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Calcium oxalate in the sputum may aid in the diagnosis of pulmonary aspergillosis: A report of two cases

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ABSTRACT

We present two cases of pulmonary aspergillosis in which calcium oxalate crystals in the sputum proved to be a useful diagnostic clue. In case 1, *Aspergillus hyphae* was not identified; however, calcium oxalate crystals were present, and chronic necrotizing pulmonary aspergillosis was diagnosed. In case 2, calcium oxalate was detected and *Aspergillus fumigatus* was identified later. Thus, the presence of calcium oxalate in the sputum may be an important indicator for an *A. fumigatus* infection.

1. Introduction

Invasive pulmonary aspergillosis is extremely difficult to diagnose, and a combination of culture tests, clinical attributes, imaging tests, and serological diagnosis must be used [1]. In approximately 40% of all cases, invasive pulmonary aspergillosis is not clinically diagnosed while the patient is still alive; it is only discovered during post-mortem examination. The diagnostic criteria for Chronic progressive necrotizing pulmonary aspergillosis is defined as follows: (1) it progresses >1 month with lower respiratory tract symptoms; (2) the clinical manifestations are exacerbated on new imaging findings; (3) the exacerbated imaging findings confirm an *Aspergillus* infection serologically or pathologically; and (4) the condition of the patient on antifungal treatment is inexplicably necessary in terms of general bacterial infection or another disorder; and (5) elevated inflammatory reactions or advanced lesions attributable to *Aspergillosis* must meet criteria 1–4 even in the absence of an elevated inflammatory response [2]. Invasive pulmonary aspergillosis is normally diagnosed on the basis of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) diagnostic criteria for invasive fungal infections [3].

The presence of calcium oxalate in sputum culture samples is regarded as potentially useful in the diagnosis of Aspergillus *niger* [4] and Aspergillus *fumigatus* [5,6]. A study comparing 65 patients who tested positive for *Aspergillus* and 60 control patients (with or without other types of infection) showed that calcium oxalate crystals are specific markers of an *Aspergillus* infection [7]. However, the value of calcium oxalate in a sputum culture for the diagnosis of pulmonary aspergillosis has very rarely been examined [7].

We treated two patients in whom calcium oxalate crystals in the sputum proved to be a useful clue in the diagnosis of *Aspergillus*. Since calcium oxalate in sputum samples aids in diagnosing *A. niger* and *A. fumigatus*, which account for most cases of pulmonary aspergillosis [8,9], this suggests that it should be added to the diagnostic criteria for all cases of aspergillosis, including *A. niger*.

2. Case reports

2.1. Case 1

A 66-year-old man presented to an outlying hospital with complaints of coughing, hemoptysis, and back pain. He had type 2 diabetes managed with kinesitherapy and dietotherapy but

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Table 1
The blood test results for case 1.

General blood tests		Blood biochemistry tests			
WBC	14,900/μL	T-bil	0.5 mg/dL	CI	107 mEg/L
Seg	88.6%	TP	7.8 g/dL	CRP	30.78 mg/dL
Eo	1.0%	Alb	3.6 g/dL	β-D glucan	(−)
Ly	5.4%	AST	29 IU/L	QFT	(−)
Mo	4.2%	ALT	29 IU/L	*Aspergillus* antibody	(−)
RBC	367×10^4/μL	Ch-E	225 IU/L	*Aspergillus* antigen	(+)
Hb	12.1 g/dL	γ-GTP	28 IU/L	*Cryptococcus* antibody	(+)
Ht	35.5%	ALP	169 IU/L	PR3-ANCA	(−)
MCV	96.7 fL	LDH	161 IU/L	MPO-ANCA	(−)
MCH	34.1 Pg	BUN	24 mg/dL	HIV antibody	(−)
MCHC	35.2%	Cre	0.66 mg/dL	TP antibody	(−)
Plt	30.4×10^4/μL	Na	140 mEq/L	RPR	(−)
		K	4.4 mEq/L		

without medication. He had no particular contact with any disease agents, no history of owning pets or of traveling North America (Los Angeles), South America (Cancun and Mexico). He smoked 40 cigarettes/day and had no history of alcohol use or allergies. He developed chest and back pain around November 8, 20XX (day −7). On November 12 (day −3), he visited a local ear-nose-throat clinic; leukocytosis and an inflammatory response were detected. On November 15 (day 0), he was referred to our hospital's emergency department for detailed testing and treatment. After imaging tests were performed, he was admitted for further testing and treatment (Table 1).

Upon arrival, the patient was hyperthermic (38.7 °C) with a fast pulse (99 beats/min). His respiration and blood pressure were 16/min and 104/76 mmHg, respectively.

His breath sounds were reduced in the left upper lung field only. He had a ground-glass shadow in the left upper lung field on

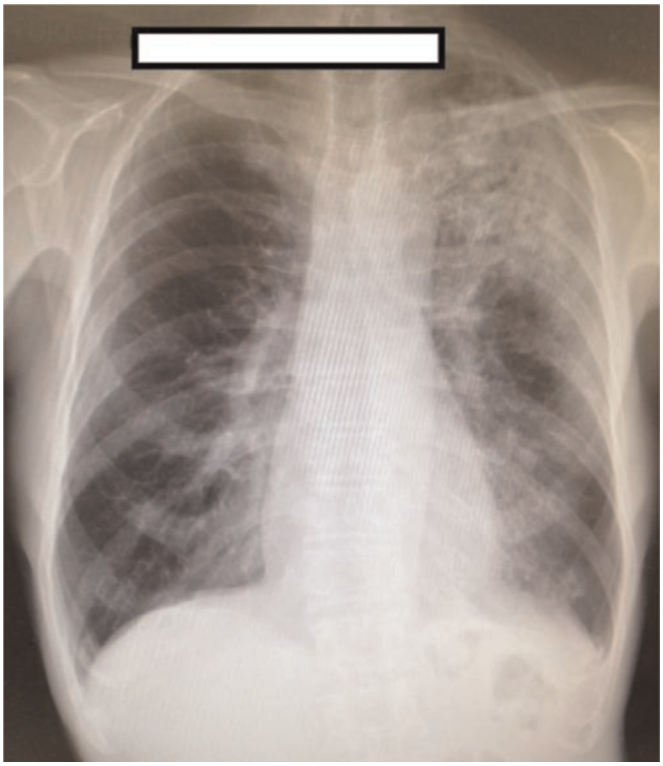

Fig. 1. Case 1: thoracic plain radiograph.

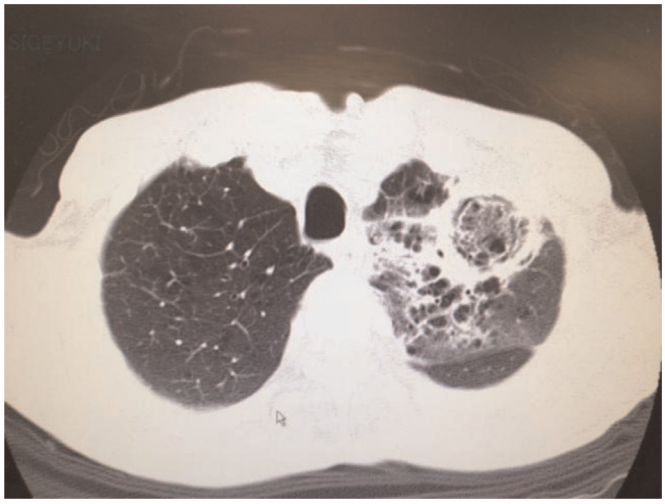

Fig. 2. Case 1: thoracic plain computed tomography scan.

thoracic plain radiograph (Fig. 1), and there was a reverse halo shadow in the left S3 with ground-glass attenuation surrounding the consolidation in S1 and S2 on the thoracic computed tomography (CT) scan (Fig. 2).

On the basis of the clinical course and imaging findings, the differential diagnosis in case 1 included community-acquired pneumonia, pulmonary mycobacteriosis (tuberculosis or non-tuberculous mycobacteriosis), pulmonary mycosis (chronic pulmonary aspergillosis, cryptococcosis, or histoplasmosis), pulmonary nocardiosis, actinomycosis, alveolar cell carcinoma, pulmonary malignant lymphoma, and cryptogenic organizing pneumonia. The samples were tested for the human immunodeficiency virus antibody, β-D-glucan, *Aspergillus* and *Cryptococcus* antigens, and sputum cytology. Sputum Gram stain test was negative; however, considering the frequency of the community-acquired pneumonia, ceftriaxone (2 g/day) was started on day 1.

In addition to the aforementioned tests, that for the *Aspergillus* galactomannan antigen yielded positive results; however, the sputum smear test (three consecutive expectorations) for *Mycobacterium* yielded negative results, and the sputum culture was negative. No significant findings were detected in the sputum culture or the cytology acquired by bronchoscopy. Given the presence of calcium oxalate crystals (Fig. 3) as well as the fact that the diagnosis of chronic pulmonary aspergillosis was consistent with the imaging findings, treatment with voriconazole (loading dose: 300 mg intravenously every 12 h on the first day; subsequent doses: 200 mg intravenously every 12 h) was started on day 8. The test for the *Aspergillus* galactomannan antigen performed on day 12 yielded positive results. Since the patient's symptoms improved, as did the blood test results, the medication was switched to oral voriconazole (600 mg/day) from day 16. The patient was discharged on day 23, and oral voriconazole was continued.

2.2. Case 2

A 66-year-old man presented to an outlying hospital with complaints of fever and a productive cough. The patient had emphysema and stomach cancer (total gastrectomy). He smoked 20 cigarettes/day for 40 years (until 6 years previously) and drank 350 mL of beer/day. He had no history of pets and never traveled to any foreign country.

The patient developed a fever, coughing, and expectoration around September 12, 20XX (day −12). He had been taking some antibiotics that were prescribed by a medical practitioner. The patient visited our hospital on September 24 (day −2).

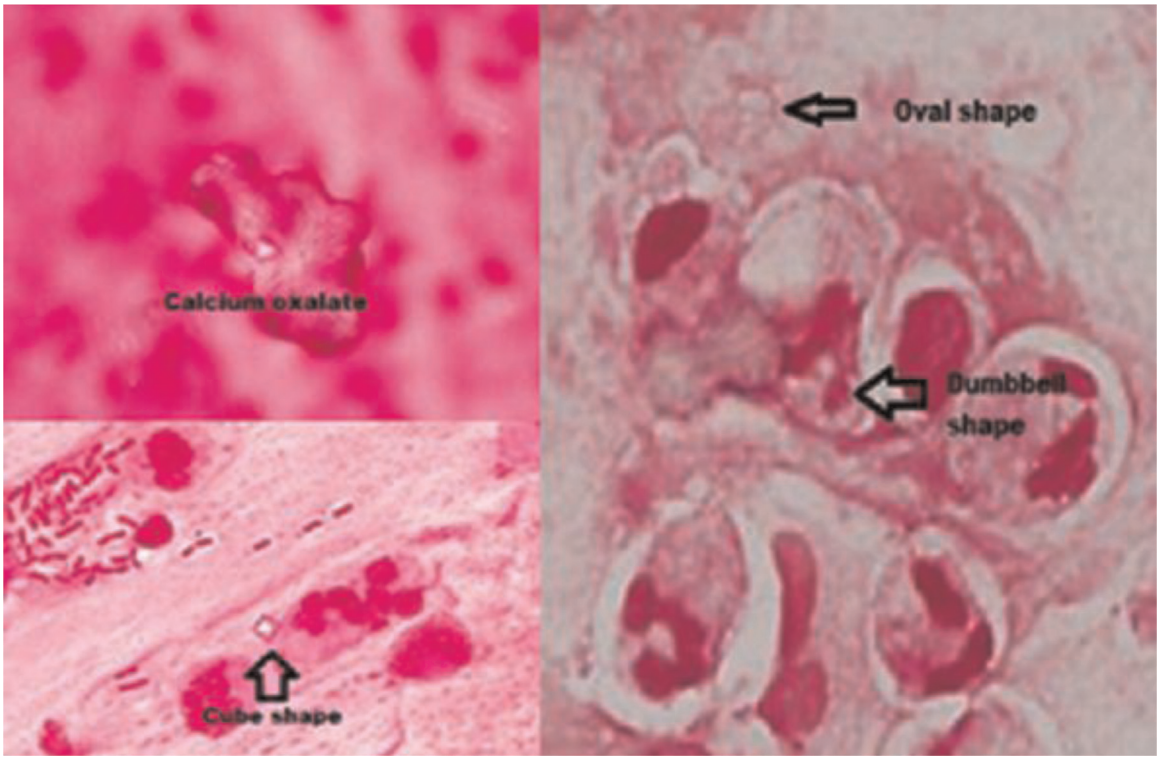

Fig. 3. The presence of calcium oxalate in the sputum of case 1.

Emphysematous changes in the upper lobe of the right lung and niveau formation in the bulla were evident, and consolidation was also present in the left upper lung. The patient was monitored as an outpatient but was eventually admitted to our hospital's Department of Respiratory Medicine on September 26 (day 0) after hemoptysis was observed (Table 2).

He had ground-glass shadow in the left upper lung field on the thoracic plain radiograph (Fig. 4) and consolidation associated with air bronchogram in the left S3 on thoracic computed tomography scan (Fig. 5).

Klebsiella pneumoniae was identified from an initial sputum sample, which was collected at day −12, and treatment with ampicillin/sulbactam (3 g intravenously every 6 h) was started on day 1. However, his symptoms failed to improve, and since the tests for the *Aspergillus* antigen and antibody were both positive on day 10, our department was consulted. The calcium oxalate crystals were also evident in the sputum culture (Figs. 6 and 7); thus, chronic necrotizing pulmonary aspergillosis was suspected and treatment with voriconazole was started (loading dose: 300 mg intravenously every 12 h on the first day; subsequent doses: 200 mg intravenously every 12 h). *A. fumigatus* was identified in a sputum culture test on day 12. However, the patient's

Table 2
The blood test results for case 2.

General blood tests		Blood biochemistry tests			
WBC	14,300/μL	T-bil	0.3 mg/dL	CI	107 mEg/L
Seg	89.0%	D-bil	0.2 mg/dL	CRP	10.57 mg/dL
Eo	0.0%	TP	7.8 g/dL	β-D glucan	(−)
Ly	4.0%	Alb	3.6 g/dL	QFT	(−)
Mo	1.0%	AST	29 IU/L	*Aspergillus* antibody	(+)
AT-Ly	2.0%	ALT	29 IU/L	*Aspergillus* antigen	(+)
RBC	298×10^4/μL	Ch-E	14 IU/L	*Cryptococcus* antibody	(−)
Hb	9.5 g/dL	γ-GTP	14 IU/L	HIV antibody	(−)
Ht	29.7%	ALP	297 IU/L	TP antibody	(−)
MCV	99.7 fL	LDH	252 IU/L	RPR	(−)
MCH	31.9 Pg	BUN	6 mg/dL		
MCHC	32.0%	Cre	0.36 mg/dL		
Plt	20.6×10^4/μL	Na	2.9 mEq/L		
		K	4.4 mEq/L		

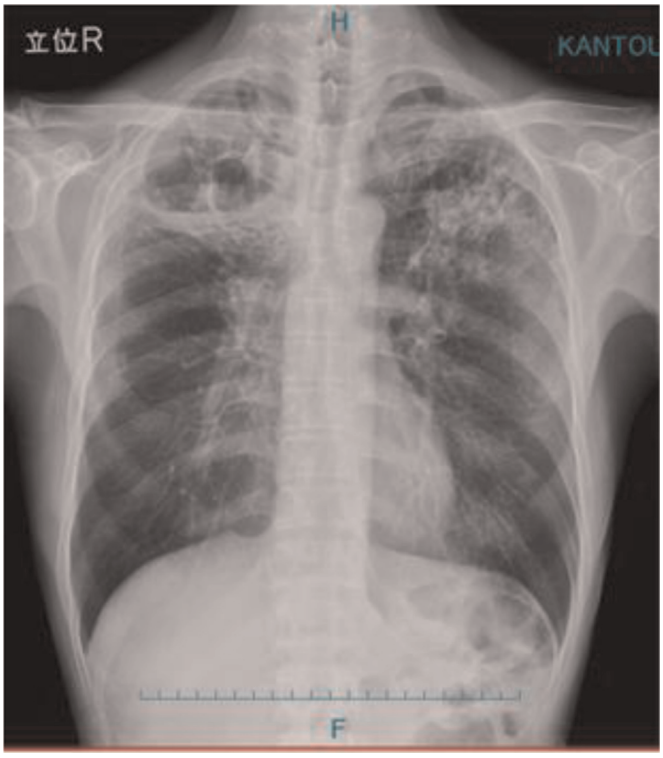

Fig. 4. The thoracic plain radiograph of case 2.

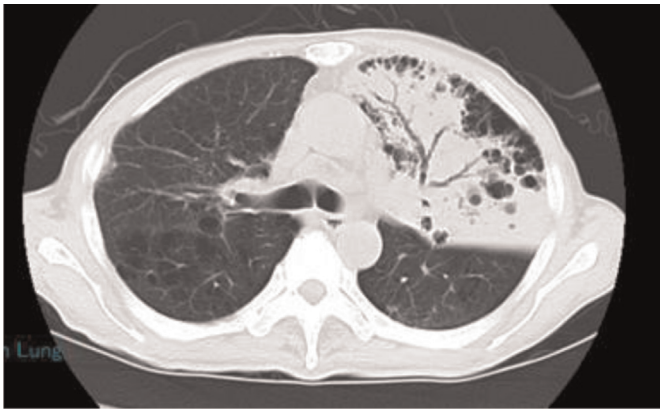

Fig. 5. The thoracic computed tomography scan of case 2.

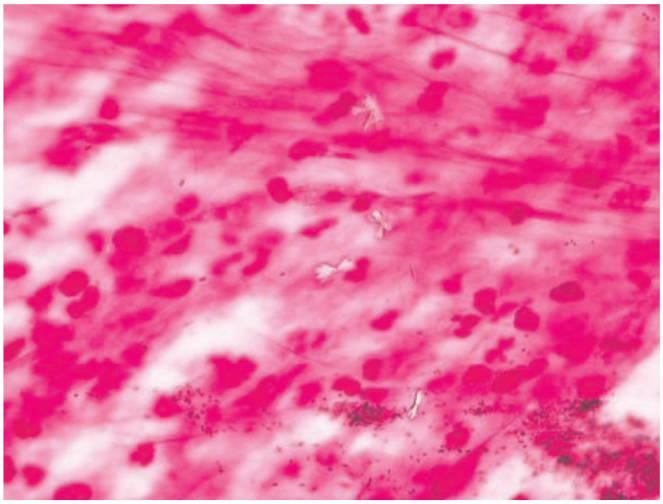

Fig. 6. The calcium oxalate observed in the Gram stain for case 2.

Fig. 7. The calcium oxalate Gram staining in case 2, as viewed through a simple polarizing filter.

general condition on admission was poor, and he died on day 14.

The study was approved by the Ethics committee of Kanto Rosai Hospital, and all the patients provided written informed consent to publish the manuscript.

3. Discussion

The main causative species of pulmonary aspergillosis are *A. fumigatus*, *A. niger*, *Aspergillus nidulans*, and *Aspergillus flavus* [10], of which *A. fumigatus* accounts for most cases [8,9,11]. Calcium oxalate is formed when oxalic acid is released into tissue as a metabolite when *Aspergillus* binds with tissue calcium [8]. Calcium oxalate itself is toxic and destroys tissue [12], causing the progression of lesions. Studies have suggested that calcium oxalate may be useful in the diagnosis of *A. niger* [4–6], and some have suggested that it may also be useful in the diagnosis of *A. fumigatus*, as it is present in the pathological samples [1]. However, no previous study has investigated the value of calcium oxalate for diagnosing pulmonary aspergillosis caused by *A. fumigatus* in living patients. Given this scenario, the cases presented here are of particular interest.

The *Aspergillus* galactomannan antigen is one of the parameters used for diagnosing chronic necrotizing pulmonary aspergillosis and is among the microbial parameters of the EORTC/MSG diagnostic criteria for invasive fungal infections. However, it has been reported that its sensitivity for *A. fumigatus* may be lower than that for non-*fumigatus* infections [13], and it is possible that the presence of calcium oxalate in particular may be a sufficient cause for suspecting *A. fumigatus* pulmonary aspergillosis in chronic lower respiratory lesions.

In conclusion, calcium oxalate may aid in the diagnosis of pulmonary aspergillosis caused by *A. fumigatus*. The exact use of calcium oxalate as a diagnostic parameter of chronic necrotizing pulmonary aspergillosis as well as a microbial parameter of the EORTC/MSG diagnostic criteria for invasive fungal infections should be confirmed through the examination of more similar cases.

Conflict of interest

There are no conflicts of interest.

Acknowledgments

There are none.

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