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Original Article

Multiple reaction monitoring mass spectrometry to identify novel plasma protein biomarkers of treatment response in cystic fibrosis pulmonary exacerbations☆,☆☆,★

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Abstract

Background: Systemic inflammation decreases with IV antibiotics during the treatment of CF pulmonary exacerbations (PEx). We used multiple reaction monitoring mass spectrometry and immunoassays to monitor blood proteins during PEx treatment to determine if early changes could be used to predict PEx outcomes following treatment.
Methods: Blood samples from 25 PEx (22 unique adults) were collected within 24 h of admission, day 5, day 10, and at IV antibiotic completion. Ninety-two blood proteins involved in host immunity and inflammation were measured.
Results: Levels of several blood proteins changed from admission to end of IV antibiotics, most increasing with treatment. Early changes (admission to day 5) in fibrinogen levels had the strongest correlation with overall improvement in CFRSD-CRISS and FEV_1% predicted by the end of treatment.
Conclusions: Several plasma proteins changed significantly with IV antibiotics. Future studies will evaluate fibrinogen as an early biomarker of PEx treatment response in CF.

Keywords: Cystic fibrosis; Pulmonary exacerbations; Systemic inflammation; Biomarkers; Treatment response

1. Introduction

Cystic fibrosis (CF) pulmonary exacerbations (PEx) are characterized by a change in respiratory symptoms beyond typical day-to-day fluctuation, necessitating additional treatment [1,2]. PEx remain common with ~50% of adults and ~25% of children with CF experiencing at least one episode requiring treatment with intravenous (IV) antibiotics each year in the United States [3]. These events can profoundly impact patients' health-related quality of life and health outcomes, including rate of lung function loss and mortality [4–8].

Novel treatment monitoring strategies are needed to improve PEx outcomes in CF patients. Blood-based biomarkers reflective of systemic inflammation have the potential to inform clinical decision-making during CF PEx treatment. Many blood protein biomarkers previously examined change significantly from the beginning to end of PEx treatment [9]. However, for a blood biomarker to impact treatment decisions, ideally it should be

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informative early in the PEx treatment course and not just change from beginning to end of treatment. The availability of a reliable and predictive biomarker of treatment response measured early during the PEx course might provide an opportunity to identify treatment non-responders earlier, thus allowing CF physicians to modify treatments in a timelier manner to improve PEx outcomes.

The objective of this study was to use multiple reaction monitoring mass spectrometry (MRM-MS) and high-performance immunoassays to identify blood protein biomarkers that change in response to PEx treatment. MRM-MS is a powerful biomarker discovery platform capable of measuring up to 100 peptides simultaneously with minimal blood volume [10]. We evaluated the same panel of peptides/proteins that was examined in a prior study to discover blood biomarkers of imminent CF PEx [11]. To increase the opportunity for clinical utility, we were particularly interested in proteins that change by the end of the first week of treatment and that correlate with overall changes in symptoms and/or lung function by the end of treatment. We hypothesized that early change (i.e. between days 0 and 5 of IV antibiotic therapy) in blood protein levels could be used to predict the extent of symptom and lung function response following treatment.

2. Methods

2.1. Study subjects and design

Adults with a confirmed diagnosis of CF based on standard criteria [12] admitted to St. Paul's Hospital (Vancouver, BC) between July 1, 2013 and June 30, 2015 for PEx treatment were eligible. The definition of a PEx used in this study was a change in respiratory symptoms and clinical status requiring IV antibiotics [2], with a score of at least 4/12 based on the modified Fuchs PEx criteria [13]. Exclusion criteria included prior solid organ transplantation and active inflammatory conditions (e.g. allergic bronchopulmonary aspergillosis) requiring chronic immunosuppressive therapy.

Participants received standard treatment with airway clearance therapies and IV antibiotics for ~14 days, but the CF physician determined the exact duration of PEx treatment based on assessment of symptoms and lung function response. Antibiotics were chosen at the discretion of the treating physician based on recent sputum microbiology results, history of antibiotic allergies, and/or history of prior clinical response to specific antibiotics. Systemic corticosteroids were also permitted at the discretion of the treating physician.

Written informed consent was obtained from participating subjects and the research protocol was approved by the University of British Columbia Providence Health Care Research Institute Research Ethics Board (UBC-PHC REB number H12-00835).

2.2. Blood collection, processing, and analysis for plasma proteins

Blood samples were collected within 24 h of hospital admission (V1), day 5 (V2), day 10 (V3), and treatment completion (V4). Venous blood was collected in potassium EDTA and serum tubes according to standard operating procedures. Blood samples were immediately placed on ice, processed for plasma collection, and stored within 2 h at −80 °C for batched analysis.

Plasma samples underwent targeted proteomic analysis using MRM-MS at the University of Victoria Genome BC Proteomics Centre (Victoria, BC, Canada) as described previously [11]. Identical to our prior study, a bio-library of 127 unique proteins (represented by 230 peptide fragments) was initially evaluated [11]. Following quality control metrics (as described previously [14]) and differences in protein abundance for exacerbation (versus stable) plasma samples, 92 proteins (represented by 134 peptide fragments) were evaluated in this study compared to 75 proteins (represented by 109 peptide fragments) in our prior publication [11]. Many of the proteins in this library are related to host immunity, inflammation, coagulation, and the acute phase response (see Appendix Table 1). Candidate proteins including serum interleukin (IL)-1β, IL-6, IL-8, tumor necrosis factor (TNF)-α, and calprotectin could not be measured with MRM-MS and thus were evaluated using commercial immunoassay kits from Mesoscale Discovery (MSD) (Carlsbad, CA). All assays were performed in duplicate and mean values were used for analysis.

2.3. Other clinical variables

Duration of symptoms prior to hospitalization for IV antibiotics (≤ or >2 weeks), sputum microbiology, white blood cell (WBC) count, initial IV antibiotic regimen, need for systemic corticosteroids, and total duration of IV antibiotic treatment were collected for each PEx.

2.4. Treatment outcomes

PEx treatment outcomes of interest included change in forced expiratory volume in 1 s (FEV_1) % predicted and Cystic Fibrosis Respiratory Symptom Diary-Chronic Respiratory Infection Symptom Score (CFRSD-CRISS) from admission to end of PEx treatment. We focused on relative change in FEV_1% predicted as opposed to absolute change as the latter can be influenced by baseline values, with a larger change observed in patients with a higher FEV_1% predicted at baseline and a smaller change observed in patients with lower FEV_1% predicted at baseline, as demonstrated in a large observational CF exacerbation study [15]. In contrast, we focused on absolute change in CFRSD-CRISS as the extent of change did not appear to vary based on baseline disease severity [15]. Spirometry and CFRSD-CRISS were obtained from subjects on the same days as blood draws. Spirometry was performed in accordance with ATS criteria [16]. CFRSD-CRISS is scaled from 0 to 100 points, with a higher score indicating greater severity of respiratory symptoms and a minimum clinically important difference of 16 points during PEx treatment [17]. The treating physicians had access to the spirometry results and WBC count during treatment but were blinded to all other research data, including blood biomarker measurements and CFRSD-CRISS, which were analyzed retrospectively.

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