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Sputum club cell protein concentration is associated with pulmonary exacerbation in cystic fibrosis☆,☆☆

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Abstract

Background: Cystic fibrosis (CF) patients exhibit a progressive decline in lung function accelerated by intermittent pulmonary exacerbations. There are urgent needs for clinically relevant biomarkers to aid in the diagnosis and management of a CF pulmonary exacerbation, in addition to providing insight into its pathophysiology. Club cell secretory protein (CCSP) is produced by bronchial epithelial cells, known to have anti-inflammatory properties and may play a role in CF pulmonary exacerbations. Our objective was to measure sputum CCSP concentration during hospitalizations for CF pulmonary exacerbation and during quarterly outpatient clinic visits for 2 years. We explored the correlations between CCSP concentration, lung function and markers of inflammation and infection.
Methods: In this prospective, longitudinal cohort study, expectorated sputum, blood and lung function data were collected from 45 CF patients during 68 hospitalizations for pulmonary exacerbation and 193 clinic visits. Sputum CCSP concentration was measured and sputum and blood were assayed with a panel of inflammatory cytokines. We used a repeated measures model to compare log transformed sputum CCSP concentrations across multiple time points and to correlate those concentrations with related clinical variables.
Results: Our population had a mean age of 29 (16–58 years), and a median FEV_1 %predicted of 60% (18–105%). Sputum CCSP concentration was significantly lower in the initial, interim and final exacerbation samples ($p = 0.0021$, $p = 0.0005$ and $p = 0.0274$, respectively) compared to outpatient visits. Sputum CCSP concentration was negatively associated with sputum neutrophil elastase concentration ($p = 0.0373$). Patients with *Pseudomonas aeruginosa* mucoid had a significantly lower sputum CCSP concentration ($p = 0.0129$).
Conclusion: Sputum CCSP concentration is associated with CF pulmonary exacerbation.

Keywords: Cystic fibrosis; Biomarker; Sputum; Inflammation; Lung function; Cytokines; Infection

1. Introduction

CF lung disease begins silently in infancy and is characterized by infection, chronic inflammation, bronchiectasis, progressive lung function decline and intermittent pulmonary exacerbations [1]. Pulmonary exacerbations are associated with an increase in pulmonary symptoms, decrease in lung function, loss of energy, weight loss and changes in physical exam findings [2]. However,

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given the lack of a universally applicable definition and the knowledge that 25% of CF patients treated for an exacerbation do not recover to their baseline lung function, significant gaps in knowledge remain [3]. There are urgent needs for clinically relevant biomarkers to aid in the diagnosis and management of a CF pulmonary exacerbation, in addition to providing insight into its pathophysiology.

Given that neutrophils and inflammatory cytokines are hallmarks of CF lung disease, biomarkers of airway inflammation have been an area of intense research focus in CF [4,5]. However, anti-inflammatory mediators may also play a role in CF airway injury and may provide valuable insight into the mechanisms of lung disease progression [6]. Club cell secretory protein (CCSP, formerly known as Clara cell secretory protein, CC10 or CC16) has anti-inflammatory and immunosuppressive properties and is primarily produced by non-ciliated Club cells (formerly known as Clara cells) in the conducting airways. Club cells secrete CCSP in very high concentrations in the epithelial lining fluid where it modulates the production and activity of phospholipase A_2 (PLA_2), interferon-γ and tumor necrosis factor-α. Its role in protecting the lung makes it an attractive candidate biomarker important to the defense against oxidative stress [7–9]. CCSP is thought to be a biomarker of lung epithelial injury, with its airway concentration reflecting epithelial cell integrity and its plasma concentration assessing the permeability/leakiness of the lung epithelial barrier. Sputum CCSP was previously identified by proteomics analysis as a potential biomarker of CF lung disease warranting further study [10]. Bronchoalveolar lavage fluid (BALF) concentration of CCSP was found to be lower in CF patients compared to those with chronic bronchitis and was inversely correlated with airway inflammation [6]. These studies suggest CCSP may play an important role in CF; however, no information is known about CCSP concentration in CF patients during pulmonary exacerbation or longitudinally during times of clinical stability.

In this prospective, longitudinal cohort study, we collected expectorated sputum, blood and lung function data from patients with CF at multiple time points during hospitalization for a pulmonary exacerbation and during quarterly outpatient clinic visits for 2 years. We hypothesized that sputum CCSP concentration would be lower during a pulmonary exacerbation and would correlate with the severity of lung disease as measured by the degree of obstructive lung disease [forced expiratory volume in one second (FEV_1)] and markers of inflammation and infection.

2. Materials and methods

2.1. Study population

Forty-five patients with a confirmed diagnosis of CF were identified on admission to the hospital for treatment of a pulmonary exacerbation and recruited into our study from 2008 to 2011 [11]. For study purposes, a pulmonary exacerbation was defined as the need for hospitalization for intravenous (IV) antibiotics and airway clearance for an increase in pulmonary symptoms, and/or a 10% decrease in FEV_1. Clinical stability was defined as no current use of IV antibiotics or lack of symptoms consistent with a pulmonary exacerbation. Patients were required to spontaneously expectorate sputum. The study protocol was approved by the University of Minnesota Institutional Review Board and informed consent and/or assent was obtained from each of the subjects and/or their parents or guardians.

2.2. Study design

This was a single-center, prospective, 2-year longitudinal cohort study of patients with CF during times of pulmonary exacerbation (hospitalization) and times of clinical stability (outpatient clinic visits). All hospitalized patients received standard-of-care therapies including airway clearance, nutritional support and IV antibiotics. Each subject provided an expectorated sputum sample and a blood sample, and underwent pulmonary function testing approximately within 24 h of the initiation of IV antibiotics (initial sample), on days 3–4 (interim sample) and on days 5–7 (final sample). Subjects at the University of Minnesota are routinely discharged after <1 week in the hospital to finish therapy at home. Patients were not required to provide samples at all three time points to be included. Upon discharge, subjects were followed at quarterly outpatient clinic visits for 2 years. An expectorated sputum sample, blood sample and pulmonary function testing data were collected at each clinic visit. If a CF patient was on IV antibiotics or reported symptoms of a pulmonary exacerbation at an outpatient visit, samples collected were excluded from the analysis. Identical samples were collected if patients had subsequent hospitalizations for pulmonary exacerbation during the study period. Sputum was processed as previously described and frozen immediately after collection at −80 °C prior to analysis [12].

2.3. Laboratory assays

2.3.1. Sputum sample analysis

Proteases (neutrophil elastase and matrix metalloproteinase [MMP-9]) were measured in thawed specimens not treated with protease inhibitors. Free neutrophil elastase activity was quantified by a spectrophotometric assay based on the hydrolysis of the specific substrate MeO-suc-Ala-Ala-Pro-Ala-*p*-nitroanilide (Sigma Chemical Co, St. Louis, MO) and MMP-9 was measured by a commercially available ELISA kit (Quantikine; R&D Systems, Minneapolis, MN). The cytokines IL-23 and TGF-β1 were measured using commercially available ELISA kits (R&D Systems, Minneapolis, MN). IFN-γ, IL-1b, IL-2, IL-6, IL-8, MCP-1 and TNF-α, were analyzed as an eight-plex (EMD Millipore Corporation, Billerica, MA). Qualitative bacterial cultures were performed on an airway specimen obtained during each admission and for each clinic visit.

2.3.2. CCSP assay

Sputum CCSP concentration was measured in the homogenized sputum supernatant treated with protease inhibitors using a human-specific competitive ELISA assay (APC Biotechnology Services, Inc. Rockville, MD). The LOD for this assay is 5 ng/ml. Four parameter logistic curves were used that ranged from 5 to 500 ng/ml. The intra-assay coefficient of variation (CV) was