SPLUNC1 is a novel marker of disease severity and airway infection in bronchiectasis

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To the Editor:

Biomarkers of disease severity are urgently needed to guide precision medicine in airway diseases [1, 2].

Short palate lung and nasal epithelial clone 1 (SPLUNC1), encoded by the BPIFA1 gene, is a 25-kDa innate immune protein expressed by non-ciliated epithelial and mucus cells that is secreted into the airway surface liquid [3, 4]. It has been shown to have multiple potentially beneficial effects in the context of chronic lung inflammation, including inhibition of the epithelial Na^+ channel (ENaC) and antimicrobial activity against Gram-negative pathogens [3, 4].

In this issue of the *European Respiratory Journal*, Khanal *et al.* [4] have demonstrated in two cohorts (n=44 and n=35) of patients with cystic fibrosis that low SPLUNC1 levels are a biomarker of exacerbation. Degradation of SPLUNC1 by neutrophil elastase along with suppression of epithelial SPLUNC1 expression by interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) result in reduced SPLUNC1 levels in sputum at exacerbation [4].

While there are important differences between cystic fibrosis and bronchiectasis [5], elevated neutrophil elastase, IL-1β and TNF-α are also reported to be increased with severity of "non-CF" bronchiectasis and at exacerbation [6–8]. It is therefore likely that if SPLUNC1 is a marker of severity and is related to airway inflammation, its value as a biomarker is not limited to cystic fibrosis.

We therefore examined levels of SPLUNC1 in patients with bronchiectasis not due to cystic fibrosis using two previously reported datasets [9]. In cohort 1, we tested the hypothesis that SPLUNC1 levels would be lower in patients with more severe bronchiectasis. 40 patients with idiopathic or post-infective bronchiectasis, (20 classified as severe and 20 as mild using the bronchiectasis severity index) were enrolled and provided spontaneous sputum samples for proteomic analysis. All patients were clinically stable, defined as at least 4 weeks free from antibiotic treatment of exacerbation, and current smokers were excluded. Further details of the cohort are reported previously [9].

In cohort 2, 20 patients with bronchiectasis were studied when clinically stable, at the onset of an acute exacerbation occurring within 6 months of the initial sputum samples, followed by review after receiving 14 days of intravenous antibiotic treatment. Patients provided spontaneous sputum samples at each visit.

Protein profiles in sputum were generated using a label-free shotgun proteomic workflow as previously described [9]. Data for SPLUNC1/BPIFA1 was log transformed, mean centred and unit variance scaled. To test differences between severe and mild disease, and between patients during stability, exacerbation and recovery we used the t-test. $p<0.05$ was considered statistically significant for all analyses.

In cohort 1, the mean±SD age was 61.1±11.4 years and 21/40 patients were female. In cohort 2, the mean±SD age was 65±11 years and 12/20 were female. 11 patients had *Pseudomonas aeruginosa* infection at exacerbation.

In the analysis of cohort 1, SPLUNC1 levels were significantly lower in patients with severe disease (n=20) compared to those with mild disease (n=20; p=0.007) (figure 1a). Individual markers of disease severity were associated with lower SPLUNC1 levels, including hospitalisations (p=0.007), Reiff score

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Having been shown to be a biomarker of severity in cystic fibrosis, this study shows SPLUNC1 is also a novel marker of severity and lung infections in non-CF bronchiectasis https://bit.ly/3ABzEdc

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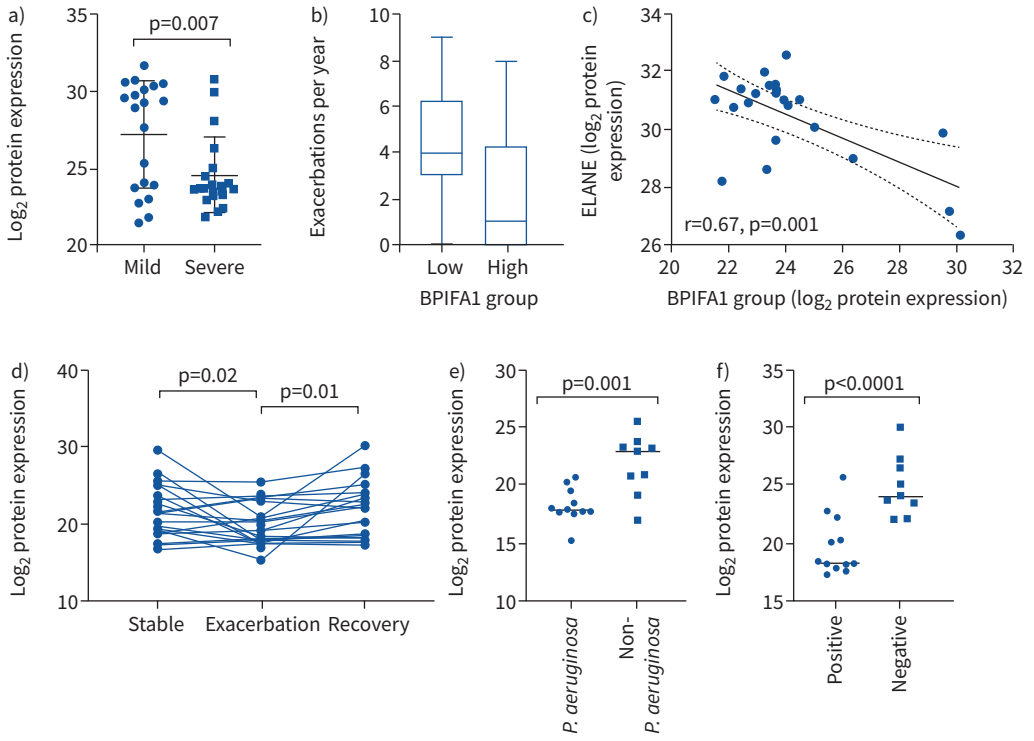

FIGURE 1 Relationship between SPLUNC1 (BPIFA1) levels measured by liquid chromatography–mass spectrometry and clinical characteristics and outcomes in bronchiectasis. a) Relationship with severity of disease classified by the bronchiectasis severity index. Comparisons by t-test. b) Relationship of SPLUNC1 (BPIFA1) group (high >25 units, low <25 units) and exacerbations over 12 months. Box plots show median with interquartile range, comparison by Mann–Whitney U-test. c) Linear regression of neutrophil elastase (ELANE) and SPLUNC1 (BPIFA1) levels in sputum. d) Individual changes in SPLUNC1 (BPIFA1) levels in stable state, exacerbation and recovery (after completion of 2 weeks of intravenous antibiotic treatment). Comparisons by paired t-test. e) SPLUNC1 (BPIFA1) levels at the onset of exacerbation in patients positive for *Pseudomonas aeruginosa* by culture (n=11) or negative for *P. aeruginosa* by culture (n=9). Comparisons by t-test. f) SPLUNC1 (BPIFA1) levels after completion of 14 days of intravenous antibiotic treatment by positive (n=11) or negative (n=9) sputum cultures for any pathogenic bacteria. Comparisons by t-test.

(r=−0.34, p=0.03) and SPLUNC1 levels were lower in patients with positive sputum cultures (n=26) *versus* those without (n=14) (mean difference 4.14, 95% CI 2.37–5.91: p<0.0001) and correlated with bacterial load (r=−0.35, p=0.028). Visual inspection of figure 1a illustrates a predominantly mild group with BPIFA1 levels greater than 25 units and a predominantly severe group lower than 25 units. Comparing subsequent exacerbations rates over 1 year in these two groups confirmed significantly different rates of exacerbation (p=0.006) (figure 1b). There was an inverse correlation between BPIFA1 and neutrophil elastase (ELANE) (figure 1c) consistent with the observations of KHANAL *et al.* [4]. From this cohort we conclude that SPLUNC1 levels are reduced in severe bronchiectasis and airway infection.

In cohort 2, at baseline SPLUNC1 levels were confirmed as a biomarker of severity of disease. Sputum SPLUNC1 was correlated with % of predicted forced expiratory volume in 1 s (r=0.65, p=0.002), and inversely related to MRC dyspnoea score (r=−0.53, p=0.015), exacerbation frequency (r=−0.67, p=0.001) and the bronchiectasis severity index (r=−0.71, p=0.0005). Patients with baseline *P. aeruginosa* infection had lower levels of SPLUNC1 (mean±SD 20.4±3.0 *versus* 23.5±3.0; p=0.03).

Changes in SPLUNC1 levels at exacerbation were investigated. SPLUNC1 levels were significantly lower at the onset of exacerbation compared to stability (p=0.02) (figure 1d). Changes in SPLUNC1 levels were heterogeneous but no clinical characteristics at baseline were associated with the level of change in SPLUNC1. SPLUNC1 levels returned to baseline levels after 2 weeks' antibiotic treatment (p=0.01) (figure 1d). Mean levels of SPLUNC1 after antibiotics were similar to those measured at stability (p=0.9). Exacerbations that were positive for *P. aeruginosa* infection on culture had lower levels of SPLUNC1

(p=0.001) (figure 1e). Among those individuals with positive sputum cultures of any pathogenic micro-organism after the completion of antibiotic treatment levels of SPLUNC1 were persistently lower (p<0.0001) (figure 1f).

Limitations of our study are acknowledged, including the relatively small sample size of both cohorts. Nevertheless, our data suggest that SPLUNC1 is a marker of severity of disease in bronchiectasis and is associated with increased neutrophilic inflammation and airway infection. Low levels of SPLUNC1 are associated with increased exacerbations and antibiotic treatment increases levels of SPLUNC1 in sputum. There is a need to identify new biomarkers of disease severity in bronchiectasis, as well as to better understand the biology of frequently exacerbating and severe bronchiectasis endophenotypes [10, 11]. This data adds to our understanding of lung inflammation in bronchiectasis, which is an emerging target for treatment [12].

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