Journal of Cystic Fibrosis 19 (2020) 49–51

Contents lists available at ScienceDirect

Journal of Cystic Fibrosis

journal homepage: www.elsevier.com/locate/jcf

Short Communication

Blood biomarkers to predict short-term pulmonary exacerbation risk in children and adolescents with CF: A pilot study

Amrit Singh[a,b,c], Mary He[c], Virginia Chen[b,c], Zsuzsanna Hollander[b,c], Scott J. Tebbutt[b,c,d], Raymond T. Ng[b,c], Bruce M. McManus[a,b,c], Don D. Sin[b,c,d], Felix Ratjen[e], Bradley S. Quon[b,c,d,*]

[a] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
[b] Centre for Heart Lung Innovation, University of British Columbia and St. Paul's Hospital, Vancouver, BC, Canada
[c] Prevention Of Organ Failure (PROOF) Centre of Excellence, Vancouver, BC, Canada
[d] Division of Respiratory Medicine, Department of Medicine, University of British Columbia, Vancouver, BC, Canada
[e] Division of Respiratory Medicine, Department of Pediatrics, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 16 April 2019
Revised 22 May 2019
Accepted 26 May 2019
Available online 5 June 2019

Keywords:
Biomarkers
Pulmonary exacerbations
Proteomics

ABSTRACT

In CF, pulmonary exacerbations (PEx) can lead to permanent loss in lung function and thus should be prevented. Previously, we identified a blood protein biosignature consisting of 6 proteins capable of predicting short-term PEx events in CF adults. In this study, we utilized blood samples from the placebo arm of a randomized controlled trial to assess whether this candidate protein biosignature was also capable of predicting short-term PEx events in CF children and adolescents. This pilot study provides preliminary evidence that blood inflammation can be monitored to predict short-term PEx risk in CF children and adolescents.

1. Introduction

Pulmonary exacerbations (PEx) are clinically impactful events for individuals living with CF [1]. Many individuals who present with a PEx do not recover baseline lung function even after the event has been diagnosed and treated [2]. Consequently, PEx can account for about half of the lung function lost over an individual's lifetime [3]. A recent study has suggested that lung function is lost prior to a PEx as opposed to after the event and therefore preventing these events is a priority [4].

Prior history of PEx is an important predictor of subsequent events but this historical information is less useful during the routine monitoring of patients [5]. Multiple studies have demonstrated that systemic inflammation is increased at the time of clinical presentation with a PEx and resolves with treatment but it remains unknown if systemic inflammation can be monitored to predict PEx [6–8]. We previously discovered and validated a 6-protein blood biomarker panel capable of predicting PEx within 4 months in adults with CF but the prognostic performance in CF children and adolescents was not examined [9]. In this pilot study, we utilized blood samples from the placebo arm of a randomized controlled clinical trial evaluating azithromycin in CF subjects between 6 and 18 years to determine if our 6-protein blood biomarker panel was also capable of predicting short-term PEx risk in children and adolescents [10].

2. Methods

Plasma samples and clinical data from 67 subjects randomized to the placebo arm of the AZ0004 study were obtained from the Cystic Fibrosis Foundation Therapeutics (CFFT) Biorepository as previously described (UBC-PHC REB H16-00208) [11].

In brief, the AZ0004 study included CF individuals with the following parameters: aged 6–18 years, percent-predicted FEV_1 (ppFEV1) ≥ 50%, and negative respiratory cultures for *P. aeruginosa* in the preceding year. Subjects were excluded if they used

Abbreviations: A. xylosoxidans, Achromobacter xylosoxidans; ANC, Absolute neutrophil count; APOC2, Apolipoprotein C-II (APOC2); AUC, Area under the ROC curve; *B. cepacia complex*, *Burkholderia cepacia complex*; CAH1, Carbonic anhydrase 1; CFFT, CF Foundation Therapeutics; CD5, Cluster of differentiation 5; CV, Cross validation; G-CSF, Granulocyte colony stimulating factor; HBA, Haemoglobin subunit alpha; *H. influenza*, *Haemophilus influenza*; hsCRP, High-sensitivity C-reactive protein; IV, Intravenous; MPO, Myeloperoxidase; MRM-MS, Multiple reaction monitoring mass spectrometry; MRSA, Methicillin-resistant *Staphylococcus aureus*; MSSA, Methicillin-susceptible *Staphylococcus aureus*; PRDX2, Peroxiredoxin-2; $ppFEV_1$, Percent-predicted forced expiratory volume in 1 s; *P. aeruginosa*, *Pseudomonas aeruginosa*; PEx, Pulmonary exacerbations; ROC, Receiver operating characteristics; SAA, Serum amyloid A; SD, Standard deviation; *S. maltophilia*, *Stenotrophomonas maltophilia*.

* Corresponding author at: Centre for Heart Lung Innovation, University of British Columbia, #166 - 1081 Burrard Street, Vancouver, British Columbia V6Z 1Y6, Canada.
E-mail address: bradley.quon@hli.ubc.ca (B.S. Quon).

https://doi.org/10.1016/j.jcf.2019.05.020
1569-1993/

antibiotics or high-dose systemic corticosteroids within 14 days of screening. Clinical evaluations were performed at days 0, 28, 84, and 168 [10].

Plasma samples were sent to the University of Victoria Genome BC Proteomics Centre (Victoria, British Columbia, Canada) for multiple reaction monitoring (MRM) mass spectrometry proteomic analysis as previously described [9]. Our previously identified 6-protein blood biosignature consisting of C-reactive protein (CRP), peroxiredoxin-2 (PRDX2), haemoglobin subunit alpha (HBA), carbonic anhydrase 1 (CAH1), cluster of differentiation 5 (CD5) and apolipoprotein C-II (APOC2) was evaluated to predict PEx requiring IV antibiotics within 84 days (3 months) of randomization. While this time frame is shorter than the 4 months evaluated in our prior study, it corresponded to a regularly scheduled visit during the AZ0004 clinical trial.

We compared the predictive performance of the 6-protein biosignature to $ppFEV_1$ and candidate blood biomarkers, including absolute neutrophil count (ANC), myeloperoxidase (MPO), high-sensitivity C-reactive protein (hsCRP), calprotectin, granulocyte colony stimulating factor (G-CSF), and serum amyloid A (SAA), as measured and reported in a prior study that also utilized samples from the AZ0004 study [11].

2.1. Statistical analysis

Given the limited sample size, penalized linear regression was used to compare the following biomarker panels: 1) a clinical panel consisting of currently available clinical biomarkers including $ppFEV_1$, ANC and hsCRP; 2) ELISA panel consisting of hsCRP, MPO, G-CSF, SAA, calprotectin and ANC; and 3) MRM panel consisting of CRP, PRDX2, HBA, CAH1, CD5L, APOC2, and 4) combined panels (1 + 2) or (1 + 3) to predict the need for IV antibiotics within 84 days of baseline blood collection. Class weights were used to account for imbalanced class sizes, whereas the use of regularization (penalized) was to estimate stable coefficients in the case of correlated predictors. Biomarker data (ELISA and MRM) was log2-transformed prior to analysis.

Classification performance for each panel was determined using 5-fold cross-validation (CV) repeated 5 times as previously described [12]. We used the AUC, sensitivity, and specificity to evaluate the performance of the biomarker panels. AUCs were compared with the DeLong test [13]. Biomarker cut-offs were chosen to maximize test sensitivity and specificity (i.e. Youden's index) [14].

3. Results

The cohort consisted of 67 subjects from the placebo group whose overall clinical characteristics were similar to the placebo group reported in the AZ0004 trial [10]. Five of 67 (8%) subjects were diagnosed with a PEx requiring IV antibiotics within 84 days. The clinical characteristics including $ppFEV_1$, BMI, and respiratory culture data were similar for subjects who did vs. did not require IV antibiotics within 84 days (Table 1).

Based on AUC, the MRM biomarker panel out-performed both the clinical and ELISA biomarker panels (AUC = 0.77 vs. AUCs = 0.58 and 0.60, respectively, DeLong's p-value <0.05, Table 2). Based on Youden's index, the sensitivity was 0.80 and specificity was 0.73. The AUC of the clinical panel improved after adding the ELISA or MRM panels, however, neither combination improved upon the MRM panel alone.

4. Discussion

In this pilot study, we have evaluated a 6-protein biomarker panel measured with MRM mass spectrometry and initially discovered in CF adults to predict short-term PEx using a well-characterized pediatric CF cohort from the AZ0004 study. The biomarker panel outperforms currently available clinical factors ($ppFEV_1$) and biomarkers (ANC, hsCRP) and candidate biomarkers measured with ELISA. This additional pilot study in subjects aged 6–18 years provides strong proof-of-concept that blood inflammation can be monitored during stable visits to identify at-risk individuals in need of intensified treatment strategies to prevent PEx.

This study has a few important limitations that should be considered. While the biomarker panel demonstrated good performance, it was not excellent as some PEx events are inherently stochastic, such as that which occurs in a patient who may have been clinically and biologically stable at the time of blood collection, but then acquires a respiratory virus (e.g. influenza) that triggers an acute PEx. Furthermore, the sample size was small due to a limited number of samples remaining in the CFF

Table 1
Baseline characteristics for children and adolescent subjects included in this pilot study from the AZ0004 trial.

Characteristic	Overall (n = 67)	PEx requiring IV antibiotics < 84 days (n = 5)	No requirement for IV antibiotics < 84 days (n = 62)	P-value[a]
Age, mean (SD), y	10.3 (3.10)	10.8 (4.6)	10.1 (2.9)	0.76
Aged 6–12y	14 (21%)	2 (40%)	12 (19%)	0.23
Aged 13–18y	53 (79%)	3 (60%)	50 (81%)	
Female	32 (48%)	3 (60%)	29 (47%)	0.58
$ppFEV_1$, mean (SD)	101.2 (13.29)	101.7 (7.7)	101.1 (13.3)	0.89
BMI, kg/m^2 mean (SD)	18.2 (2.95)	18.8 (3.6)	18.1 (2.9)	0.69
Respiratory culture (+)				
MSSA	50 (75%)	5 (100%)	45 (73%)	0.32
H. influenza	16 (24%)	2 (40%)	14 (23%)	0.61
MRSA	7 (10%)	1 (20%)	6 (10%)	0.44
S. maltophilia	5 (7%)	0 (0%)	5 (8%)	1.00
A. xylosoxidans	4 (6%)	0 (0%)	4 (6%)	1.00
P. aeruginosa	0 (0%)	0 (0%)	0 (0%	1.00
B. cepacia complex	0 (0%)	0 (0%)	0 (0%)	1.00
Chronic medications				
Dornase alpha	50 (75%)	4 (80%)	46 (74%)	1.00
Inhaled tobramycin	8 (12%)	0 (0%)	8 (13%)	1.00
Ibuprofen	5 (7%)	1 (20%)	4 (6%)	0.33
Hypertonic saline	20 (30%)	2 (40%)	18 (29%)	0.63

Abbreviations: A. xylosoxidans = Achromobacter xylosoxidans; B. cepacia complex = *Burkholderia cepacia* complex; H. influenza = Haemophilus influenza; MRSA = methicillin-resistant *Staphylococcus aureus*; MSSA = methicillin-susceptible *Staphylococcus aureus*; $ppFEV_1$ = percent-predicted FEV_1; PEx = pulmonary exacerbation; P. aeruginosa = *Pseudomonas aeruginosa*; SD = standard deviation; S. maltophilia = Stenotrophomonas maltophilia.

[a] Baseline characteristics were normally distributed and statistical significance was assessed using the Student's t-test for continuous variables and Fisher's Exact test for categorical variables.

Table 2
Performance characteristics of the clinical, ELISA, and MRM panels to predict pulmonary exacerbations requiring IV antibiotics within 84 days.

Biomarker panel	AUC (95% CI)	Sensitivity[a]	Specificity[a]
Clinical[b]	0.58 (0.31, 0.85)	60%	71%
ELISA[c]	0.60 (0.34, 0.85)	40%	89%
MRM[d]	0.77 (0.57, 0.96)	80%	73%
Clinical + ELISA	0.64 (0.44, 0.84)	100%	45%
Clinical + MRM	0.72 (0.49, 0.94)	100%	48%

Abbreviations: ANC = absolute neutrophil count; APOC2 = apolipoprotein C2; AUC = area under the receiver operating characteristic curve; CAH1 = carbonic anhydrase 1; CALP = calprotectin; CD5L = CD5 ligand; CRP=C-reactive protein; ELISA = enzyme-linked immunosorbent assay; GCSF = granulocyte colongy stimulating factor; HBA = haemoglobin subunit alpha; hsCRP = high-sensitivity CRP; IV = intravenous; MPO = myeloperoxidase; MRM = multiple reaction monitoring mass spectrometry; $ppFEV_1$ = percent-predicted FEV_1; PRDX2 = peroxiredoxin 2; SAA = serum amyloid A.

[a] Sensitivity and specificity based on an optimal cut-off defined by Youden's index.

[b] Clinical panel = $ppFEV_1$, ANC and hsCRP.

[c] ELISA panel = hsCRP, MPO, G-CSF, SAA, calprotectin and ANC.

[d] MRM panel = CRP, PRDX2, HBA, CAH1, CD5L, APOC2.

biorepository from the placebo group of the AZ0004 trial (67 of 129 participants). While this represents a convenience sample, the study characteristics of the participants with sample remaining are similar to the overall AZ0004 placebo group [10]. The PEx event rate was low in terms of the number of PEx events requiring IV antibiotics within 84 days in this pediatric cohort. A priori, we chose to evaluate PEx requiring IV as opposed to oral antibiotics to improve the specificity of PEx diagnosis as the proportion of participants with a PEx requiring oral antibiotics within the first 84 days of the clinical trial was high (32 of 67 participants) given the close monitoring that occurs during a clinical trial. In a post-hoc analysis evaluating PEx requiring oral or IV antibiotics within 84 days, the performance of the biomarker signature was weaker (AUC 0.62; 95% CI 0.44 to 0.80) potentially related to a lower threshold to initiate oral (vs. IV) antibiotics as part of the clinical trial. As a result, we cannot generalize our findings to PEx requiring oral antibiotics. Lastly, history of PEx requiring IV antibiotics in the prior year is an important predictor of future PEx events but unfortunately this data was not collected as part of the AZ0004 clinical trial to perform a comparison on the predictive performance of historical PEx data relative to our blood biomarker panel but this should be considered in future studies [5].

In conclusion, this pilot study provides further proof-of-concept that monitoring systemic inflammation in CF patients might provide an opportunity to identify high risk individuals at imminent risk of PEx. However, further studies are required to determine if the MRM biomarker panel performance can be replicated in a larger pediatric cohort prior to the evaluation of a biomarker-guided strategy to guide intervention to prevent PEx in a randomized controlled clinical trial.

Funding

Cystic Fibrosis Foundation Therapeutics (CFFT) provided clinical samples and grant support for this study (QUON15U0). B.S.Q. is supported by a Michael Smith Foundation for Health Research Scholar Award.

Author contributions

AS, BQ, ST, RN, MH, VC, ZH contributed to the design of the study, interpretation of the analysis, drafting of the manuscript, and final approval. FR, DS, BM contributed to the interpretation of the analysis, drafting of the paper, and final approval.

Summary of conflict of interest statement

None of the study authors report personal or financial relationships with other people or organizations that could have inappropriately influenced this study's results or conclusions.

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Acknowledgements

We would like to thank the U.S. CF Foundation Therapeutics for providing clinical samples and grant support for this study (QUON15U0). We would also like to acknowledge Dr. Lisa Saiman, Dr. Nicole Mayer-Hamblett, Dr. Sonya Heltshe, and members from the CFF Therapeutics Development Network and CFF Biorepository for assisting with study planning and execution.

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