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Features of Lung Cyst in Birt-Hogg-Dubé Syndrome from Patients with Multiple Lung Cysts

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Abstract

Background: High-resolution chest computed tomography (CT) is a crucial assessment tool for diagnosing Birt-Hogg-Dubé (BHD) syndrome. This study aimed to analyze differences of lung cysts between BHD and other cystic lung diseases.
Methods: From January 2020 to December 2022, patients with multiple lung cysts who underwent chest CT at Gangnam Severance Hospital were included.
Results: Over a 3-year period (from January 2020 to December 2022), out of 52,823 patients who underwent a chest CT scan, 301 (0.6%) patients with multiple lung cysts were enrolled in this study. Of enrolled patients, 24 (8.0%) were diagnosed with BHD. In patients with BHD, 95.8% exhibited bilateral cysts, and 83.3% showed basal predominance. The cysts' maximal diameter averaged 32.1 mm (interquartile range, 26.5 to 43.5). Additionally, 95.8% of patients with BHD had diverse cyst sizes and morphologies. Multivariate logistic regression analysis revealed that bilateral cysts (odds ratio [OR], 12.393; 95% confidence interval [CI], 1.613 to 274.682; p=0.038), basal predominance (OR, 8.511; 95% CI, 2.252 to 39.392; p=0.002), maximum diameter (OR, 1.053; 95% CI, 1.009 to 1.108; p=0.032), and diversity of morphology (OR, 19.513; 95% CI, 2.833 to 398.119; p=0.010) were significant factors associated with BHD diagnosis. A multivariate prediction model for BHD diagnosis demonstrated a sensitivity of 95.83%, a specificity of 81.22%, and an area under the receiver operating characteristic curve of 0.951 (95% CI, 0.914 to 0.987).
Conclusion: Distinguishing features of lung cysts from other cystic lung diseases include bilateral cysts, basal dominance, large size, and irregular shape.

Keywords: Birt-Hogg-Dube Syndrome; Cystic Lung Disease; Chest Computed Tomography; FLCN Mutation

Introduction

Birt-Hogg-Dubé (BHD) syndrome is a rare genetic condition first described in 1977. Characterized by mutations in the folliculin (*FLCN*) gene, BHD syndrome has various clinical manifestations, including skin lesions, renal tumors, and pulmonary cysts[1,2]. While the exact incidence of BHD syndrome remains unknown, its prevalence has been estimated to be around 1 in 200,000 individuals[3]. With increasing awareness of this disease, the incidence of BHD syndrome is also gradually increasing[4]. However, it is believed that many patients remain undiagnosed[5].

High-resolution chest computed tomography (CT) is a crucial diagnostic tool in the initial assessment of BHD syndrome[2,6]. For patients with BHD syndrome,

their lung cysts are typically bilateral and preferentially located in the lower and medial lung, often around the costophrenic sulci[6]. These lung cysts demonstrate significant variabilities in their sizes, with some exceeding 2 cm, despite the majority having sizes less than 1 cm[7]. Furthermore, the morphology of lung cysts in BHD syndrome can range from oval, round, and lenticular, to irregular[7].

Although numerous studies have been conducted on BHD syndrome in other countries, characteristics of BHD syndrome-related chest CT findings in the Korean population remain insufficiently elucidated. Recently, we have described radiological features of lung cysts in Korean patients with BHD syndrome[4,8]. However, these studies had limitations due to their small sizes of patient cohort and the selection of patients with multiple lung cysts according to their study design, rather than all patients with multiple lung cysts.

Therefore, this study aimed to analyze radiological differences between BHD syndrome and other cystic lung diseases in patients who underwent chest CT scans over a 3-year period at a single-center. Based on results of analysis, we aim to develop a model for screening BHD patients using CT image findings.

Materials and Methods

1. Patients

From January 2020 to December 2022, a retrospective screening was conducted for all patients who underwent chest CT scans at Gangnam Severance Hospital with their scans interpreted by radiologists. All patients with multiple lung cysts were included. The electronic medical record portal system (Severance Clinical Research Analysis Portal program [SCRAP] 2.0) and Order Communication System within the clinical data retrieval system for all patients visiting Gangnam Severance Hospital were used to search for information. Both radiologists' reports and descriptions were screened for the term 'cyst' in all chest CT scans. From these, records of CT findings indicating the presence of multiple lung cysts were re-screened and images were ultimately reviewed to finalize the enrollment.

Clinical data, including demographic details (age, sex, height, weight), medical history (comorbidities classified by Charlson comorbidity index using Quan et al.'s algorithm[9]), median laboratory results (complete blood count, blood urea nitrogen [BUN], creatinine, aspartate aminotransferase [AST], alanine aminotransferase [ALT], C-reactive protein [CRP]), and pulmonary function test (PFT) results were collected. Additionally, results of next-generation sequencing were analyzed for diagnosing BHD. Diagnoses of lymphangioleiomyomatosis (LAM) and pulmonary Langerhans cell histiocytosis (PLCH) were made based on the International Classification of Diseases, 10th Revision codes, confirmed biopsy results, or gene mutations (e.g., *TSC* mutations for LAM) according to guidelines or literature for each disease[10,11].

2. CT protocol and analysis

Chest CT scans were conducted with either a 64-slice Multi-Detector CT scanner (Somatom Sensation 64, Siemens Medical Solutions, Erlangen, Germany) or a 128-slice Multi-Detector CT scanner (Somatom Sensation AS+, Siemens Solutions; or Ingenuity Core 128, Philips Healthcare, Cleveland, OH, USA). Initial scout images were captured to establish the field-of-view, employing a mediastinal window setting with a reconstruction interval of 1 to 3 mm. Scanning parameters were uniformly set at 120 kVp, with mA levels adjustable from 100 to 200 and slice thickness from 1 to 3 mm. The image reconstruction process for standard CT scans was performed using the scanner's workstation. All CT images were stored and managed with a picture archiving and communication system (Centricity 4.0, GE Medical Systems, Mountain Prospect, IL, USA).

A lung cyst was defined as an air-filled lesion identifiable by its wall, which would not exceed 3 mm in thickness. The presence of multiple or diffuse cysts was noted when numerous cysts appeared to be distributed across both lungs. The number of lung cysts was categorized as follows: <10, 10–20, 20–40, ≥40. Additionally, the shape of a lung cyst was categorized as round, oval, or irregular. A term of 'diversity of morphology' was used to denote the presence of all three shapes within a single lung.

3. *FLCN* gene mutation analysis

For detecting mutations in the *FLCN* gene, whole blood samples were collected from patients using tubes that contained ethylenediaminetetraacetic acid (EDTA). Genomic DNAs were then isolated using an Easy-DNA Kit (Invitrogen, Carlsbad, CA, USA). To evaluate genomic DNA's purity and concentration, a nanodrop device (ND-1000, Thermo Fisher Scientific, Wilmington, DE, USA) was utilized. Primers targeting *FLCN* exons 4–14 and their adjacent intronic regions were designed for performing polymerase chain reaction (PCR) amplification. PCR-generated products were purified with a QIAquick Gel Extraction Kit (Qiagen, Dusseldorf, Germany). Purified PCR products were then sequenced using appropriate PCR primers and a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Bio-

systems, Foster City, CA, USA).

Sequenced outputs were then aligned and compared against the reference sequence via Sequencher software (Gene Codes, Ann Arbor, MI, USA). To identify any pathogenic variations, Sanger sequencing was performed. If pathogenic variants were not initially detected, multiple ligation probe amplification was employed as a supplementary step to ensure the absence of large deletions within the *FLCN* gene.

4. Statistical analysis

Categorical variables are presented as frequencies (percentages). Continuous variables are presented as mean±standard deviation for normally distributed variables or as median (interquartile range [IQR]) for non-normally distributed variables. Normality assumptions for continuous variables were confirmed using the Shapiro-Wilk test. To compare baseline characteristics among three or more distinct patient groups, one-way analysis of variance (ANOVA) or Kruskal-Wallis test was employed. Tukey's honest significant difference test served as a *post hoc* procedure following ANOVA, while Dunn's test was applied after the Kruskal-Walli's test. Fisher's exact test was employed for analyzing categorical data.

Logistic regression was conducted to examine the predictive power of radiological features for BHD syndrome. Additionally, diagnostic accuracy of the prediction model was analyzed using receiver operating characteristic (ROC) curves and area under the curve (AUC). Youden's J statistic was utilized to establish the optimal diagnostic threshold. Statistical significance was considered when a p-value was less than 0.05.

Statistical computations were executed with R software version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). ROC curve analysis was performed using 'ROCR' and 'pROC' packages. For constructing a nomogram, the 'rms' package in R was utilized.

5. Ethics

This study was approved by the Institutional Review Board (IRB) of Gangnam Severance Hospital, Yonsei University Health System (IRB approval number: 3-2024-0076). The requirement for written consent was waived by the IRB due to the retrospective nature of this study.

Results

1. Baseline characteristics of enrolled patients

For 3 years, 533,074 patients either visited the hospital or were admitted. Among them, 52,823 patients underwent chest CT scans. Of these 52,823 patients, 2,186 (4.1%) were found to have at least one lung cyst and 301 (0.6%) patients were found to have multiple lung cysts. They were included in our analysis.

Among the 301 patients with multiple lung cysts, 24 (8.0%) were diagnosed with BHD syndrome due to *FLCN* mutations. However, none of the patients was diagnosed solely based on clinical symptoms of BHD. LAM and PLCH were diagnosed in seven (2.3%) and two (0.7%) patients, respectively.

Regarding baseline characteristics, the BHD group (75.0%) and the LAM group (100.0%) had significantly higher proportions of females than the group with other forms of multiple lung cysts (the other group) (44.4%; both p<0.001) (Table 1). BHD and LAM groups were younger (mean age: 52.4 and 46.0 years, respectively) than the other group (mean age: 60.5 years, both p<0.001). However, BHD and LAM groups did not show a significant difference in mean age (p=0.267). There was no difference in height and weight between the BHD group and the other group, whereas the LAM group had significantly lower height and weight compared to the other group (height: 160.0 cm [IQR, 155.5 to 166.2] for the BHD group, 159.0 cm [IQR, 156.5 to 161.4] for the LAM group, and 165.0 cm [IQR, 158.2 to 171.8] for the other group, p=0.050 and p=0.025, respectively; weight: 59.3±10.3 kg for the BHD group, 52.9±13.7 kg for the LAM group, and 63.8±11.7 kg for the other group, p=0.080 and p=0.016, respectively). Regarding smoking history, never smokers were significantly more common in the BHD group than in the other group (23 patients [95.8%] vs. 162 patients [60.4%], p<0.001).

Regarding underlying diseases, chronic pulmonary disease was more prevalent in BHD group (41.7%) than in the other group (19.8%, p=0.026). Malignancy was more common in the other group (38.3%) than in BHD groups (4.2%, p=0.002). However, myocardial infarction, congestive heart failure, and diabetes mellitus showed no significant differences among BHD, LAM, and other groups (Table 1).

Among the 268 patients in the other group, 105 (39.2%) were lost to follow-up, 10 (3.7%) died, 12 (4.5%) underwent diagnostic testing but were not diagnosed, and seven (2.6%) refused diagnostic testing. In addition, 12 (4.5%) patients had an advanced malignancy and 29 (10.8%) patients aged more than 70 years, neither of whom underwent further testing.

2. Clinical characteristics of BHD and other cystic lung diseases

Table 2 describes clinical characteristics of patients

Table 1. Baseline characteristics of patients according to the cause of multiple lung cysts

Characteristic	Total (n=301)	BHD (n=24)	LAM (n=7)	PLCH (n=2)	Others (n=268)	p-value
Age, yr	59.5±13.0	52.4±12.7	46.0±14.5	59.8 ±18.5	60.5±12.7	0.001*
Male sex	156 (51.8)	6 (25.0)	0	2 (100.0)	148 (55.2)	0.001*
Height, cm	164.4 (158.0–171.0)	160.0 (155.5–166.2)	159.0 (156.5–161.4)	159.5 (159.5–159.5)	165.0 (158.2–171.8)	0.030*
Weight, kg	63.1±11.8	59.3±10.3	52.9±13.7	74.2±NA	63.8±11.7	0.013*
BMI, kg/m^2	23.1 (21.0–25.0)	22.4 (21.0–24.6)	19.9 (19.6–22.2)	29.2 (29.2–29.2)	23.2 (21.1–25.1)	0.085
History of smoking						0.037*
Never smoker	192 (63.8)	23 (95.8)	6 (85.7)	1 (50.0)	162 (60.4)	
Ex-smoker	50 (16.6)	1 (4.2)	0	0	49 (18.3)	
Current smoker	26 (8.6)	0	0	0	26 (9.7)	
Unknown	33 (11.0)	0	1 (14.3)	1 (50.0)	31 (11.6)	
Underlying disease						
Myocardial infarction	4 (1.4)	1 (4.2)	0	0	3 (1.2)	0.693
Congestive heart failure	6 (2.2)	1 (4.2)	0	0	5 (2.1)	0.882
Peripheral vascular disease	18 (6.5)	1 (4.2)	0	0	17 (7.0)	0.817
Cerebrovascular disease	52 (18.8)	4 (16.7)	1 (14.3)	0	47 (19.3)	0.879
Dementia	9 (3.3)	0	0	1 (50.0)	8 (3.3)	0.002*
Chronic pulmonary disease	63 (22.8)	10 (41.7)	4 (57.1)	1 (50.0)	48 (19.8)	0.009*
Rheumatic disease	4 (1.4)	1 (4.2)	0	0	3 (1.2)	0.693
Peptic ulcer disease	17 (6.2)	0	0	0	17 (7.0)	0.483
Mild liver disease	38 (13.8)	2 (8.3)	1 (14.3)	1 (50.0)	34 (14.0)	0.420
Diabetes without chronic complication	40 (14.5)	1 (4.2)	0	1 (50.0)	38 (15.6)	0.136
Diabetes with chronic complication	14 (5.1)	0	0	1 (50.0)	13 (5.3)	0.018*
Paraplegia and hemiplegia	3 (1.1)	0	1 (14.3)	0	2 (0.8)	0.008*
Renal disease	8 (2.9)	1 (4.2)	1 (14.3)	0	6 (2.5)	0.310
Any malignancy[†]	96 (34.8)	1 (4.2)	1 (14.3)	1 (50.0)	93 (38.3)	0.005*
Moderate or severe liver disease	1 (0.4)	0	0	0	1 (0.4)	0.987
Metastatic solid tumor	24 (8.7)	0	0	0	24 (9.9)	0.312
AIDS/HIV	1 (0.4)	0	0	0	1 (0.4)	0.987

Values are presented as mean±standard deviation, number (%), or median (interquartile range).
*p<0.05. [†]Including lymphoma and leukemia, except malignant neoplasm of the skin.
BHD: Birt-Hogg-Dubé; LAM: lymphangioleiomyomatosis; PLCH: pulmonary Langerhans cell histiocytosis; NA: not available; BMI: body mass index; AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus.

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Table 2. Clinical features of multiple cystic lung disease

Variable	Total (n=301)	BHD (n=24)	LAM (n=7)	PLCH (n=2)	Others (n=268)	p-value
Laboratory data						
Hemoglobin	13.6 (12.2 to 14.8)	13.2 (12.2 to 14.4)	13.8 (12.4 to 14.2)	13.1 (11.2 to 15.1)	13.6 (12.3 to 14.9)	0.818
White blood cell	6.0 (4.9 to 7.4)	5.8 (4.6 to 6.9)	9.9 (9.8 to 10.4)	17.5 (5.2 to 29.7)	5.9 (4.9 to 7.3)	0.007*
Platlet	230.0 (193.0 to 269.0)	222.5 (210.0 to 245.0)	254.5 (204.0 to 258.5)	316.8 (183.5 to 450.0)	230.0 (193.0 to 270.5)	0.948
Blood urea nitrogen	14.4 (12.2 to 17.0)	13.5 (12.4 to 16.9)	13.2 (12.6 to 14.5)	12.3 (10.8 to 13.8)	14.4 (12.2 to 17.0)	0.707
Creatinine	0.8 (0.6 to 0.9)	0.7 (0.6 to 0.8)	0.7 (0.7 to 0.8)	0.8 (0.8 to 0.9)	0.8 (0.6 to 0.9)	0.781
AST	27.5 (23.5 to 34.0)	25.0 (21.5 to 27.0)	22.0 (20.0 to 24.0)	31.5 (29.0 to 34.0)	28.0 (23.5 to 34.0)	0.125
ALT	23.0 (18.0 to 32.0)	20.0 (17.5 to 25.0)	24.0 (18.0 to 29.0)	21.5 (19.0 to 24.0)	23.0 (18.0 to 32.5)	0.756
CRP	0.9 (0.4 to 7.2)	0.6 (0.2 to 2.2)	6.5 (3.5 to 19.8)	3.8 (0.2 to 7.4)	0.9 (0.4 to 8.1)	0.539
Pulmonary function test						
FVC, L	3.7±1.0	3.0±0.7	2.4±0.3	2.7±NA	3.8±1.0	0.007*
FVC, z-score	0.1 (−0.7 to 1.1)	−0.5 (−1.2 to −0.3)	−1.8 (−2.4 to −1.2)	−1.4 (−1.4 to −1.4)	0.3 (−0.6 to 1.3)	0.009*
FEV_1, L	2.7±0.8	2.3±0.6	1.7±0.4	2.1±NA	2.8±0.8	0.025*
FEV_1, z-score	−0.5±1.4	−0.9±0.9	−2.2±0.9	−1.2±NA	−0.4±1.4	0.145
FEV_1/FVC, %	74.0±7.7	77.5±5.3	70.0±5.7	78.0±NA	73.6±8.0	0.300
FEV_1/FVC, z-score	−0.9±1.2	−0.8±0.8	−1.7±0.7	0.1±NA	−0.9±1.2	0.594
$FEF_{25\%-75\%}$, L	2.0 (1.5 to 2.7)	1.9 (1.6 to 2.5)	1.1 (0.9 to 1.4)	1.9 (1.9 to 1.9)	2.1 (1.5 to 2.7)	0.371
$FEF_{25\%-75\%}$, z-score	2.1±0.9	−0.7±0.9	−1.7±0.5	−0.0±NA	−0.6±1.1	0.474

Values are presented as median (interquartile range) or mean±standard deviation.
*p<0.05.
BHD: Birt-Hogg-Dubé; LAM: lymphangioleiomyomatosis; PLCH: pulmonary Langerhans cell histiocytosis; AST: aspartate aminotransferase; ALT: alanine aminotransferase; CRP: C-reactive protein; FVC: forced vital capacity; NA: not available; FEV_1: forced expiratory volume in 1 second; FEF: forced expiratory flow.

Table 3. The characteristics of enrolled patients stratified by the number of cysts

Variable	Number of cysts					p-value
	Total (n=301)	<10 (n=146)	10–20 (n=71)	20–40 (n=42)	>40 (n=42)	
Age, yr	59.5±13.0	60.5±12.1	57.3±13.3	60.4±14.9	58.9±13.6	0.363
Sex						0.997
Male	156 (51.8)	76 (52.1)	36 (50.7)	22 (52.4)	22 (52.4)	
Female	145 (48.2)	70 (47.9)	35 (49.3)	20 (47.6)	20 (47.6)	
Height, cm	164.4 (158.0 to 171.0)	164.5 (157.5 to 172.0)	165.0 (160.0 to 171.0)	164.3 (159.0 to 169.5)	162.2 (156.0 to 169.0)	0.604
Weight, kg	62.0 (55.0 to 72.0)	63.0 (55.0 to 72.0)	60.4 (54.0 to 73.2)	63.5 (56.2 to 69.0)	58.2 (52.0 to 72.0)	0.649
BMI, kg/m^2	23.1 (21.0 to 25.0)	23.4 (21.5 to 25.1)	22.6 (20.9 to 24.9)	23.2 (21.1 to 24.6)	22.0 (20.1 to 25.6)	0.647
Cause of multiple lung cysts						<0.001*
BHD	24 (8.0)	1 (0.7)	3 (4.2)	7 (16.7)	13 (31.0)	
LAM	7 (2.3)	1 (0.7)	1 (1.4)	1 (2.4)	4 (9.5)	
PLCH	268 (89.0)	0	0	0	2 (4.8)	
Others	2 (0.7)	144 (98.6)	67 (94.4)	34 (81.0)	23 (54.8)	
FVC, z-score	0.1 (−0.7 to 1.1)	0.4 (−0.7 to 1.1)	0.2 (−0.4 to 1.6)	−0.7 (−1.5 to −0.3)	−0.5 (−1.4 to 0.2)	0.048*
FEV_1, z-score	−0.5±1.4	−0.3±1.4	−0.4±1.5	−1.2±0.9	−0.7±1.3	0.333
Bilateral lung cysts	202 (67.1)	63 (43.2)	57 (80.3)	40 (95.2)	42 (100.0)	<0.001*
Basal predominance	79 (26.2)	26 (17.8)	15 (21.1)	10 (23.8)	28 (66.7)	<0.001*
Maximal diameter of lung cyst, mm	12.2 (8.3 to 20.3)	10.4 (7.1 to 15.1)	10.6 (8.0 to 17.1)	15.3 (10.4 to 30.2)	25.9 (17.6 to 36.8)	<0.001*
Diversity of lung cyst size	99 (32.9)	21 (14.4)	20 (28.2)	23 (54.8)	35 (83.3)	<0.001*
Irregularity of lung cyst shape	95 (31.6)	26 (17.8)	17 (23.9)	19 (45.2)	33 (78.6)	<0.001*

Values are presented as mean±standard deviation, number (%), or median (interquartile range).
*p<0.05.
BMI: body mass index; BHD: Birt-Hogg-Dubé; LAM: lymphangioleiomyomatosis; PLCH: pulmonary Langerhans cell histiocytosis; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 second.

with BHD syndrome, LAM, or other forms of multiple lung cysts. Laboratory results revealed that the white blood cell (WBC) count was significantly elevated in the LAM group (9.9 [IQR, 9.8 to 10.4]) compared to that in the BHD group (5.8 [IQR, 4.6 to 6.9]; p<0.001) and the other group (5.9 [IQR, 4.9 to 7.3]; p=0.001). However, WBC counts showed no significant difference between BHD and other groups (p=0.530). In addition, hemoglobin level, platelet count, and levels of BUN, creatinine, AST, ALT, and CRP were not significantly different among the three groups.

PFT showed that patients in both BHD and LAM groups had lower forced vital capacity (FVC) (3.0±0.7 and 2.4±0.3 L, respectively) than those in the other group (3.8±1.0 L; p=0.006 and p=0.046, respectively). After converting these values to z-scores based on references of Global Lung Initiative (GLI) 2012, patients in BHD continued to display significantly lower FVC values group (–0.5 [IQR, –1.2 to –0.3]) than those in the other group (0.3 [IQR, –0.6 to 1.3]; p=0.010). However, FVC z-scores were not significantly different between BHD and LAM groups (p=0.153).

Forced expiratory volume in 1 second (FEV_1) was lower in the BHD group (2.3±0.6 L) and LAM (1.7±0.4 L) than those in the other group (2.8±0.8 L; p=0.031 and p=0.039, respectively). However, differences of FEV_1 z-scores between groups did not reach statistical significance (Table 2).

3. Characteristics of lung cysts among patients

Among the 301 patients with multiple lung cysts, 146 (48.5%) had fewer than 10 cysts, while 42 (14.0%) had more than 40 cysts. Patient characteristics stratified by the number of cysts are summarized in Table 3. Bilateral cysts were observed in 202 (67.1%) patients. Cyst with a basal predominance was found in 79 (26.2%) patients. The mean maximal diameter of all cysts was 12.2 mm (range, 8.3 to 20.3).

In PFT, an increase in the number of cysts correlated with a significant decline in FVC z-score based on GLI 2012 reference (number of cysts <10, FVC z-score: 0.4 [IQR, –0.7 to 1.1]; number of cysts=10–20, FVC z-score: 0.2 [IQR, –0.4 to 1.6]; number of cysts=20–40, FVC z-score: –0.7 [IQR, –1.5 to –0.3]; number of cysts >40, FVC z-score: –0.5 [IQR, –1.4 to 0.2]; p=0.048) (Table 3).

Features of lung cysts in BHD, LAM, PLCH, and other groups are described in Table 4. Regarding the number of lung cysts, 54.2%, 57.1%, and 100.0% of patients in BHD, LAM, and PLCH groups, respectively, had more than 40 cysts. Moreover, 95.8%, 100%, and 100% of patients in BHD, LAM, and PLCH groups, respectively, exhibited bilateral cysts. Basal predominance was a prominent feature in BHD cases (BHD, LAM, PLCH, and other groups: 20 [83.3%], 4 [57.1%], 0 [0%], and 55 patients [20.4%], respectively; p<0.001). These patients typically had larger cysts with a larger diameter (average: 32.1 mm [IQR, 26.5 to 43.5]) than patients in the LAM group (average diameter: 17.0 mm [IQR, 13.2 to 19.1], p<0.001), the PLCH group (average diameter:

Table 4. The characteristics of lung cysts between BHD and non-BHD group

Characteristic	Total (n=301)	BHD (n=24)	LAM (n=7)	PLCH (n=2)	Others (n=268)	p-value
Number of cysts						<0.001*
<10	146 (48.5)	1 (4.2)	1 (14.3)	0	144 (53.7)	
10–20	71 (23.6)	3 (12.5)	1 (14.3)	0	67 (25.0)	
20–40	42 (14.0)	7 (29.2)	1 (14.3)	0	34 (12.7)	
>40	42 (14.0)	13 (54.2)	4 (57.1)	2 (100.0)	23 (8.6)	
Bilateral lung cysts	202 (67.1)	23 (95.8)	7 (100.0)	2 (100.0)	170 (63.4)	0.002*
Basal predominance	79 (26.2)	20 (83.3)	4 (57.1)	0	55 (20.5)	<0.001*
Maximal diameter of lung cyst, mm	12.2 (8.3–20.3)	32.1 (26.5–43.5)	17.0 (13.2–19.1)	16.3 (15.0–17.6)	11.2 (7.9–17.0)	<0.001*
Diversity of lung cyst size	99 (32.9)	23 (95.8)	5 (71.4)	2 (100.0)	69 (25.7)	<0.001*
Irregularity of lung cyst shape	95 (31.6)	23 (95.8)	3 (42.9)	2 (100.0)	67 (25.0)	<0.001*

Values are presented as number (%) or median (interquartile range).
*p<0.05.
BHD: Birt-Hogg-Dubé; LAM: lymphangioleiomyomatosis; PLCH: pulmonary Langerhans cell histiocytosis.

16.3 mm [IQR, 15.0 to 17.6], p=0.012), and the other group (average diameter: 11.3 mm [IQR, 7.9 to 17.0], p<0.001). Furthermore, the BHD group had a higher percentage of patients demonstrating diverse cyst sizes and morphologies than LAM and other groups (diverse cyst size: 23 [95.8%], 5 [71.4%], and 71 patients [26.3%], respectively, p<0.001; diverse morphologies in BHD, LAM, and other groups: 23 [95.8%] patients, 3 [42.9%], and 69 patients [25.6%], respectively; p<0.001) (Table 4). However, the percentage of patients with diverse cyst sizes and morphologies did not show significant differences between BHD and PLCH groups (23 patients [95.8%] vs. 2 patients [100.0%], p=1.000) (Table 4).

4. Correlation between cystic features and *FLCN* gene mutations

In the univariate logistic regression model, several factors, including the number of cysts >40, bilateral cysts, basal predominance, larger maximum diameter, diverse cyst sizes, and diverse morphologies, showed positive correlations with the presence of *FLCN* gene mutations (odds ratios [ORs], 10.107, 12.592, 18.475, 1.083, 60.829, and 65.486, respectively; all p<0.05) (Table 5). Conversely, age was inversely associated with the presence of *FLCN* gene mutations (OR, 0.955; 95% confidence interval [CI], 0.922 to 0.986; p=0.006). Additionally, being a male showed a significant correlation with FLCN mutations (vs. female, OR, 0.282; 95% CI, 0.010 to 0.696; p=0.009).

In the multivariate logistic regression model, some associations observed in the univariate analysis persisted, including bilateral cysts (OR, 12.393; 95% CI, 1.613 to 274.682; p=0.038), basal predominance (OR, 8.511; 95% CI, 2.252 to 39.392; p=0.002), maximum diameter (OR, 1.053; 95% CI, 1.009 to 1.108; p=0.032), and diverse morphologies (OR, 19.513; 95% CI, 2.833 to 398.119; p=0.010) that remained to be significantly associated with *FLCN* gene mutations in the multivariate logistic regression analysis. However, age and sex were not significantly associated with *FLCN* gene mutations in the multivariate analysis (OR, 0.970; 95% CI, 0.926 to 1.013; p=0.183 and OR, 0.370; 95% CI, 0.085 to 1.474; p=0.164, respectively). In addition, the number of cysts greater than 40 was not significantly associated with *FLCN* gene mutations in the multivariate model (OR, 0.788; 95% CI, 0.191 to 3.003; p=0.732), although it had a strong association in the univariate analysis.

5. BHD syndrome prediction model derived from chest CT scans

Using the stepwise selection method, a multivariate

Table 5. Logistic regression between radiologic features of lung cysts and *FLCN* mutations

Variables	Univariate analysis		Multivariate analysis		Multivariate prediction model	
	OR (95% CI)	p-value	OR (95% CI)	p-value	Coefficient	p-value
Age, yr	0.955 (0.922–0.986)	0.006*	0.970 (0.926–1.013)	0.183	–0.044	0.037*
Sex (vs. female)	0.282 (0.010–0.696)	0.009*	0.370 (0.085–1.474)	0.164		
Number >40	10.107 (4.160–25.095)	<0.001*	0.788 (0.191 –3.003)	0.732		
Bilateral cyst	12.592 (2.593–226.995)	0.014*	12.393 (1.613–274.682)	0.038*	2.350	0.039*
Basal predominance	18.475 (6.688–65.385)	<0.001*	8.511 (2.252–39.392)	0.002*	2.252	<0.001*
Maximum diameter, mm	1.083 (1.054–1.117)	<0.001*	1.053 (1.009–1.108)	0.032*	0.047	0.044*
Diversity of size	60.829 (12.484–1,097.780)	<0.001*	Omitted†			
Diversity of morphology	65.486 (13.428–1,182.181)	<0.001*	19.513 (2.833–398.119)	0.010*	2.896	0.010*

*p<0.05. †Omitted due to highly correlation with maximum diameter.
FLCN: folliculin; OR: odds ratio; CI: confidence interval.

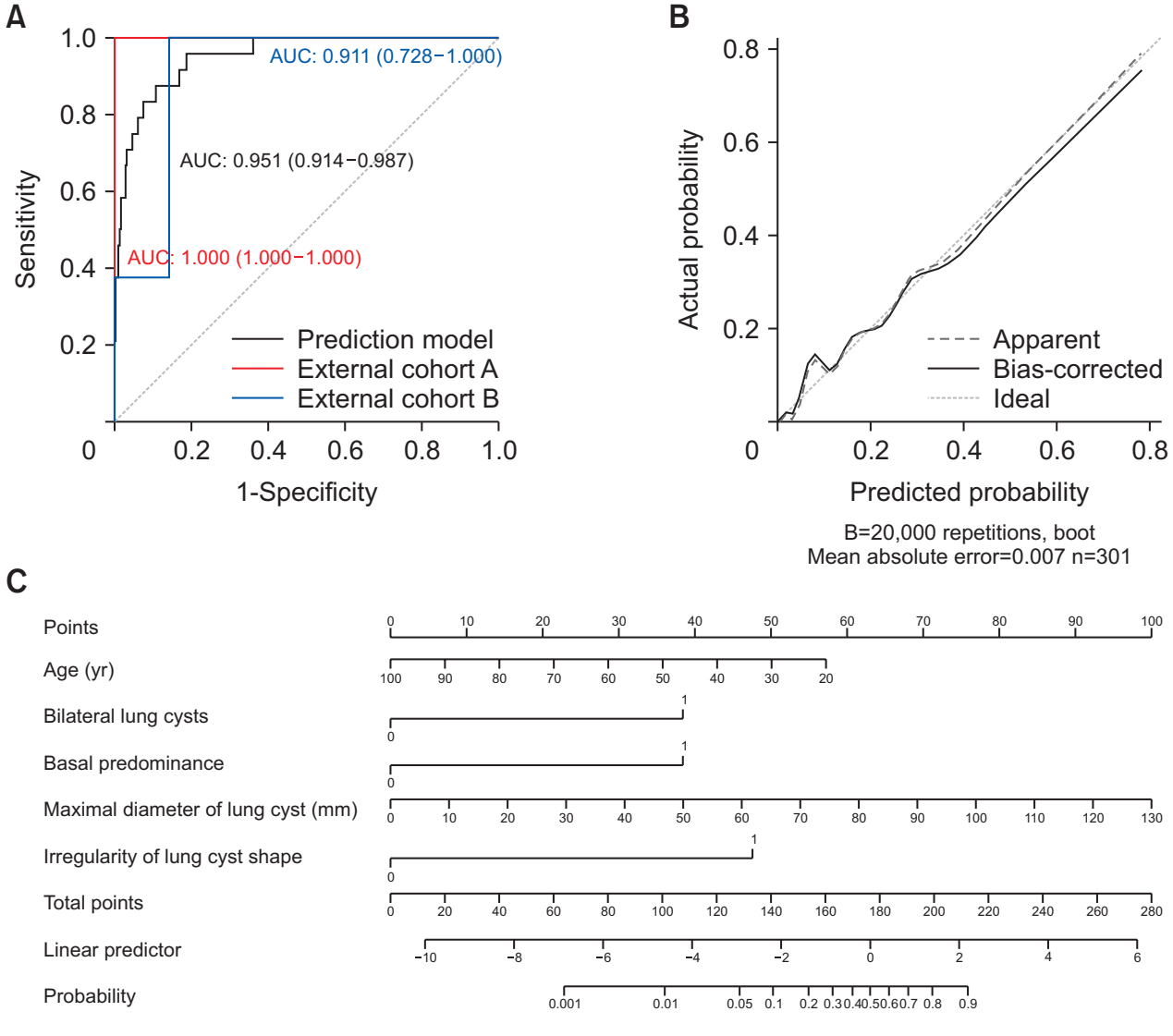
prediction model for BHD syndrome was derived. It is described in Table 5. The model achieved a high diagnostic accuracy with a threshold of 0.165, a sensitivity of 95.83%, a specificity of 81.22%, and an AUC of 0.951 (95% CI, 0.914 to 0.987). For external validation of the BHD prediction model in cohorts from two separate studies[4,8], after excluding four overlapping patients (two patients from each cohort), the model yielded area under the receiver operating characteristic curves (AUROCs) of 1.000 and 0.911, respectively (Figure 1A, B). For clinical application, the prediction model was translated into a user-friendly nomogram as depicted in Figure 1C.

Discussion

This 3-year study revealed the frequency and complexity of multiple lung cysts. In patients who underwent chest CT scans, 4.1% had at least one lung cyst and 0.6% had multiple cysts. In the current study, the presence of multiple bilateral and basally located lung cysts, variability in cyst morphology, and the maximal diameter of cysts were key indicators for diagnosing BHD syndrome, consistent with findings of a previous research study[6].

The presence of numerous lung cysts is a significant characteristic of BHD syndrome. However, the number of lung cysts does not constitute a criterion for diagnosing BHD syndrome. Similarly, in this study, while the

Figure 1. Receiver operating characteristic analysis for diagnosis of Birt-Hogg-Dubé (BHD) syndrome. (A) Receiver operating characteristic analysis. (B) Calibration of prediction model. (C) Nomogram of prediction model for diagnosis of BHD. AUC: area under the curve.



number of lung cysts showed a significant association with BHD syndrome in the univariate logistic regression, it did not demonstrate a significant correlation with BHD syndrome in the multivariate analysis. The reported number of cysts in patients with BHD varied across studies. Toro et al.[12] reported that BHD patients could have between 0 and 166 lung cysts, whereas Agarwal et al.[13] found that only 33% had more than 20 cysts. This variability in the number of cysts suggests that the number of cysts is not specific to BHD diagnosis.

The distribution, shape, and size of cysts are more important than the number of cysts for BHD syndrome diagnosis. Menko et al.[2] have included the distribution of lung cysts on chest CT as a minor criterion for diagnosing BHD syndrome, describing bilateral, basally located lung cysts as key characteristics. Agarwal et al.[13] have reported that 87% of patients exhibit bilateral cysts and that 87% of patients have basally located cysts. In a single-center study, Park et al.[4] found that 100% of patients with BHD had bilateral, basally located cysts. Similarly, in a previous research study, we found that 100% of patients with BHD syndrome had bilateral, basal cysts[8]. In the present study, 95% and 83.3% of patients with BHD syndrome had bilateral cysts and basal cysts, respectively, consistent with findings of previous studies[8].

Lung cysts of patients with BHD syndrome commonly show diverse morphologies within a single lung, including oval, round, lenticular, or irregular shapes[6,14]. This observation might be associated with the occurrence of large cysts in patients with BHD syndrome[6]. These large cysts are characteristically multiseptated and irregular in form. They are predominantly located at bases of lungs[7,13].

The presence of large lung cysts coexisting with smaller cysts is a unique characteristic that can distinguish BHD syndrome from other diseases with multiple lung cysts. The presence of lung cysts larger than 2 cm has been reported in both our prior research and other studies[4,6-8]. Recent advances have revealed key roles of folliculin protein in crucial cellular pathways and cell adhesion, including its role in affecting lung structure and function[15]. Previous studies, particularly those using mouse models, have shown that defects in these pathway due to *FLCN* deficiency can lead to enlarged alveolar spaces[16,17]. The "stretch hypothesis" suggests that BHD-related cysts can form from adhesion defects, causing alveoli to expand under the stress of breathing, particularly in more vulnerable areas of lungs[15]. This mechanism can explain why patients with BHD syndrome have larger cysts than patients with other multiple lung cysts. However, further research is still needed to fully elucidate this phenomenon.

Our data showed that patients with BHD syndrome had a distinct demographic profile, showing a significantly higher prevalence of females than those with other cystic lung diseases. BHD syndrome is an autosomal dominant disorder. It is often reported to be more common in females[13], particularly in studies conducted in East Asia[4,8,18,19]. Reasons for its gender discrepancy remain unclear, with no established explanations. Possible factors could include racial differences, selective bias, and/or other unidentified variables. Further research is necessary to explore and understand underlying causes of this observed trend.

This study has several strengths. First, it addressed limitations noted in our previous research by elucidating unique characteristics of BHD syndrome in patients with multiple lung cysts. Specifically, it provides a clinically useful prediction tool that can aid in diagnosing BHD syndrome based solely on CT findings and age in patients with multiple lung cysts. Particularly, by adding variables such as age, cyst size, and morphology to one of the minor criteria—CT findings—this study provides crucial evidence for creating a scoring diagnostic criterion for BHD.

This study also had several limitations. First, the number of BHD patients was small. In addition, it was a single-center study. These might have led to statistical insignificance. Additionally, geographical and medical characteristics of the center itself might have influenced our study results. Second, a significant number of patients with multiple lung cysts remained undiagnosed for their underlying causes. Notably, among those undiagnosed, seven patients exhibited CT findings suggestive of BHD. However, further evaluation was not performed due to patients' refusal of additional tests. These factors might have impacted this study's results. In addition, due to the retrospective nature of this study, there were limitations in data collection for certain variables, especially bronchodilator reversibility (BDR) in PFTs. Consequently, BDR was excluded from this study. This exclusion is a limitation as BDR is an important factor in interpreting PFT results. Lastly, external cohort validation was conducted on a distinct patient group from the same institution, with a total of four overlapping patients identified and subsequently removed. Data from the same institution often share similar artifacts. This process might have introduced unintended selection bias, potentially affecting our study results.

In conclusion, BHD syndrome is a principal form of cystic lung disease characterized by multiple lung cysts. Age, bilateral cysts, basal dominance, large size, and irregular shape major factors for differentiating

BHD syndrome from other cystic lung diseases. The multivariate predictive model for diagnosing BHD can aid in identifying undiagnosed patients.

Authors' Contributions

Conceptualization: Choi YJ, Byun MK. Methodology: Park HJ, Kim CY, Jung BM, Cho JH. Formal analysis: all authors. Data curation: all authors. Project administration: Byun MK. Writing - original draft preparation: all authors. Writing - review and editing: all authors. Approval of final manuscript: all authors.

Conflicts of Interest

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