15	6	
44	35	M	S	exon 13	c.1533_1536delGATG	21	8	
45	38	M	N	exon 13	c.1533_1536delGATG	4	3	
47	29	M	N	exon 14	c.1539-?_c.1740+?del	8	8	
48	46	M	N	exon 14	c.1539-?_c.1740+?del	6	6	
49	31	F	N	exons 9 - 14	c.872-?_c.1740+?del	6	2	
50	58	F	N	exons 9 - 14	c.872-?_c.1740+?del	1	1	

Abbreviations used are: S, current smoker; N, never smoker; and U, unknown.

Table 2. Comparison of the number of cyst in the lung specimen between BHDS and PSP.

Histological findings	Cysts with BHDS (n = 229)	Cysts with PSP (n = 117)	χ^2 test
Cysts located at			
subpleural area	116 (50.7%)	115 (98.3%)	P < 0.001
intrapulmonary area	113 (49.3%)	2 (1.7%)	
Cysts abutting on			
interlobular septa	202 (88.2%)	16 (13.7%)	P < 0.001
bronchiole	11 (4.8%)	42 (35.9%)	P < 0.001
Intracystic septa	31 (13.6%)	0 (0%)	P < 0.001
Venules protruding into the cyst	90 (39.5%)	2 (1.7%)	P < 0.001
Cyst without inflammation			
total	125/229 (54.6%)	2/117 (1.7%)	P < 0.001
subpleural area	37/116 (31.9%)	2/115 (1.7%)	P < 0.001
intrapulmonary area	88/113 (77.9%)*	0/2 (0%)	NS (P = 0.177)

*P < 0.001 as comparing number of the cysts without inflammation between the location at subpleural and intrapulmonary area.

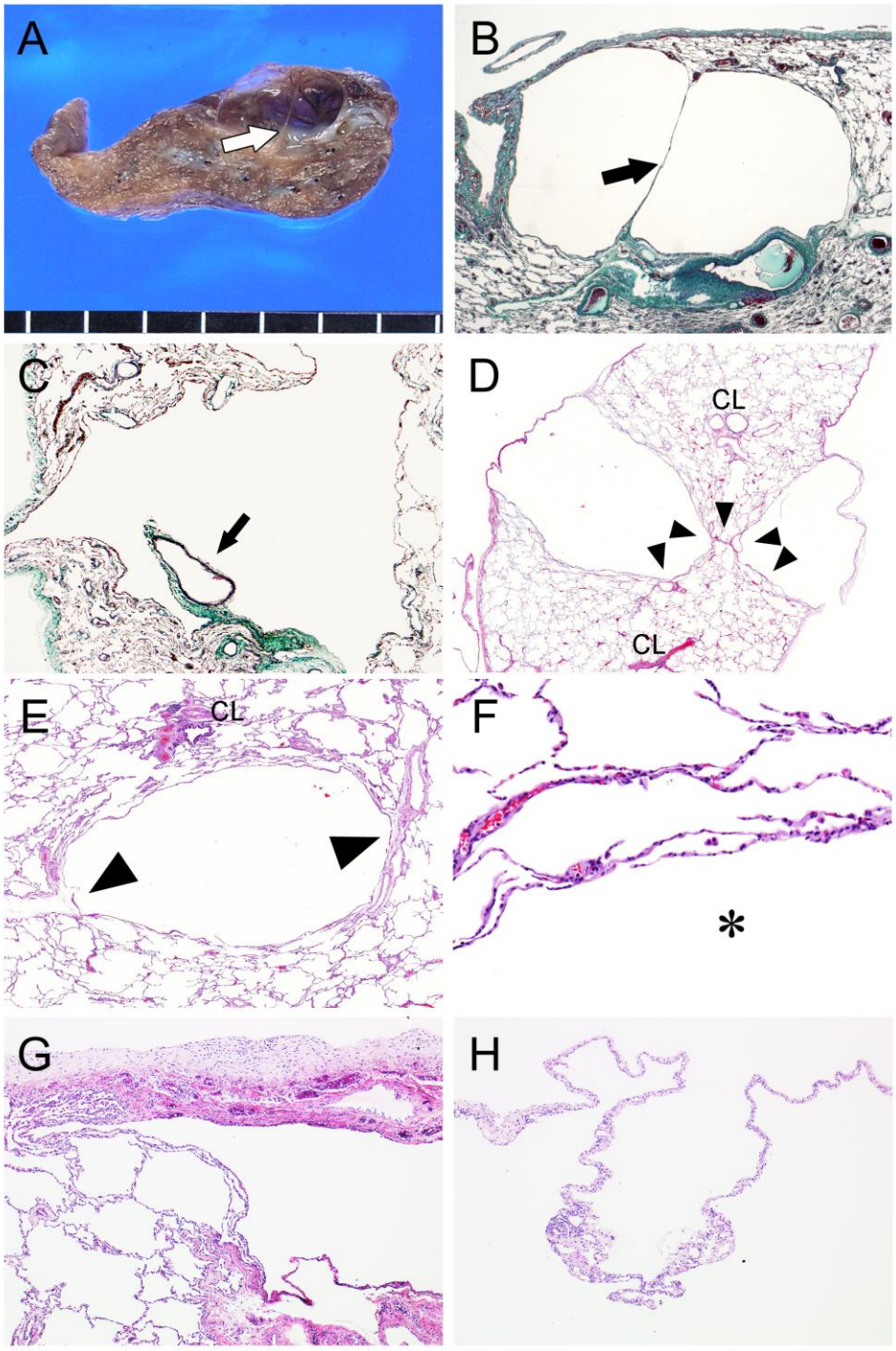
NS: Not significant

Table 3. Comparison of the number of subpleural cysts with inflammation in BHDS and PSP

	The number of subpleural cysts examined	The number of cysts with inflammation	Inflammation at pleural site		Inflammation at basal site	
			Cellular	Fibrous	Cellular	Fibrous
BHDS	116	79 (68.1%)	75 (64.7%)	55 (47.4%)	19 (16.4%)	6 (5.2%)
PSP	115	115 (100%)	73 (63.5%)	115 (100%)	56 (48.7%)	106 (92.2%)
χ^2 test		P < 0.001	P = 0.852	P < 0.001	P < 0.001	P < 0.001

Inflammation was examined with regard to the part of cyst (pleural or basal site) and type of inflammation (cellular or fibrous).

The number in parenthesis indicates the percentage of cyst with specified feature in BHDS and PSP, respectively.



Flg. 1

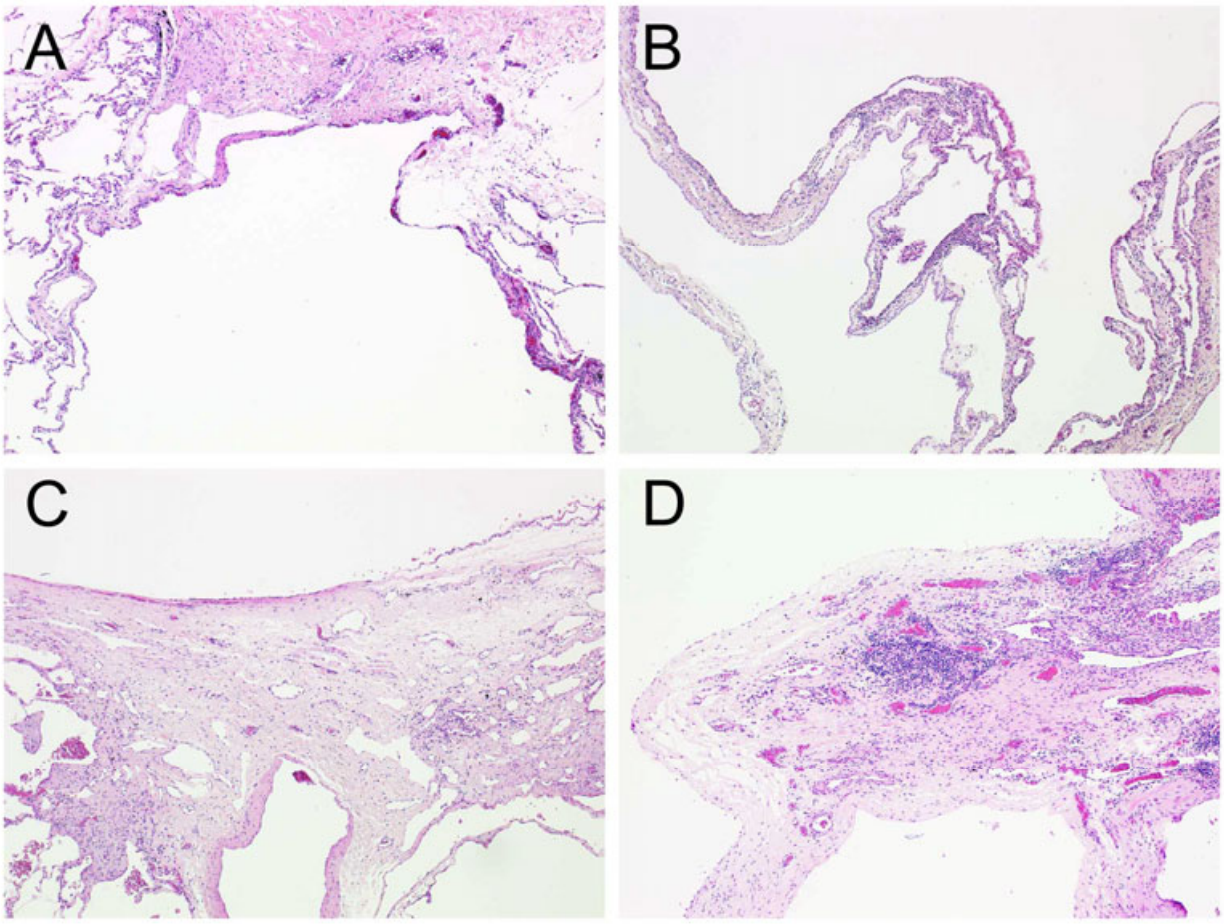


Fig. 2

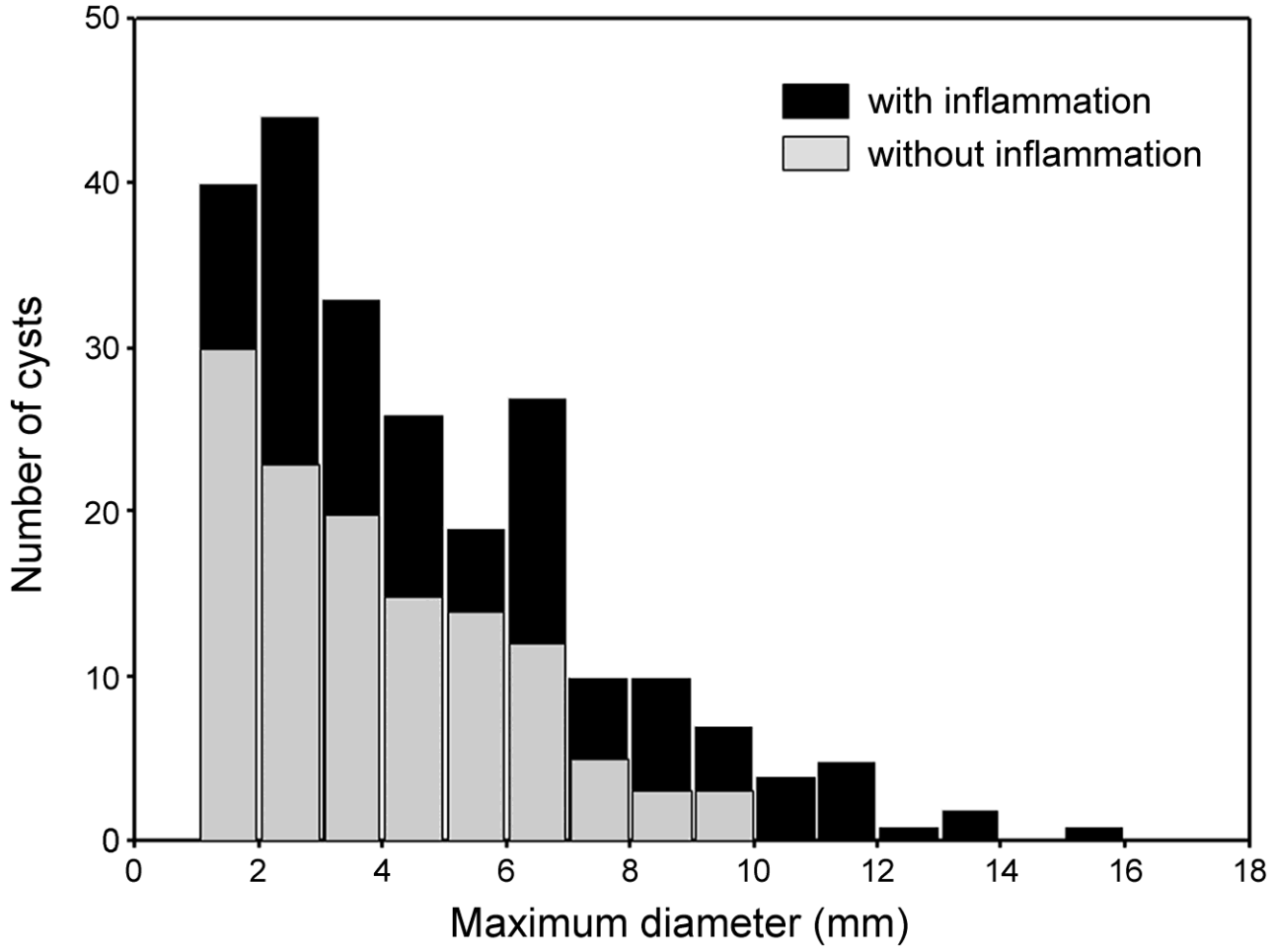


Fig. 3

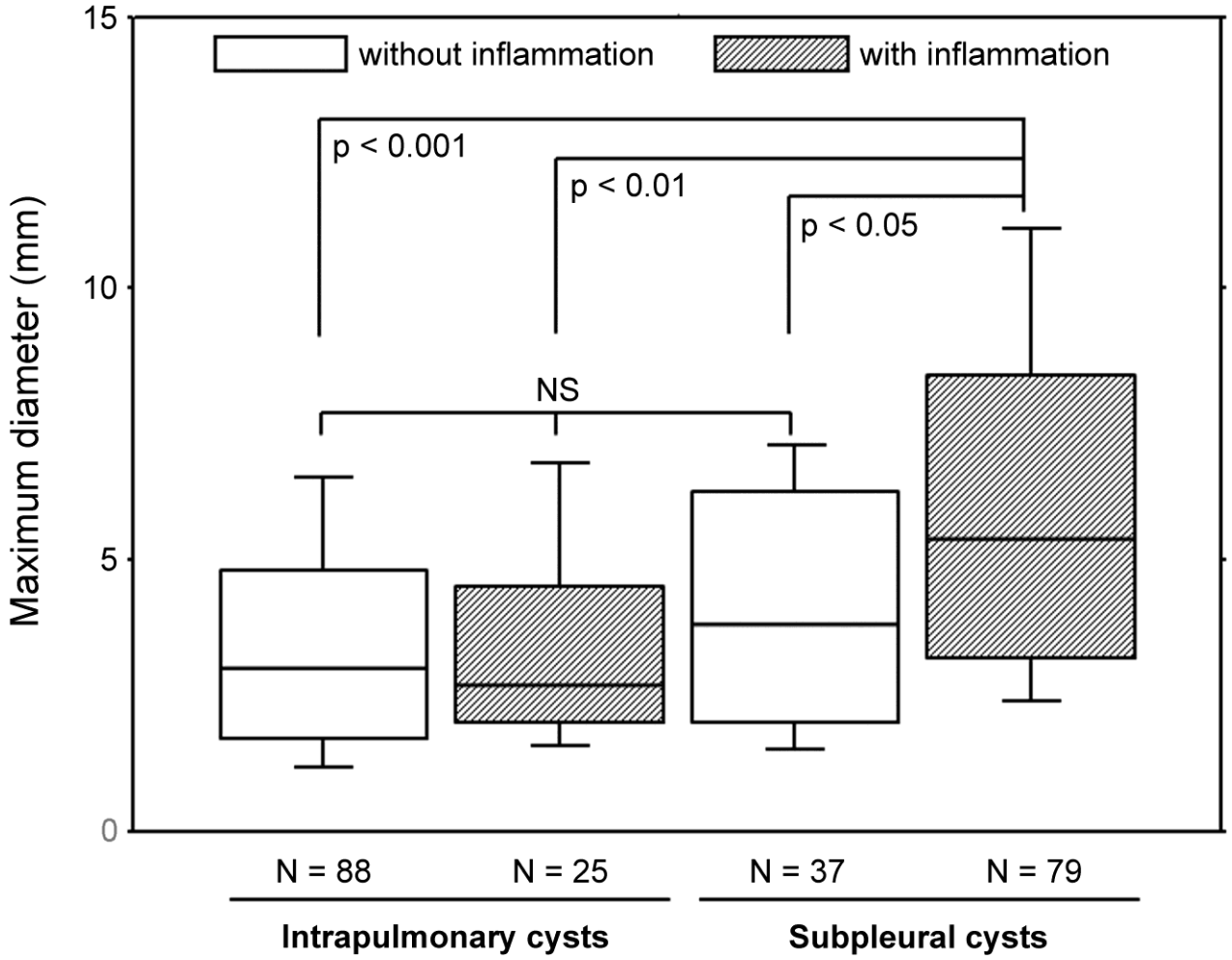


Fig. 4