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Cholesteryl palmitate crystals in bronchoalveolar lavage fluid smears as a possible prognostic biomarker for chronic interstitial pneumonia: A preliminary study

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ABSTRACT

Background: We observed cholesterol-like crystals (Crystal X) in the bronchoalveolar lavage fluid (BALF) smears of patients with diffuse pulmonary disease. We analyzed the clinical data of patients with and without crystals, and elucidated the structure of Crystal X and its concentration in the BALF.

Methods: Two hundred eighty-nine patients with diffuse pulmonary disease who underwent bronchoalveolar lavage (BAL) were analyzed. The relationships between the presence and number of Crystal X in BALF smears and clinical parameters were investigated. Furthermore, structure determination and quantitative analyses of the crystals were performed.

Results: Seventy-five (26.0%) patients had Crystal X in their BALF. The crystals were frequently observed in patients with chronic interstitial pneumonia (CIP, 60/160=35.3%). Patients with Crystal X exhibited significantly higher serum Kerbs von Lungren 6 antigen and surfactant protein-D levels ($P<0.01$) and lower percentage vital capacity ($P<0.05$) than patients without Crystal X. The number of crystals was significantly correlated with these parameters. The presence of crystals was also associated with a lower survival rate at 1 year after the BAL. The interfacial angles of the crystals were $126\pm2^{\circ}$ and $144\pm2^{\circ}$, different from those of cholesterol monohydrate crystals. Infrared absorption spectrometry showed Crystal X was cholesteryl palmitate. Its concentration was significantly higher in BALF with crystals than in BALF without crystals ($P<0.01$).

Conclusions: Crystal X in the BALF of patients with diffuse pulmonary disease was identified as cholesteryl palmitate, which may be a useful prognostic biomarker for CIP.

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1. Introduction

Various types of crystals have been identified in biologic samples. The presence of these crystals sometimes has a close correlation with the pathogenesis of a disease and is a useful marker of its diagnosis. For example, cholesterol (i.e., cholesterol monohydrate) crystals have been identified in the urine of patients with nephrotic syndrome, which may be associated with the hypercholesterolemia that occurs as a component of the syndrome. In patients with hyperuricemia, uric acid crystals are observed in joint fluids, which potentially cause gout attacks, and are an important maker for the diagnosis [1]. In addition, various types of crystals have often been observed in histopathological samples. Cholesterol crystals are often present as cholesterol crystal clefts in arteriosclerotic lesions. They have been identified in the lungs of patients with cholesterol pneumonitis, hypersensitivity pneumonitis, sarcoidosis, pulmonary hypertension, and silicosis [2]. The presence of calcium oxalate crystals has been reported in patients with pulmonary aspergillosis [3]. In addition to having a diagnostic significance, these crystals are directly involved in inflammatory pathology [4].

We observed cholesterol-like crystals (hereinafter referred to as Crystal X) in the bronchoalveolar lavage fluid (BALF) smears of some patients with diffuse pulmonary disease. To determine whether Crystal X is a clinically useful biomarker for chronic interstitial pneumonia (CIP), we analyzed the clinical data of patients with and without the crystals, and determined the chemical structure and the concentration of Crystal X in the BALF.

2. Materials and methods

2.1. Patients

Two hundred eighty-nine patients with diffuse pulmonary disease underwent BAL for further examination at our department between January 2009 and March 2012 (Table 1). The BALF smears were retrospectively reviewed. The presence and number of Crystal X in the smears were examined. Crystal X occurred at a high frequency in patients with CIP (e.g., idiopathic interstitial pneumonia [IIP] and interstitial pneumonia associated with collagen tissue disease [IP-CTD]); therefore, the association between the presence and number of Crystal X and the clinical parameters were evaluated in 148 patients with CIP. This study was conducted with the approval of the ethics committee of Fukushima Medical University (Fukushima, Japan; approval number, 1887; approval date, January 30, 2014).

Table 1 – Analysis of the crystals in the bronchoalveolar lavage fluid of patients with diffuse pulmonary disease.

	Total	Crystal (−)	Crystal (+)	
	(*N*)	*N* (%)	*N* (%)	Number of crystals
IIP	130	85 (65.4)	45 (34.6)	6.8±1.9
IP-CTD	40	25 (62.5)	15 (37.5)	17.6±6.8
Sarcoidosis	38	34 (89.5)	4 (10.5)	6.7±5.7
Hypersensitivity pneumonitis	7	3 (42.9)	4 (57.1)	11.2±3.9
Organizing pneumonia	11	8 (72.7)	3 (27.3)	1.0±0.0
Alveolar proteinosis	3	1 (33.3)	2 (66.7)	31.0±2.0
Pneumocystis pneumonia	11	10 (90.9)	1 (9.1)	1
Radiation pneumonitis	3	2 (66.7)	1 (33.3)	1
Drug-induced pneumonia	10	10	0	0
Eosinophilic pneumonia	10	10	0	0
Pneumonia	7	7	0	0
Others	19	19	0	0
Total	289	214 (74.0)	75 (26.0)	

IIP, idiopathic interstitial pneumonia; IP-CTD, interstitial pneumonia associated with connective tissue disease.

2.2. Assessment of crystals in patients with diffuse pulmonary disease

First, the frequency of the presence of Crystal X in the BALF smears was examined in patients with diffuse pulmonary disease. The patients were then classified into two groups: the CIP group and the non-CIP group. The frequency of the presence of Crystal X was compared between the two groups. The CIP group was divided into two subgroups: the crystal (+) group and the crystal (−) group. Serological data, pulmonary functional data, and the results of BAL (i.e., total and differential cell counts) were compared between the two subgroups. In addition, the correlations between the number of crystals and the values of the aforementioned parameters were evaluated. The survival rate at 1 year after BAL was also compared between the two subgroups. The diagnosis of IIP was made based on the diagnostic criteria of the 2010 American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association (ATS/ERS/JRS/ALAT) statement, and IIP was divided

Abbreviations: ABCA, ATP-binding cassette transporter A; BALF, bronchoalveolar lavage fluid; CIP, chronic interstitial pneumonia; Crystal X, cholesterol-like crystal; Elovl 6, elongation of very long chain fatty acid member 6; IIP, idiopathic interstitial pneumonia; IP, interstitial pneumonia; IP-CTD, interstitial pneumonia associated with connective tissue disease; IPF, idiopathic pulmonary fibrosis.

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