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Calcium stone lithoptysis in primary ciliary dyskinesia

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Summary
Background: An association between lithoptysis and primary ciliary dyskinesia (PCD) has not been previously reported. However, reports of lithoptysis from 2 older patients (>60 yr) prompted a study of this association.
Methods: We performed a prospective study of all PCD patients presenting to our institution between August 2003 and March 2006, seeking the symptom of lithoptysis or calcium deposition on radiology. A retrospective analysis of all PCD patients presenting prior to August 2003 was also performed. Patients age $\geqslant 40$ previously reviewed were recontacted. If a history of lithoptysis or calcium deposition was present, we further reviewed radiographic, microbiologic, and biochemical data, including serum calcium and phosphate. Broncholiths were analyzed by light and electron microscopy- and electron-dispersive X-ray analysis.
Results: In total, 142 patients ($n = 28$ age $\geqslant 40$) were included, 41 in the prospective and 91 in the retrospective study. Lithoptysis was reported in 5 patients (all age $\geqslant 40$). Chest CT scans identified calcification (4/5), involving bronchiectatic airways in 3 patients and focal nodular calcification in 1 patient. Two other patients (age 46, 59) were identified with airway calcification without lithoptysis. Available broncholiths from 2 of these patients were composed of calcite, whereas a broncholith from 1 patient with focal nodular calcification contained calcium phosphate. Sputum was positive for *Pseudomonas aeruginosa* in all 7 patients, but negative for mycobacterial and fungal cultures.
Conclusion: There is an association between lithoptysis and PCD in patients age $\geqslant 40$. We hypothesize that calcite stone formation is a biomineralization response to chronic airway inflammation and retention of infected airway secretions in PCD in a subset of PCD patients.

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Introduction

Primary ciliary dyskinesia (PCD) is a clinical disease of the sino-pulmonary system, caused by defective ciliary structure and function, leading to abnormalities in mucociliary clearance. It is a genetically heterogeneous, autosomal recessive trait, with a prevalence of ~1/12 000–1/17 000.[1] Clinical manifestations of the disease include recurrent sino-pulmonary infections, otitis media and eventual bronchiectasis with associated male and female subfertility. Abnormalities of organ lateralization lead to situs inversus, and therefore Kartagener's Syndrome, (situs inversus, bronchiectasis and recurrent middle ear disease) in approximately 50% of patients.[1,2] A clear diagnosis currently relies on an accurate clinical phenotype, with electron microscopic analysis of ciliary ultrastructure.

The University of North Carolina at Chapel Hill, in conjunction with other US research sites, has established the largest database of patients with PCD in North America. To date, rigorous evaluation of 141 patients from 110 families has allowed stratification of patients by phenotype (ciliary defect), with the ultimate goal of identification of the genetics of PCD.[3,4] In August and November 2003, we identified 2 new adult PCD patients (ages 60 and 65) with the symptoms of coughing up stones or lithoptysis and calcium deposition in the lung, a novel and unexpected observation. An association between PCD, lithoptysis and calcium deposition involving the airways has not previously been identified. Thus, we tested the hypothesis that lithoptysis related to airway calcium deposition might occur in other patients with PCD. We also compared pulmonary function as reflected by FEV_1 in PCD patients with and without pulmonary calcium deposition and lithoptysis.

Materials and methods

After 2 PCD patients (age > 60) reported lithoptysis upon presentation to UNC in August and November 2003, we investigated the hypothesis that there was an association between PCD, lithoptysis and calcium deposition in the lung. A prospective analysis of all subsequent PCD patients ($n = 41$) until April 2006 (29 months) was performed with direct questioning for the symptom of lithoptysis (spitting up a hard concretion, a firm stone-like structure in the sputum, a gritty sensation in the sputum). Patients were also asked if they had been advised of calcium deposition on prior chest radiographic investigations. Available radiographic studies, including CT chest studies were reviewed for evidence of calcification. If a history of lithoptysis or calcium deposition was identified, clinical, radiographic, and microbiologic and biochemistry data were further reviewed including serum renal profile, calcium and phosphate. Patients were also questioned, and charts were reviewed seeking potential acute or surgical complications of broncholithiasis including hemoptysis, lung collapse, mediastinal abscess and post-obstructive pneumonia.

A retrospective analysis of all PCD patients reviewed at our institution prior to August 2003 was also performed ($n = 91$). Available medical records and radiographic studies (including CT chest studies) were reviewed for evidence of lithoptysis and lung calcium deposition. Due to the observation that both initial patients identified were age > 60, follow-up telephone contacts were made to all patients age > 40, the symptoms of lithoptysis were described and patients were asked if they had experienced these symptoms. Patients were also asked if they had been advised of calcium deposition on prior chest radiographic investigations. If a history of lithoptysis or calcium deposition was identified, further questioning and review of clinical records was performed as described above.

If recovered, broncholiths were examined by light and scanning electron microscopy. Energy-dispersive X-ray analysis (EDAX) was conducted utilizing a Kevex 7000 EDX unit with a Li drifted Si detector. Spectrum acquisition was under control of a 4 pI acquisition system. Software for the acquisition was Desktop Spectrum Analyzer from the National Institute of Standards and Technology. The scanning electron microscope was a Cambridge S200 operated at 20 kV. The working distance was 20 mm. The tilt angle was 40°. Acquisition time was 100 s. An area of the stone was magnified to fill the entire scanning roster and a spectrum was acquired for 100 s. A similar area away from the stone on the carbon substrate was also analyzed for 100 s. Calibration of the detector was accomplished utilizing a pure Al/Cu standard. Broncholiths were also decalcified and stained for fungi.

As an index of the impact of pulmonary calcium deposition on lung function, we compared the FEV_1 (best ± 1 yr of when the CT scan was performed) between PCD patients age > 40 with and without (control) pulmonary calcium deposition on CT scan, using a two-tailed *t*-test.

Results

Including both index cases, 51 patients (8 age ⩾ 40) were included in the prospective study. CT scans were available in 23 of these 51 patients. Three

patients were identified with lithoptysis plus calcium deposition and 1 patient with calcium deposition alone.

The retrospective study included 91 patients (20 age $\geqslant$ 40). Seven patients age $\geqslant$ 40 were not available for questioning; however, medical records did not indicate a history of lithoptysis or calcium deposition on available chest radiography. CT studies were available in 19 of these 91 patients. The retrospective study identified 1 patient with lithoptysis and calcium deposition, 1 patient with lithoptysis alone and 1 patient with calcium deposition alone. Fig. 1 combines both the prospective and retrospective studies.

Table 1 shows the phenotypic, radiographic and laboratory data for all 7 patients with a history of lithoptysis and/or evidence of calcium deposition on chest CT. Mean age was 56 $\pm$ 7 yr; 3 were female. All patients had a history of chronic sino-pulmonary disease and middle ear disease, typical of PCD.[1] Consistent with prior reports, neonatal respiratory symptoms were present in 86%.[1] The FEV_1 was 44 $\pm$ 13%. In all PCD patients age $\geqslant$ 40 with chest CT studies available ($n = 13$), there was no statistical difference between the mean FEV_1 in PCD patients with ($n = 7$, 44 $\pm$ 13%) or without ($n = 6$, 55 $\pm$ 27%) calcium deposition and/or lithoptysis ($P = 0.33$).

Table 2 also summarizes data pertaining to PCD diagnosis. In all but 1 patient (patient 3), clinical diagnosis was confirmed by ciliary ultrastructural analysis. However, diagnosis was reinforced by situs inversus, low nasal nitric oxide and sperm immotility. Genotyping thus far revealed pathogenic mutations in *DNAH5* in 2 patients.[4]

No patient had a history of renal failure and serum calcium, phosphate and serum alkaline phosphatase were normal in all patients. No male had fathered children, and 2 reported prior fertility testing revealing sperm immotility. One female had 2 biological children. Two patients (patients 5 and 6) had siblings ($n = 3$) with primary dyskinesia including 1 patient with an identical twin (patient 6). All siblings were contacted ($n = 2$ post-lung transplantation) and had available CT images for review, however none reported a history of lithoptysis and calcium deposition was not identified in CT images pre- or post-lung transplantation. The pathology of explanted lungs was reviewed in both siblings, the only 2 PCD patients who had undergone lung transplantation at UNC since our transplant program was initiated. There was no evidence of pulmonary calcium deposition in either patient.

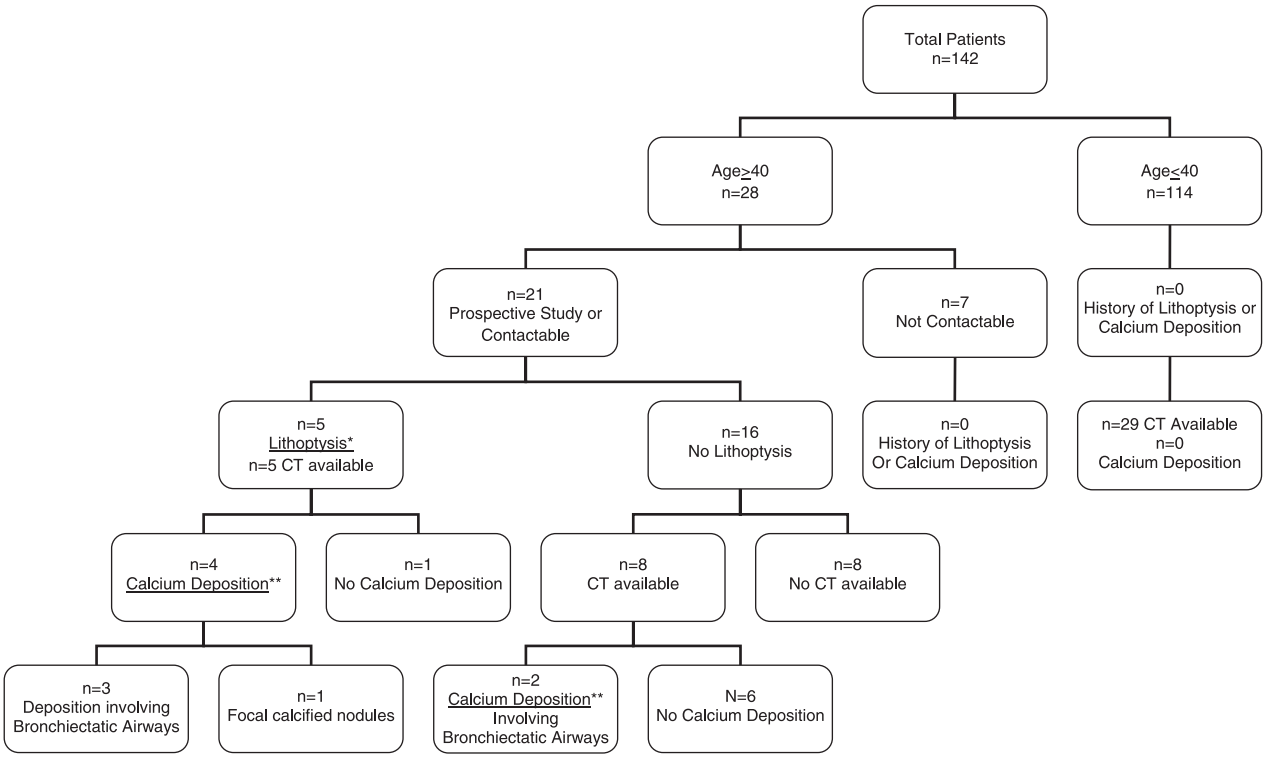


Figure 1 Number of total primary ciliary dyskinesia population ($n = 142$) with reported lithoptysis* ($n = 5$) $\pm$ calcium deposition** ($n = 6$) identified on chest computed tomography imaging.

Table 1 Demographic, clinical and radiographic information of 7 patients with primary ciliary dyskinesia and associated lithoptysis and/or radiographic pulmonary calcification.

Patient	Sex	Age (yr)	Situs	Ciliary Defect	Nasal NO (nl/min)	FEV_1 (% pred)	Previous lobectomy	Radiographic calcium deposition	Broncholith
1	F	61	SI	IDA	12	35	LUL, lingula	Bilat lower (Fig. 2a)	Frequent[a] (Fig. 2a)
2	M	59	SI	ODA[b]	20	24	RLL,LLL	Bilat lower (Fig. 2b)	Frequent[a] (Fig. 2b)
3	M	66	SI	UNK[b]	34	56	RLL,LLL	RML	3 episodes
4	F	60	SS	ODA	32	57	None	RUL (Fig. 2c)	Frequent[a] (Fig. 2c)
5	M	47	SS	IDA	20	53	None	None	Once
6	F	46	SS	IDA	13	30	None	RML	Never
7	M	59	SS	CA	20	48	RML	Lingula	Never
Average		56 ± 7			22 ± 9	44 ± 13			

Ciliary ultrastructural analysis revealed inner dynein arm defect (IDA) in 43%, outer dynein arm defect (ODA) in 29%, central apparatus defect (CA) 14% and poor quality sample/unknown (UNK) 14%.

Normal nasal nitric oxide (NO) = 376 ± 124 nl/min.[1]

SI = situs inversus, SS = situs solitus, FEV_1 = Forced expiratory volume in 1 s (% predicted), LUL = left upper lobe, LLL = left lower lobe, RML = right middle lobe, Bilat = Bilateral.

[a]Broncholith specimens were analyzed.

[b]Pathogenic mutations in *DNAH5*.[4]

Microbiology ($n = 7$)

All 7 patients were chronically infected with *Pseudomonas aeruginosa* and 1 patient cultured positive for *Stenotrophomonas maltophilia*. Mycobacterial infection was unlikely given negative acid-fast bacilli cultures (average $n = 2$ samples per patient) and negative acid-fast purified protein derivative (PPD) skin test in all 7 patients. No patients had a history of, or cultured positive, for fungal infection.

Radiographic findings ($n = 7$)

Situs inversus totalis was identified in 3/7 patients and 4/7 had prior lobectomies (the pathology report from lobectomy was available in only 1 patient (patient 2) and no calcium deposition was reported). Bronchiectasis was identified in all 7 patients (average = 4.5 lobes, presuming surgically removed lobes were bronchiectatic). Bronchiectasis was distributed predominately in lower lobes (14/14 lobes), middle lobe (6/7 lobes) and lingula (6/7 lobes) as compared to upper lobes (5/14 lobes).

Calcium deposition was identified in 4 patients with lithoptysis and 2 other patients without lithoptysis (Table 1). In 5 of these 6 patients, calcium deposition was predominately in a peri-and endo-bronchial location in bronchiectatic lobes (Figs. 2a and b). No calcium deposition was identified in upper lobes and in all patients calcium deposition occurred in lobes displaying bronchiectasis. The pattern of calcium deposition was different in patient 4, with two discrete calcified granuloma identified in the right upper lobe (Fig. 2c).

Other radiographic findings included emphysema in 2 patients (29%, $n = 1$ smoker) and kyphoscoliosis in 1 patient (patient 2). Patient 1 had one solitary calcified right infra hilar lymph node and thus was the only patient with lymph node calcification on review of CT images available. There was no splenic or hepatic calcium deposition on available upper abdomen images.

Lithoptysis ($n = 5$)

Five patients reported lithoptysis. No acute or surgical complications of lithoptysis were identified. The patients' description of lithoptysis varied from a gritty sensation in their sputum to a firm stone-like object in the back of the throat. Two patients reported continuous lithoptysis up to 4 times a week for up to 10 yr (patient 1 and 4).

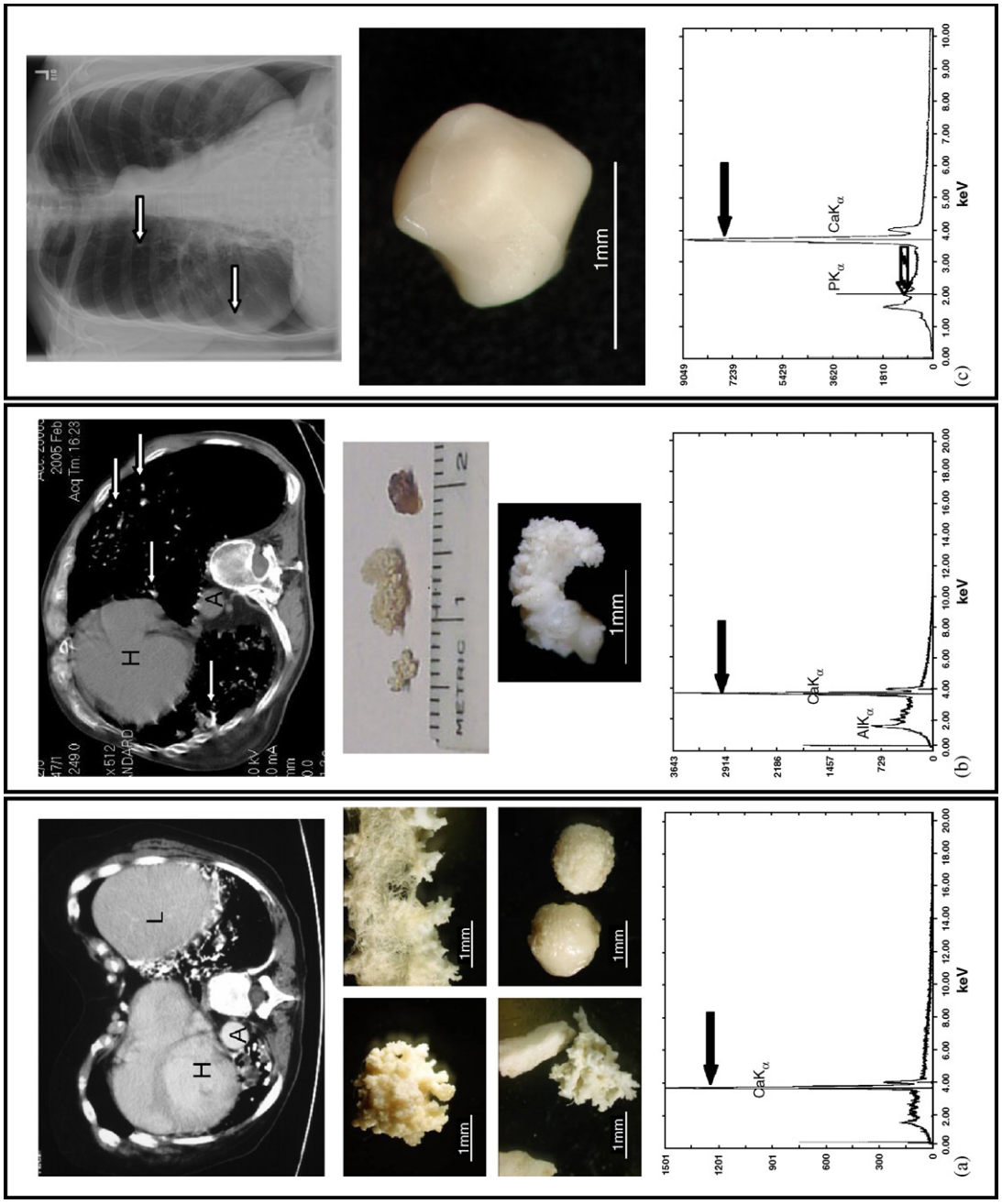
One patient, without radiographic calcium deposition, reported one single episode of lithoptysis (patient 5). Broncholith specimens were available for analysis in 3 patients (Fig. 2 and Table 1, patients 1, 2 and 4), and were examined by routine and electron microscopy. Multiple samples were available in patients 1 and 2, and there were three morphological patterns, round, spiculated, and longer branching liths (Figs. 2a and b). Only one specimen was available in patient 4 (round and smooth (2c)). EDAX revealed only a signal for carbon in samples from both patients 1 and 2 (Figs. 2a and b). Morphology coupled with EDAX result defined the material to be calcium carbonate (calcite). EDAX revealed a signal for both calcium and phosphate in patient 4 (Fig. 2c). Decalcification and silver staining of broncholiths revealed no evidence of fungal infection in patient 1.

Discussion

The identification of this novel association of pulmonary calcium deposition and calcium stone lithoptysis in a subset of older well-characterized PCD patients provokes a number of questions: why calcium deposition occurs, what type(s) of calcium stones develop and is calcium deposition in PCD clinically significant?

Calcium is deposited in the lung via two different mechanisms—calcification and ossification.[5] Pulmonary calcification involves the deposition of

Figure 2 Radiologic–pathologic correlation of 3 patients with primary ciliary dyskinesia, pulmonary calcium deposition and lithoptysis. (a) Contrast-enhanced axial CT scan of 61-yr-old woman with Kartagener's syndrome demonstrates extensive bibasilar calcification. Note the presence of situs inversus totalis and pectus excavatum. (H = heart, L = liver, A = aorta). Numerous broncholiths are displayed under light microscopy. EDAX analysis revealed a signal only for calcium (black arrow) and coupled with morphology identified that the specimens were composed of calcite. (b) Noncontrast axial CT scan of 59-yr-old man with Kartagener's syndrome demonstrates bibasilar calcification (white arrows). Note the presence of situs inversus totalis and kyphoscoliosis. (H = heart, A = aorta). Numerous broncholiths are displayed under light microscopy (including an image sent by the patient [upper photograph]). EDAX analysis revealed a signal only for calcium (black arrow) and coupled with morphology identified that the specimens were composed of calcite. (c) Posterior–anterior chest radiograph of a 60-yr-old woman with primary ciliary dyskinesia demonstrates two small calcified right lung nodules (white arrows). One smooth broncholith is displayed under light microscopy. EDAX analysis revealed signals for both calcium (black arrow) and phosphate (striped arrow).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(a)
(b)
(c)

calcium salts without ossification. It is categorized into two subtypes, metastatic calcification and dystrophic calcification.[5,6] Metastatic calcification manifests in previously normal lung parenchyma and occurs in both benign (dialysis patients) and malignant processes, often involving systemic calcium imbalance and is invariably deposited as calcium phosphate.[5] Dystrophic calcification occurs in previously injured lung parenchyma, often following an inflammatory process and usually deposits as calcium phosphate,[6] although dystrophic calcification secondary to deposition of calcium carbonate also occurs.[7–9] It may be associated with infection, bleeding and pulmonary infarction and often occurs secondary to granulomatous inflammation. In contrast to pulmonary calcification, pulmonary ossification refers to the formation of bone tissue (hydroxyapatitie) with or without marrow elements. A separate entity, pulmonary alveolar microlithiasis, leads to intraalveolar deposition of laminated calcium phosphate concretions.[5]

Broncholith formation from dystrophic calcification of granulomatous inflammation is the most common reported cause of lithoptysis; with *Mycobacterium tuberculosis* and histoplasmosis the most cited causes.[10,11] Broncholiths have been reported secondary to noninfectious conditions including malignancy and silicosis (calcium phosphate),[12] entities which are unlikely in this patient group given negative prior medical and occupational histories.

To address the question of why calcium deposition occurred in our cohort, we reviewed the distribution of calcium deposition on radiographic imaging, and identified two patterns: peribronchial and endobronchial distribution involving bronchiectatic airways ($n = 5$), and focal nodules ($n = 1$). Focusing on the larger group with calcium deposition involving the airways, broncholiths were available in 2 patients. In both, the presence of calcium without phosphate on EDAX, coupled with morphologic analysis identified only calcium carbonate (calcite) ruling out ossification. Dystrophic rather than metastatic calcification is more likely because of radiographic distribution and the negative renal and malignant history. Ciliary dysmotility in PCD predisposes to chronic pulmonary infections. Thus, granulomatous infections need therefore to be considered. However, fungal and mycobacterial infections are unlikely given negative PPD, cultures, staining for fungal elements and no evidence of lymph node (except one infrahilar node in patient 1), splenic or hepatic calcification. Furthermore, analysis of broncholiths related to such granulomatous disease in prior reports usually reveals calcium phosphate,[5,12,13] although calcite stones have been expectorated by patients with tuberculosis.[9]

The pattern of calcium deposition in patient 4 (pulmonary nodules) was possibly related to an unidentified granulomatous pulmonary disorder (e.g. endemic mycosis or tuberculous infection), where focal calcified pulmonary nodules are a well-recognized finding. Consistent with this hypothesis, we identified that the lith specimen expectorated from this patient contained calcium phosphate.

We hypothesize that calcification in a peri- and endo-bronchial distribution in these 5 PCD patients resulted from chronic airway inflammation, which is highlighted by the association only with bronchiectatic lobes. The youngest patient (patient 6) was 46, suggesting that chronicity of disease plays a role in the development of bronchiectatic airway related calcification in PCD. It is unclear why pulmonary calcium deposition does not occur in similar diseases of defective airway host defense including cystic fibrosis. A search of the literature identified a single case report associating cystic fibrosis with calcium deposition in the form of diffuse pulmonary ossification.[14] Possibilities include a milder phenotype in PCD[1] and the low pH of CF airway surface liquid may not be inductive to calcification.[5]

Pulmonary calcium deposition associated with idiopathic bronchiectasis has been previously identified. A case report from Japan[15] identified a 49-yr-old fertile male with normal situs who died from respiratory failure secondary to idiopathic bronchiectasis without prior history of pulmonary mycobacterial or fungal infection or other granulomatous disease. Autopsy revealed bibasal bronchiectasis, with multiple branching endobronchial broncholiths found in bronchial lumen. Histological analysis identified ''mucus retention derived calcite broncholithiasis''. This report cited eleven prior Japanese cases of idiopathic bronchiectasis with pulmonary calcification secondary to ''retained mucus''.

Broncholithiasis as a result of calcium deposition in bronchiectatic airways in this cohort of PCD patients was not associated with severe complications of broncholithiasis previously reported.[12,16–19] Broncholithiasis has been associated with chronic cough and recurrent pneumonia[13,20,21] both of which are invariably associated with PCD. The fact that pulmonary function as reflected by FEV_1 in this subgroup compared to PCD patients age >40 without pulmonary calcium deposition was not statistically significant suggests that calcium deposition is not causative or associated with more severe limitation in pulmonary function as reflected by FEV_1.

In conclusion, we have identified a novel association of lithoptysis secondary to bronchiectatic airway calcification in older patients with PCD. We hypothesize that calcification may be associated with chronic airway infection and inflammation in this disorder. From a therapeutic point of view, we advise all patients to continue aggressive airway clearance techniques with regular sputum analysis to reduce chronic airway inflammation secondary to the retention of infected secretions.

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