ORIGINAL ARTICLE

Changes in physiological, functional and structural markers of cystic fibrosis lung disease with treatment of a pulmonary exacerbation

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ABSTRACT

Background Clinical trials in cystic fibrosis (CF) have been hindered by the paucity of well characterised and clinically relevant outcome measures.

Aim To evaluate a range of conventional and novel biomarkers of CF lung disease in a multicentre setting as a contributing study in selecting outcome assays for a clinical trial of *CFTR* gene therapy.

Methods A multicentre observational study of adult and paediatric patients with CF (>10 years) treated for a physician-defined exacerbation of CF pulmonary symptoms. Measurements were performed at commencement and immediately after a course of intravenous antibiotics. Disease activity was assessed using 46 assays across five key domains: symptoms, lung physiology, structural changes on CT, pulmonary and systemic inflammatory markers.

Results Statistically significant improvements were seen in forced expiratory volume in 1 s ($p<0.001$, n=32), lung clearance index ($p<0.01$, n=32), symptoms ($p<0.0001$, n=37), CT scores for airway wall thickness ($p<0.01$, n=31), air trapping ($p<0.01$, n=30) and large mucus plugs ($p=0.0001$, n=31), serum C-reactive protein ($p<0.0001$, n=34), serum interleukin-6 ($p<0.0001$, n=33) and serum calprotectin ($p<0.0001$, n=31).

Discussion We identify the key biomarkers of inflammation, imaging and physiology that alter alongside symptomatic improvement following treatment of an acute CF exacerbation. These data, in parallel with our study of biomarkers in patients with stable CF, provide important guidance in choosing optimal biomarkers for novel therapies. Further, they highlight that such acute therapy predominantly improves large airway parameters and systemic inflammation, but has less effect on airway inflammation.

Key messages

What is the key question?

- What are the optimal biomarkers to track clinical improvement in patients with cystic fibrosis (CF) following treatment of an acute exacerbation?

What is the bottom line?

- In this three-centre observational study we report on a range of novel and conventional measures of CF disease activity across all the key domains (symptoms, lung physiology, lung structure and pulmonary and systemic inflammation) in response to a standard intervention (intravenous antibiotic course). We found major improvements in large airway parameters (spirometry, CT measures of mucus load) and systemic inflammation, with more subtle improvements in lung clearance index. Response in pulmonary markers of inflammation was more variable and showed less consistent correlation with other measures.

Why read on?

- This study represents an important step in biomarker assessment, presents data on a wide range of novel and conventional measurements, and offers potential insights into the underlying pathophysiology of response to treatment in CF.

INTRODUCTION

The issue of how best to measure response to therapies in cystic fibrosis (CF) is not a new one.[1 2] Clinical trial outcome measures should optimally fulfil a number of requirements: a clear difference between patients with CF and healthy controls; relevance to the underlying pathology; capable of being undertaken at multiple sites; an intra-subject and inter-subject variability which would allow a clinical trial to be performed in a pragmatically achievable number of patients with CF; and showing changes with conventional treatment (ie, a

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positive control).[2] Currently, the only primary pulmonary endpoint recommended by the European Medicines Agency for CF clinical trials is the forced expiratory volume in 1 s (FEV_1),[3] yet the limitations of this measurement as a trial outcome have been recognised by CF researchers for many years.[1]

The UK CF Gene Therapy Consortium (http://www.cfgenetherapy.org.uk) conducted this study to aid identification of optimal trial outcome measures. We assessed a panel of conventional and novel assays in response to treatment for a pulmonary exacerbation with intravenous antibiotics. Most CF exacerbation studies have included relatively small numbers of subjects (n=7–32) and a restricted number of biomarkers.[4–14] We considered these findings too limited to inform our understanding of the potential effects of pulmonary gene therapy on the CF airway. This study provides a comprehensive and coordinated assessment of all five key domains of CF lung disease: symptoms, physiology, structure, and pulmonary and systemic inflammation.

Our aims were to assess the response to treatment of an exacerbation in a broad range of outcomes to establish those that changed appropriately and might be used in future clinical trials. In addition, we hoped to explore relationships between different domains of CF lung disease to broaden our understanding of the pathophysiology and effects of pulmonary exacerbations.

METHODS

This study was performed at three university hospital sites: Royal Brompton and Harefield NHS Foundation Trust, London; Western General Hospital, Edinburgh; and Royal Hospital for Sick Children, Edinburgh. This was a longitudinal analysis of patients with CF, aged 10 years and over, treated for a pulmonary exacerbation with intravenous antibiotics. The decisions on when to commence treatment, the choice of antibiotics and any additional therapies, and the duration of treatment were made by the clinical CF team, independent of the research group. Patients were excluded if FEV_1 was less than 30% predicted, or if they received systemic corticosteroids during the study or preceding month (to avoid confounding influences on inflammatory markers). Full inclusion and exclusion criteria are provided in the online supplement.

Participants were requested to complete a series of assessments (table 1) in a structured order at two time points: visit 1 (V1), within 72 h of commencing intravenous antibiotics for a pulmonary exacerbation, and visit 2 (V2), within 5 days of completion of therapy.

The study was approved by the Lothian Research and Ethics Committee, and the Royal Brompton, Harefield and NHLI Research Ethics Committee. All subjects signed informed consent and paediatric subjects gave their assent for inclusion.

Clinical assays

Full details of all the assays and techniques are given in the online supplement.

Symptoms

Symptoms were assessed on a five-point scale developed for this study and designed to reflect intra-subject acute change in major respiratory symptoms. Patients scored each of seven symptom-related questions from –2 (much worse than normal) to +2 (much better): the final summed score thus ranged from –14 to +14.

Table 1 Summary of assays performed at start and end of exacerbation in order of sequence performed

Domain	Assay
Symptoms and clinical observations	▶ Symptom score ▶ Pulse ▶ Respiratory rate ▶ SpO_2 ▶ Temperature ▶ Blood pressure ▶ Weight
Lung physiology	▶ Lung clearance index ▶ Spirometry
Pulmonary markers of inflammation	▶ Exhaled breath condensate pH, ammonia, nitrite ▶ Sputum 24 h weight, solid content, DNA content and rheology ▶ Total and differential sputum cell count ▶ Sputum calprotectin, IL-1β, IL-6, IL-8, IL-12, IFN-γ, RANTES, TNF-α, MMP-9, MPO, neutrophil elastase, TIMP-1 ▶ Microbiological culture
Systemic markers of inflammation	▶ Blood white cell count ▶ Serum IL-1β, IL-6, IL-8, IL-10, TNF-α, Calprotectin, CRP
CT assessment of lung structure*	▶ Extent of bronchiectasis ▶ Severity of bronchiectasis ▶ Airway wall thickness ▶ Small mucus plugs ▶ Large mucus plugs ▶ Air trapping ▶ Consolidated lung ▶ Ground glass lung

*The order in which the CT was performed was not fixed, some patients having this prior to the other assessments.
CRP, C-reactive protein; IL, interleukin; IFN-γ, interferon γ; MMP9, matrix metalloprotease 9; MPO, myeloperoxidase; RANTES, regulated upon activation, normal T-cell expressed and secreted; SpO_2, oxygen saturations; TNF-α, tumour necrosis factor α.

Lung physiology

Spirometry

FEV_1 and mid-expiratory flows were expressed as SD scores, or z scores, using the modified National Health and Nutrition Examination Survey III reference ranges.[15] For comparison, FEV_1 was also expressed as percent predicted using separate reference ranges for adults (≥17 years)[16] and children (≤16 years).[17]

In nine cases V2 spirometry was not recorded using the EasyOne spirometer. For these patients, we substituted both FEV_1 values with those obtained from a portable spirometer previously provided to the patient (Piko-6, Ferraris Respiratory, Hertford, UK). This substitution was only performed if spirometry had been recorded on the portable device at both study visits and furthermore these readings had been shown to be reliable (ie, absence of outliers defined by >2 SD from within-patient means on repeated measures analysis of variance (ANOVA); see online supplement). If portable spirometer data could not be used to substitute for incomplete spirometry, FEV_1 for that patient was treated as missing.

Lung clearance index

Multiple breath washout was performed as previously described[18] using a modified Innocor (Innovision, Odense, Denmark) gas analyser and 0.2% sulfur hexafluoride (SF_6) as the tracer gas.

Pulmonary markers of inflammation

Sputum was expectorated spontaneously or induced as previously described.[19] Sputum plugs were harvested and processed

in dithiothreitol before storage at −80°C. Details of individual assays are given in the online supplement.

Systemic markers of inflammation

Venous blood was analysed locally for full blood count and C-reactive protein (CRP). Serum was separated from whole venous blood by centrifugation and stored at −80°C. Details of individual assays are given in the online supplement.

CT assessment of lung structure

Contiguous thin-section chest CT images were acquired at inspiration without contrast. Anonymised images were scored by two independent radiologists blinded to clinical details, based upon a previously described grading methodology (see online supplement for details).[20]

Statistical analysis

Data were analysed using Prism and SPSS version 19. Normal distribution was assessed using the D'Agostino and Pearson omnibus normality test. Results are quoted as mean (SD) or median (IQR) values unless otherwise stated. No attempt was made to substitute missing data.

Skewed data were log transformed prior to analysis. A paired t test was used for comparison of change in variables between paired visits and comparisons between multiple groups were performed using a one-way ANOVA and Tukey's honestly significant difference test. Biomarkers reported as below the lower limit of the assay have all been ascribed a value equal to the lower limit of detection (see online supplementary table E1).

Correlations between different assays were performed on assessments performed at V1, and included all those with valid assessments at that visit even if subsequent assessments were missing or excluded because of protocol violation. Correlations were assessed using the Pearson correlation coefficient (normally distributed data) or Spearman rank correlation (skewed data). Change in assays was calculated as the V2 value minus the V1 value. A p value of below 0.05 was considered statistically significant.

Multiple correlations are presented in the online supplement (see tables E5–E11). These are intended to assist generation of hypotheses about the pathophysiology of CF and response to therapy and are therefore presented in full, with no correction for multiple comparisons.

RESULTS

Patient demographics and clinical characteristics

Forty-six patients consented to participate in the study. Two patients were subsequently excluded for concomitant use of oral corticosteroids; cross-sectional data correlations from V1 were therefore performed on 44 patients. Longitudinal data are presented on 38 patients: six V2 assessments were excluded because of excessive time delay (n=2) or non-attendance (n=3) at V2, or because of commencing oral corticosteroids between assessments (n=1) (see online supplementary figure E2).

Demographic data are summarised in table 2. Twenty-six patients (59%) were chronically colonised with *Pseudomonas aeruginosa* (see online supplement for further details). Details on treatments are given in the online supplement. Thirty-six (95%) V1 assessments were performed within 24 h of starting intravenous antibiotics and 31 (82%) V2 assessments within 48 h of completion of intravenous antibiotics.

Table 2 Demographics and symptoms at start of treatment

Number of subjects	44
Sex (m/f)	24/20
Median age (IQ range) (years)	23 (18–28)
Characteristics of exacerbation, n (%)	
Increased cough	43 (98)
Increased dyspnoea	41 (93)
Change in sputum	39 (89)
Malaise	37 (84)
Fall in FEV_1 >10%*	24 (55)
Mean (SD) FEV_1 at start of treatment, z score (% predicted)	−4.29 (1.03) 52.1 (12.2)

*Represents a fall in forced expiratory volume in 1 s (FEV_1) (litres) of over 10% compared with recent baseline (within 6 months).

Change with treatment of exacerbation

A summary of the changes in individual assays is given in table 3.

Symptoms and clinical observations

Following treatment, total symptom score improved by an average of 9.5 points (figure 1). Mean symptom score at V2 (2.8) was significantly higher than zero ($p<0.01$).

Consistent with previous observations on haemodynamic response to treatment of an exacerbation, there were small but statistically significant decreases in mean HR, relative risk and diastolic blood pressure with treatment.[21]

Lung physiology

There were significant improvements in FEV_1 and forced vital capacity (figure 2A). FEV_1 percent predicted increased by a mean of 9.6 absolute percent predicted points to 64.6 (16.8) percent predicted at end of treatment, corresponding to a relative improvement of 20.6% ($p<0.001$). FEV_1 became normal (z score >−2) with treatment in six subjects (19%).

There was significant improvement in lung clearance index (LCI) with treatment of 0.8 units (figure 2B), but no significant change in functional residual capacity (FRC). LCI fell (ie, improved) in 22 (69%) subjects. The lowest LCI at V2 was 9.4, significantly greater than the upper limit of normal LCI described in healthy controls of 7.5.[18]

Pulmonary markers of inflammation

Sputum was expectorated spontaneously in 100% of patients at V1 and 85% of patients at V2. There was a significant reduction in median 24 h sputum weight, though no significant change in the proportion of solids (percent dry weight). Total sputum cell count also fell, but there was no significant change in sputum differential cell counts expressed as percentage of total. There were significant changes in the level of sputum inflammatory markers matrix metalloprotease 9, interleukin (IL)-1β and tissue inhibitor of metalloproteinases 1 (see figure 3), but no significant change was seen in the other sputum markers (neutrophil elastase (NE), myeloperoxidase, regulated upon activation, normal T-cell expressed and secreted, tumour necrosis factor (TNF)-α, IL-8 and IL-12). In contrast to serum, there was no significant change in sputum calprotectin. IL-6 and interferon γ were generally undetectable in sputum at both time points. No significant change was observed in DNA content, sputum viscosity or elasticity.

Table 3 Summary of changes after antibiotic treatment

Disease domain	Assay	No. with paired values	Visit 1 mean (SD)	Visit 2 mean (SD)	Mean (SD) change after treatment	p Value
Clinical observations and symptoms	Weight (kg)	33	57.4 (11.9)	58.1 (11.2)	0.7 (1.8)	0.040*
	Heart rate (min^{-1})	38	90.5 (14.3)	82.7 (15.9)	−7.8 (17.3)	0.008**
	Respiratory rate (min^{-1})	35	20.9 (3.5)	18.5 (4.2)	−2.4 (4.0)	0.001**
	O_2 saturation (%)	38	95.6 (1.9)	96.0 (1.4)	0.3 (1.9)	0.272
	Systolic BP (mm Hg)	38	113.3 (12.6)	110.6 (14.4)	−2.7 (13.6)	0.231
	Diastolic BP (mm Hg)	38	71.8 (8.7)	67.0 (9.3)	−4.8 (7.8)	0.0005***
	Total symptom score	37	−6.7 (3.0)	2.8 (5.6)	9.5 (6.4)	<0.0001***
Function	FEV_1 (litres)	32	1.93 (0.66)	2.25 (0.76)	0.32 (0.48)	0.0006***
	FEV_1 SDS	32	−4.03 (1.10)	−3.23 (1.42)	0.80 (1.23)	0.0009***
	FEV_1 (% predicted)	32	55.0 (13.1)	64.6 (16.8)	9.6 (14.6)	0.0008***
	FVC SDS	23	−2.79 (1.27)	−1.86 (1.47)	0.93 (1.36)	0.003**
	$FEF_{25–75}$ SDS	15	−3.70 (0.85)	−3.30 (1.29)	0.40 (0.97)	0.130
	LCI	32	14.6 (2.7)	13.8 (2.4)	−0.8 (1.4)	0.003**
	FRC (litres)	32	2.32 (0.58)	2.33 (0.60)	0.01 (0.24)	0.795
Structure (expressed as % of maximum possible score)	Extent of bronchiectasis	30	83.2 (16.2)	80.0 (14.3)	−3.2 (10.6)	0.1
	Severity of bronchiectasis	31	64.9 (15.2)	65.3 (14.3)	0.3 (6.8)	0.8
	Airway wall thickness	31	54.0 (11.3)	49.5 (10.8)	−4.5 (8.7)	0.008**
	Air trapping	31	48.5 (16.1)	40.8 (13.4)	−7.7 (13.6)	0.004**
	Small mucus plugs	31	78.5 (16.8)	69.6 (20.6)	−8.9 (19.7)	0.018
	Large mucus plugs	31	72.0 (22.0)	59.0 (23.5)	−13.0 (16.4)	0.0001***
	Lung consolidation	31	1.9 (2.4)	1.0 (1.7)	−0.9 (2.2)	0.005
	Ground glass lung	31	0.9 (1.4)	0.5 (0.8)	−0.4 (1.7)	0.2
Serum inflammatory markers	WCC (10^6 ml)	32	10.2 (2.6)	8.7 (3.2)	−1.5 (3.5)	0.022
	CRP (mg/ml)†	34	16 (9–39)	2 (1–12)	−13.5	<0.0001***
	Calprotectin (μg/ml)†	31	27.5 (19.4–50.7)	13.9 (6.3–21.0)	−13.8	<0.0001***
	IL-6 (pg/ml)†	33	64.0 (53.6–78.0)	51.2 (48.5–54.8)	−11.7	0.0001***
	IL-8 (pg/ml)†	30	3.9 (2.5–5.1)	3.3 (2.5–4.7)	−0.3	0.709
	TNF-α (pg/ml)	33	175.8 (30.9)	178.2 (34.2)	2.3 (13.7)	0.340
Airway markers	Total cell count (×10^6)†	23	5.3 (2.7–10.8)	2.1 (0.8–10.5)	−1.6	0.005**
	Calprotectin (mg/ml)†	33	1.0 (0.45–1.50)	0.6 (0.20–1.35)	−0.1	0.066
	IL-1β (pg/ml)†	32	1032 (415–1972)	410 (51–1066)	−299	0.012*
	IL-8 (ng/ml)	31	13.8 (9.2)	15.4 (13.0)	1.6 (11.2)	0.441
	IL-12 (pg/ml)	32	223 (119)	190 (97)	−32 (93)	0.060
	RANTES (pg/ml)†	32	6.90 (3.50–11.75)	7.50 (5.75–11.55)	0.49	0.246
	NE (U/litre)	32	595 (384)	698 (574)	103 (584)	0.435
	MPO (μg/ml)†	31	18.4 (7.6–27.8)	30.8 (15.1–45.7)	7.6	0.257
	MMP9 (ng/ml)†	32	471 (157–1243)	214 (100–477)	−62.2	0.006**
	TIMP1 (ng/ml)†	32	5.20 (2.65–11.15)	7.25 (2.95–23.55)	1.15	0.022*
	24 h weight (g)†	15	60.3 (31.1–73.6)	34.0 (17.3–45.3)	−14.5	0.035*
	Dry weight (%)	15	4.67 (2.49)	4.11 (1.85)	−0.58	0.241
	DNA content (μg/mg)	15	1.15 (0.41)	0.96 (0.57)	0.19 (0.36)	0.057
	Sputum viscosity 1–10 Hz (Pa s)	14	0.10 (0.09–0.18)	0.12 (0.07–0.16)	−0.03	0.227
	Sputum elasticity 1–10 Hz (Pa)	14	8.92 (6.88–15.51)	10.72 (5.54–16.17)	−2.175	0.299
	EBC pH	37	5.9 (5.6–6.25)	6.1 (5.8–6.4)	0.20	0.016*
	EBC nitrite (μM)	35	5.99 (3.19–7.70)	6.04 (3.92–9.20)	0.87	0.106
	EBC ammonia (ppm)†	36	2.45 (1.33–5.04)	1.78 (1.00–3.93)	−0.07 (4.5)	0.242

†Statistics performed using log-transformed data; these data quoted as median (IQ range), and median change.
*p<0.05; **p<0.01; ***p<0.001.
Lower limits for detection for all cytokine assays are given in online supplementary table E1.
Levels of serum IL-10 and IL-1β and sputum IL-10 and IFN-γ were below the sensitivity of the assays for the majority of samples, and are not presented here. See online supplement for details.
BP, blood pressure; CRP, C-reactive protein; EBC, exhaled breath condensate; $FEF_{25–75}$, forced expiratory flow between 25 and 75% FVC; FEV_1, forced expiratory volume in 1 s; FRC, functional residual capacity; FVC, forced vital capacity; IFN-γ, interferon γ; IL, interleukin; LCI, lung clearance index; MMP9, matrix metalloprotease 9; MPO, myeloperoxidase; NE, neutrophil elastase; RANTES, regulated upon activation, normal T-cell expressed and secreted; SDS, SD score (z score); TIMP1, tissue inhibitor of metalloproteinases 1; TNF-α, tumour necrosis factor α; WCC, white cell count.

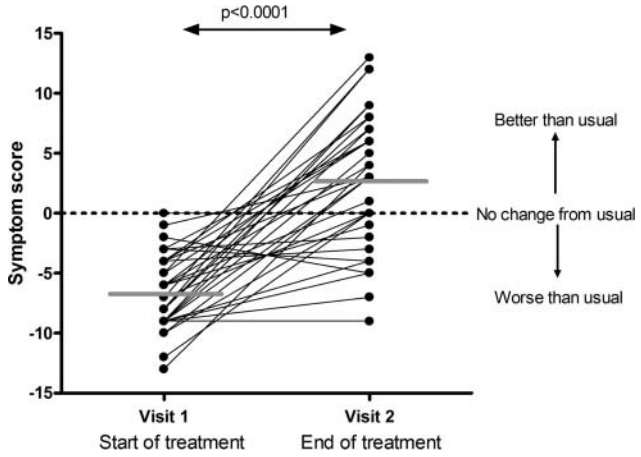

Figure 1 Effect of antibiotics on total symptom score. Each pair of points represents a single subject. Horizontal grey lines represent group means. A symptom score of 0 represents no change from usual baseline for that patient.

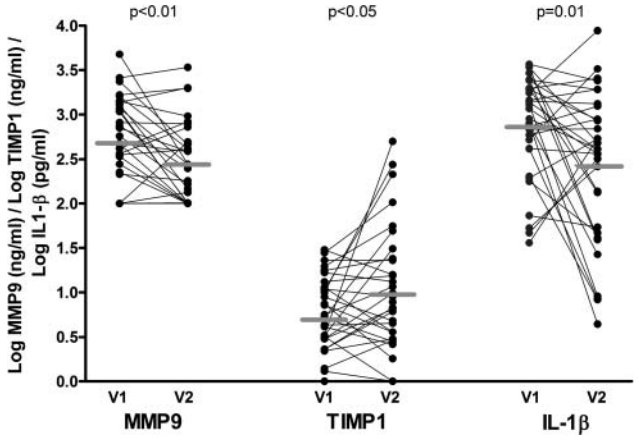

Figure 3 Change in sputum matrix metalloproteinase 9 (MMP9), tissue inhibitor of metalloproteinases 1 (TIMP1) and interleukin (IL)-1β in patients with cystic fibrosis treated for an exacerbation. Each pair of points represents a single patient before and after treatment with intravenous antibiotics. Grey bars represent group means.

There was a small but significant increase in exhaled breath condensate pH, but no change in levels of nitrite or ammonia.

Systemic markers of inflammation

Significant reductions in four markers of systemic inflammation were seen following treatment: white cell count, CRP, IL-6 and calprotectin (table 3; figure 4). No changes were observed for IL-8 or TNF-α levels. Serum IL-10 and IL-1β were generally undetectable at both time points.

Lung structure

Significant improvement was observed on CT for airway wall thickness, mucus plugs and air trapping (figure 5). Although lung consolidation score fell significantly ($p<0.05$), this was not a prominent feature of the CT scans, with an average score of only 1.9% at V1. No significant changes were observed for ground glass opacification, and extent and severity of bronchiectasis.

Correlations between measurements

In the online supplement we present cross-sectional correlation 'mileage charts', divided by assay domain, for all assays at V1. In addition, we have presented a second correlation chart comparing change in assays between visits.

DISCUSSION

This is the first study to simultaneously assess such a comprehensive range of biomarkers in CF. The aim of the study was to provide clues towards biomarker optimisation alongside a subsequent longitudinal study of these biomarkers in patients with stable disease (the gene therapy 'run-in' study), and to help harmonise working across multiple sites. The findings may also provide fresh insights into CF pathophysiology.

Researchers have long recognised the problems of using spirometry in monitoring response to therapy in CF and sought alternative endpoints which either show improved sensitivity or

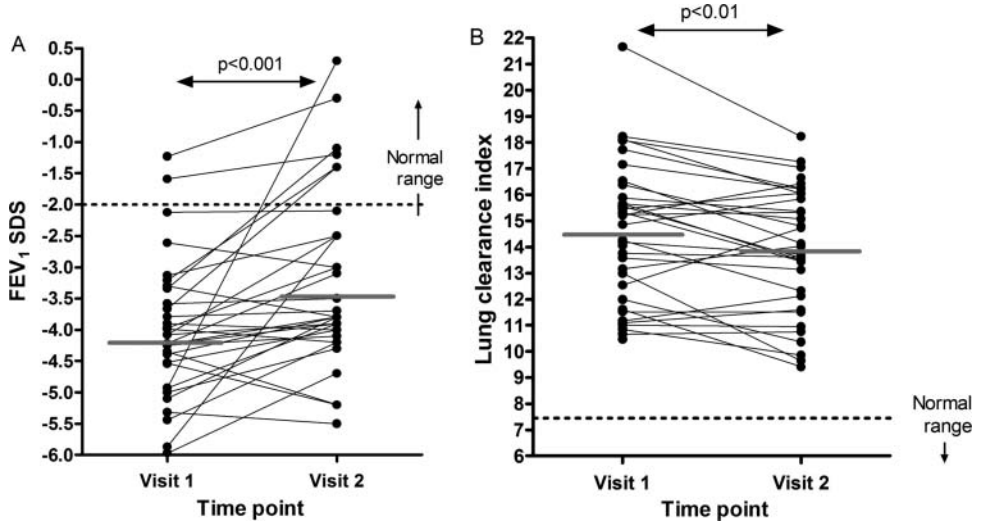

Figure 2 Change in lung physiology. (A) Change in forced expiratory volume in 1 s (FEV_1) with treatment. FEV_1 is expressed as SD scores (SDS); values greater than -2 (horizontal dotted line) are considered to be within the normal range. (B) Change in lung clearance index (LCI) with treatment. The horizontal dotted line represents the upper limit of normal LCI in a healthy control population.[19] Each pair of points represents a single subject. Horizontal grey lines represent group means.

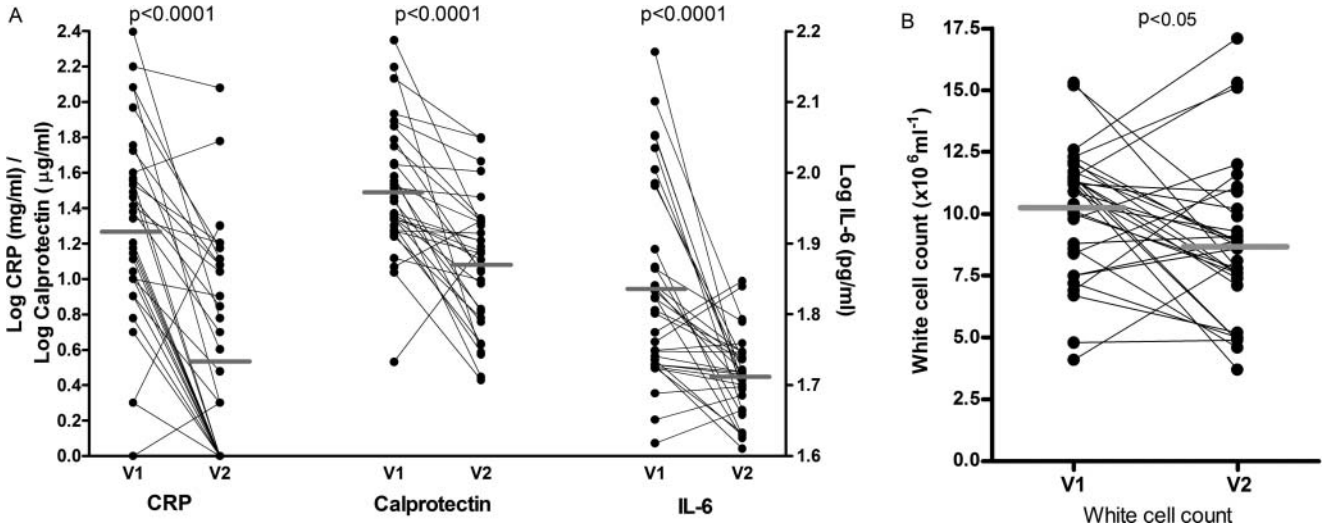

Figure 4 Change in serum inflammatory markers (A) and white cell count (B) in patients with cystic fibrosis treated for an exacerbation. Each pair of points represents a single patient before (V1) and after (V2) treatment with intravenous antibiotics. Group means are shown as horizontal grey bars. CRP, C-reactive protein; IL-6, interleukin 6.

are more closely aligned with the underlying pathophysiology.[1] We hypothesised that if a therapeutic signal was not observable in this acute context, it is reasonable to anticipate that the biomarker is unlikely to prove optimal for a trial in patients with stable disease in whom a smaller positive change might be anticipated. This issue affects all clinical trials in CF and is not limited to gene therapy. We have therefore presented the assay data and accompanying correlations in full (see online supplement), so that others can access these data when selecting biomarkers for their own research. We will consider the changes observed in each domain separately.

Symptoms

The importance of assessing patient-reported outcomes is now well established in CF clinical trial methodology.[22] The symptom score used here was devised by our group and, unlike conventional quality of life assessments, was specifically designed to assess response to acute change in major respiratory symptoms. Although different scores had been used previously to assess acute change,[5 23] when this study was initiated none had been subjected to a formal evaluation process and there was no accepted gold standard. The score we used was appropriate for the current study and provided a simple and effective method of confirming clinical response against which to compare assay performance. We recognise however that it is less well suited to long-term monitoring of patients with stable disease, or indeed to repeated delivery of gene therapy, when changes may be more subtle and multidomain. Symptom and quality of life assessments are key endpoints in our run-in study and gene therapy trials, and we have selected the Cystic Fibrosis Questionnaire Revised for these assessments.[24]

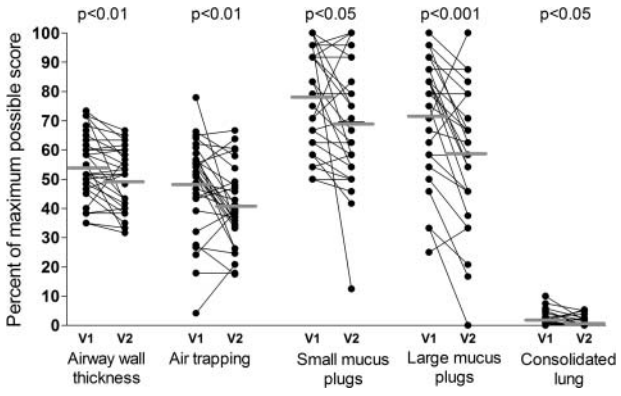

Figure 5 Change in features of cystic fibrosis (CF) lung disease at CT with treatment of a CF exacerbation. Each pair of points represents a single subject assessed before (V1) and after (V2) treatment of a CF exacerbation. Each CT feature was independently assessed by two radiologists, and the final score represents an average of their scores. Horizontal grey bars represent group means.

Lung physiology

Tackling disease in smaller airways is an important objective of CF therapies, but may not be easily correlated to change in FEV_1 or symptoms.[25] LCI is one of the major emerging endpoints in CF clinical trials.[18 26 27] As a measure of overall ventilation heterogeneity, LCI will be affected by fixed airway abnormalities due to fibrotic and destructive processes, and modifiable differences in inflammation and mucus retention. Subjects with mild (and potentially reversible) airways disease are not well represented in the current cohort—only six had FEV_1 within the normal range at V2, and all had abnormalities on CT and considerable elevation in LCI. As previously described,[5] there was considerable heterogeneity of LCI response. Less well ventilated lung regions may be revealed as mucus is cleared, increasing overall inhomogeneity, and thus LCI. In vivo, the effects on LCI and FRC of mucus clearance are likely to be complex and unpredictable,[28] and this test may be best suited to those with milder disease.

Pulmonary markers of inflammation

Sputum is an abundant source of inflammatory markers. Assays that accurately reflect endobronchial infection or inflammation are clinically and biologically relevant, and have considerable potential as pulmonary outcome measures for clinical trials.[29] All the sputum inflammatory markers selected here have previously been reported to be elevated in CF populations, and are amongst several candidate biomarkers of CF airways

inflammation.[29] Sputum IL-8 and NE in particular have been shown to correlate with FEV_1 in a large cross-sectional analysis.[30] Despite the fall in sputum total cell count, we however found no change in sputum IL-8 or NE following treatment, and little correlation with other non-sputum assays. The validity of sputum biomarkers depends on reproducible measurements that also reflect other measures of health or lung function. These data cast doubt over the applicability of many of these potential biomarkers in interventional studies. We also recognise that this study alone is insufficient to dismiss most of the sputum biomarkers entirely, and we have continued to measure the majority in our subsequent longitudinal study. We have however discontinued assessments of sputum rheology and the biomarkers that were only poorly detectable (see online supplement).

Systemic markers of inflammation

The most significant changes in inflammation were observed in serum rather than sputum: CRP, a non-specific marker of inflammation, and calprotectin, a marker of neutrophilic inflammation previously shown to be elevated in CF.[14 31] Both markers showed greater change than either sputum or blood cell counts, or any sputum soluble markers, and calprotectin showed correlations with a number of other measures of severity, including symptom score, spirometry and LCI (see online supplement). Whether these prove useful in monitoring responses to treatment in patients with stable disease is being addressed in our longitudinal study.

Structure

The CT scoring assessed individual morphological abnormalities, rather than using a single composite score.[32] This allows separation of fixed (eg, bronchiectasis) from potentially reversible (eg, wall thickness parameters) features, preventing signal from a change in the latter being diluted by a lack of change in the former. Three previous studies have investigated CT changes following antibiotic treatment,[4 10 11] demonstrating improvements in peribronchial thickening, mucus plugging and air trapping, although no single study demonstrated improvements in all three features. We observed significant improvements in mucus plugging, air trapping and bronchial wall thickness. The grading of the latter two features was designed to maximise the chances of demonstrating small changes over a short time frame by increasing the number of grades within the severity score. Inter-observer reproducibility of the scoring ranged from good to excellent, which we believe justifies the use of the scoring method[33] (see online supplement). This score has now been adopted for the run-in and gene therapy studies.

Limitations

Some potential limitations with the current study deserve discussion. Interventional trials usually seek improvement from stable baseline. This study however addresses a complementary objective: that of demonstrating response to a positive intervention. In this regard, treatment of pulmonary exacerbation is an appropriate and pragmatic model against which to evaluate assays. Although the definition of exacerbation in this study was not protocol predefined, the decision to treat was made by the clinician independent of this study, reflecting standard clinical care. Likewise, treatment is not limited to intravenous antibiotics alone, and will include additional nebulised and physical therapies as appropriate, maximising the impact of the intervention. Although data are incomplete for some analyses, the majority contained data on at least 30 pairs, making this one of the largest CF exacerbation studies reported.

In addition to the practical benefits of the study, this multidomain collection of data may provide useful insights into CF pathophysiology. Correlations will require verification in subsequent studies. A potentially interesting pathophysiological outcome was the predominance of large airway changes during treatment. Thus, some of the most statistically significant improvements were seen in FEV_1 and large airway plugs. In contrast to systemic inflammation, lung inflammation assessed by a range of sputum biomarkers altered little. Short-term reassurance provided by normalisation of symptoms may therefore not reflect longer-term pulmonary inflammation. Novel therapies aimed at the underlying defect, rather than the consequences of it, would clearly be beneficial.

Our overarching aim was to identify and optimise outcome measures for a gene therapy trial. Several airway inflammatory and mucus markers were below the limits of detection even at the start of an exacerbation, while others failed to improve with intravenous antibiotics. In addition we have established the use of LCI in a multicentre setting and refined our understanding of its role as an outcome measure. We are in the process of analysing data from our parallel run-in study of biomarkers in patients with stable CF. Preliminary indications suggest that spirometry, LCI, CT scores and quality of life scores also feature prominently.[34] Data from these studies have played an important role in the selection of biomarkers for our recently started multidose CF gene therapy trial.

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Contributors ARH, JCD, RDG, KAM, JD, ZA, NJB, MR, SM-I, NV, MHD, CS, JSG, JP-L, MDL, SJ, SS, YB, MGM, PT, AD, DH, DA, SCH, DRG, APG, DJP, JAI, ACB, UG, SC and EWFWA all made substantial contributions to the study conception and design, acquisition of data, and analysis and interpretation of data; revised the article critically for important intellectual content; and gave final approval of the version to be published. In addition, ARH, RDG, KAM, JD, ZA, NJB, MR, CM, DH, APG, ACB and UG made substantial additional contributions to assay development and interpretation. ARH, JCD and SC wrote the first draft of the manuscript. EWFWA conceived the study, jointly raised the funding and oversaw its completion.

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Data sharing statement All relevant study data are presented in this paper.

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Changes in physiological, functional and structural markers of cystic fibrosis lung disease with treatment of a pulmonary exacerbation

ONLINE SUPPLEMENT

Version 2

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METHODS

Subjects

The study was performed at three University Hospital sites: Royal Brompton & Harefield NHS Foundation Trust (RBHT), London; Western General Hospital (WGH), Edinburgh; and Royal Hospital for Sick Children (RHSC), Edinburgh. This was a longitudinal study of patients with cystic fibrosis (CF) who were commenced on intravenous (IV) antibiotics for treatment of a pulmonary exacerbation. Patients were assessed between August 2006 and May 2007.

Inclusion criteria:

- Age >10yrs
- Male or Female
- Diagnosis of CF confirmed by a characteristic phenotype in conjunction with sweat test and/or genotyping.
- A pulmonary exacerbation defined by increase in symptoms, increase in sputum production or a decrease in forced expiratory volume in 1 second (FEV_1) requiring intravenous antibiotic therapy
- FEV_1 >=30% predicted at the time of presentation with exacerbation

Exclusion criteria:

- FEV_1 was < 30% predicted at the time of presentation with exacerbation
- Receiving systemic corticosteroids at study entry, during the study or preceding month.
- Patient too unwell to perform study investigations
- Pregnant or breastfeeding

- Lung transplant

Study protocol

Subjects completed a series of non-invasive assessments of disease activity in a fixed order at two separate time points (see Figure E1):

1- Within 72 hours of commencing IV antibiotics for a pulmonary exacerbation.

2- Within 5 days of completion of antibiotic therapy

The decision to commence treatment, choice of antibiotics and duration of treatment was made by the clinical CF team independent of the research group.

Patients were also asked to record their FEV_1 daily using a pocket electronic spirometer (Piko-6, Ferraris Respiratory, Hertford, UK). Details of the individual assessments are given below, and they are listed in Table 1 (main manuscript). The order of the assays was fixed with the exception of the CT scan; some patients had this prior to the other assessments and some afterwards, though all on the same day.

This study was approved by the Lothian Research and Ethics Committee and the Royal Brompton, Harefield and NHLI Research Ethics Committee. All subjects signed informed consent (and assent for pediatric subjects).

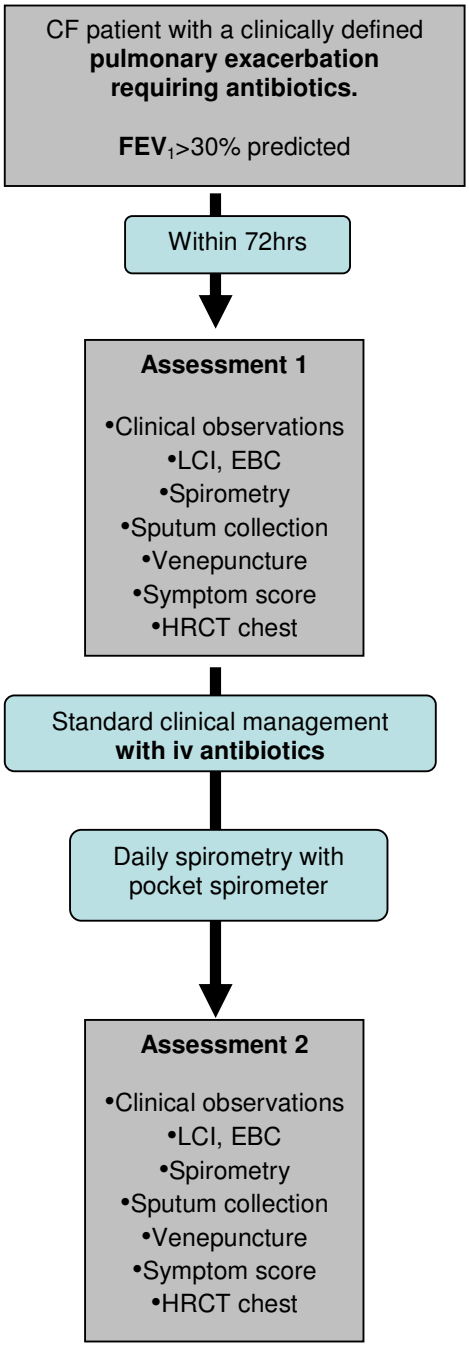

Figure E1: Summary of study flow and assessments.

LCI: lung clearance index. EBC: exhaled breath condensate. HRCT: high resolution computed tomography.

1. Symptoms and clinical observations

Clinical observations

Pulse rate, blood pressure, respiratory rate, pulse oximetry, body temperature and weight were recorded at every visit.

Symptom score

A symptom score sheet was developed to allow patients to self grade their symptoms in response to seven questions relating to different aspects of respiratory function. For each question subjects were required to tick one of five boxes, scored from -2 (much worse/dark/thicker than usual) to +2 (much better than usual). The questions asked were based upon the usual symptoms reported by patients during an exacerbation and were:

1. How severe is your cough?
2. How severe is your breathlessness?
3. How tired or lethargic are you?
4. How far can you walk easily?
5. How much sputum are you producing?
6. Has the shade of sputum changed?
7. How thick is your sputum?

Individual question scores were summed to produce a final symptom score with a range from -14 to +14. No overall change from usual is represented by a score of 0.

2. Lung physiology

Spirometry

Baseline spirometry was measured according to American Thoracic Society/ European Respiratory Society guidelines [1] using an EasyOne spirometer (ndd Medizintechnik AG,

Zurich, Switzerland). FEV_1 and mid-expiratory flows were expressed as standard deviation scores (SDS), or z scores, using reference ranges derived from the modified NHANES III database, as described by Stanojevic *et al.* [2]. For comparison, FEV_1 was also expressed as percent predicted using reference ranges provided by the European Community for Coal and Steel (adults≥17 years) [3] and Rosenthal *et al.* (children≤16 years) [4]. Three reproducible measures were required for a satisfactory result. The best of the three manoeuvres, defined as the result with the greatest sum of FEV_1 and FVC, was recorded. Measurements were performed without a nose clip.

In addition, patients were provided with a portable spirometer (Piko-6™, Ferraris Respiratory, Hertford, UK) with which to record spirometry daily at home. This device recorded the FEV_1 and FEV_6 in its memory, which was then downloaded at the subsequent study visit. Prior to being issued with the handheld device, subjects were instructed in how to use it and how to perform spirometry unsupervised at home.

In nine cases, due to a communication error the spirometry at visit 2 (V2) was not recorded using the EasyOne spirometer. For these patients, we substituted both the FEV_1 values at visit 1 (V1) and V2 with the FEV_1 obtained from the portable spirometer. We only substituted incomplete EasyOne spirometry FEV_1 if: a) spirometry had been recorded on the portable device at both study visits and b) the portable spirometer readings had been shown to be reliable. Portable-spirometer FEV_1 was considered reliable if readings at V1 and V2 were not outliers. Outliers were identified by performing repeated measures analysis of variance on the daily FEV_1 values. Any observation that fell outside two within-patient standard deviations away from the within-patient means was then defined as an outlier.

If the portable spirometer data could not be used to substitute for incomplete spirometry, FEV_1 for that patient was treated as missing. Since the portable device does not record forced vital capacity (FVC) or forced expiratory flows over the middle portion of a forced expiration

(FEF_{25-75}), these data are missing from longitudinal analysis when the portable spirometer has been used. Portable spirometer results were only used for the assessment of longitudinal change, and EasyOne spirometry data have been retained for cross-sectional analysis of V1 data.

Lung Clearance Index

Multiple breath washout was performed as previously described, using a modified Innocor^{TM} gas analyzer and 0.2% sulfur hexafluoride (SF_6) as the tracer gas [5]. Washout tests were performed with the subject seated and suitably distracted by watching television. A nose clip was applied and tidal breathing established whilst the subject breathed through a mouthpiece attached to a filter and flowmeter.

During the first part of the test, the wash-in, the subject inspired 0.2% SF_6 in air from a flow-past circuit attached to the end of the mouthpiece and flowmeter apparatus. Wash-in gas was supplied from a compressed gas cylinder (BOC, Guildford, UK), with the gas flow rate adjusted to ensure that rebreathing did not occur. The wash-in phase was continued until inspiratory and expiratory SF_6 concentrations differed by less than 0.004% (absolute difference in SF_6 concentration). Once wash-in was complete, the flowpast circuit was manually detached during expiration, and the washout commenced.

During the washout the subject breathed room air until the end tidal SF_6 concentration had fallen to less than 0.005% (1/40th of the SF_6 concentration during wash-in). Each subject completed three wash-outs. Functional residual capacity (FRC) was calculated from the total volume of expired tracer gas, and end tidal tracer gas concentrations at start and end of the washout [6], and adjusted for BTPS. LCI is defined as the cumulative expired volume required to reduce the end tidal tracer gas to 1/40th of the starting concentration divided by the FRC.

LCI is quoted as the mean of at least two reproducible repeats from washouts of satisfactory quality. As an additional quality control measure, washouts whose FRC differed by more than 10% from both of the other two repeats were excluded from analysis. Washout analysis was performed at the WGH site by three experienced operators (AH, KM, NB), with cross checking of analyses to ensure consistent and reproducible results.

3. Pulmonary markers of inflammation

Sputum collection and processing

Sputum was expectorated spontaneously in 43/44 (98%) of patients at V1 and 33/38 (87%) of patients at V2. Hypertonic saline sputum induction was performed on five patients unable to expectorate spontaneously at V2, and sputum successfully obtained from two of these patients. Sputum induction was performed to a standard methodology [9], modified as previously described [10]. Subjects were pre-treated with 2.5mg nebulised albuterol. After a wait of 20 minutes, spirometry was repeated, and the patient was then administered 3% saline via an ultrasonic nebuliser (Devilbiss, Sunrise Medical, CA, USA). After 4 minutes of nebulisation, subjects were asked to blow their nose and rinse their mouth with water before attempting to expectorate. This was repeated to a maximum of three saline nebulisations. Subjects repeated spirometry after every saline nebulisation to ensure no adverse effect of the procedure. Each sputum sample was collected in a fresh, pre-chilled tube, but all samples were pooled for processing, which was identical for both spontaneous and induced sputum.

Freshly expectorated sputum (spontaneous or induced) was stored on ice for a maximum of 2 hours and processed using a method modified from that described by Pavord et al. [9]. Whole sputum was transferred to a sterile Petri dish and the sputum plugs separated out into a pre-weighed Falcon tube. The sputum plugs were treated with freshly prepared 0.1% dithiotreitol (Sigma-Aldrich, Dorset, UK) in Dulbecco's phosphate buffered saline (D-PBS), at a ratio of

4ml:1g. Each aliquot was then briefly vortexed and rotated for 15 minutes at 4°C. After dilution in an equal volume of D-PBS, the sample was filtered through pre-moistened 48µm nylon gauze (Seva, Bury, UK) to remove solid debris. The sputum sol phase was obtained by centrifugation (1200rpm for 10 minutes at 4°C), and the supernatant transferred to cryovials for storage at -80°C.

The cell pellet was re-suspended in 0.9% D-PBS. Total cell counts were obtained by counting cells in an improved Neubauer counting chamber. For differential cell counts, four spots (25, 50, 75 and 100µl) were pipetted onto glass slides for cytology. The slides were spun at 400 rpm for 5 minutes to draw the cells onto the slides. These were then fixed and stained using a commercially available kit based on May-Grünewald Giemsa stain (Surgipath Industries, Richmond, IL, USA) or using a standard hematoxylin and eosin stain. Cell differentials were obtained by inspecting the slide with the optimal cell density at a magnification of 100 times, under oil. 300-500 cells were identified and counted from each slide from two different regions, and the final percentage is the mean of these two measurements. Cell counts were performed by a single operator at each site.

Sputum solids content

Aliquots of fresh sputum (~0.6 g) were frozen within 2 hrs of collection until further processing. Sputum was placed into three pre-weighed tubes (~0.2 g/tube) and the exact wet weight was calculated by re-weighing the tubes. Sputum was freeze-dried overnight (Edwards EF4 Modulyo freeze dryer, West Sussex, UK) to obtain the dry weight. Data was expressed as % dry weight and data from triplicate measurements were averaged for each sample.

Sputum total DNA content

DNA was extracted from freeze-dried sputum samples (see above) using the QIAamp DNA Mini Kit (Qiagen, Crawley, UK) according to manufacturer's recommendations. Prior to DNA extraction, freeze-dried sputum samples were re-dissolved in 200µl of DNase free water. 200µl of NALC solution (3% N-acetyl-L-cysteine and 4% sodium hydroxide) were then added and samples incubated at 56ºC for 30 min. The DNA concentration was determined using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Paisley, UK) according to manufacturer's recommendations. Data from triplicate measurements were averaged for each sample.

Sputum inflammatory markers and proteases

All assays were validated as suitable for use with DTT, as recommended by the ERS guidelines [11].

IL-8 assays were performed using a commercial kit (IL-8 Easia Kit, Biosource, Invitrogen, CA, USA). Mini-complete protease inhibitor (Roche, Burgess Hill, Sussex, UK) was added to the aliquot used for analysis of the remaining sputum cytokines (other than IL-8). Sputum IL-1β, IL-6, TNFα, and RANTES were measured using Bio-plex cytokine assay reagents (Bio-Rad Laboratories, Hemel Hempstead, Hertfordshire UK) analysed on a Luminex 100 analyser (Luminex Corporation, Oosterhout, The Netherlands). Sputum IL-12 and INF-γ were measured by commercial ELISA (Biosource, Invitrogen, CA, USA).

Commercial ELISA kits were employed to measure MMP-9, TIMP-1 (both from GE-healthcare, Amersham, Buckinghamshire, UK) and MPO (Assay Designs, Michigan, USA). Kits were used as per manufacturers' instructions but with the addition of 0.05% DTT to the provided sample buffer to equilibrate standard curve to the DTT levels present in native sputum samples. Neutrophil elastase (NE) activity was measured in samples diluted 1:10 in

assay buffer (0.3M TRIS-HCl, containing 1.5M NaCl, pH 8.0) by spectrophotometric assay. 10µl of sputum samples and NE standards (human leukocyte elastase (Sigma, Poole, UK)) were pre-incubated on a 96-well microtitre plate for 1 minute at 37°C. 90µl substrate (0.56mM N-methoxysuccinyl-ala-ala-pro-val-p-nitroanilide (Sigma, UK) in assay buffer) was added and the plate incubated for a further 5 minutes at 37°C. Colour change was read as an increase in absorbance at 410nm using a microtitre plate reader (Biochrom, UK). Elastase activity in the samples was calculated against a standard curve on each plate. Calprotectin assay is described below.

With the exception of the calprotectin, IL8 and MPO assays, sputum inflammatory marker assays were all conducted at RBH.

Lower limit of detection for sputum assays are presented in Table E1.

Sputum rheology

Sputum for rheological analysis was frozen within 2 hrs of expectoration and was defrosted before rheology measurements were performed. We have previously shown that one freeze/thaw cycle does not alter rheological properties (manuscript in preparation). Sputum linear visco-elasticity was measured with a CSL 100 rheometer (TA Instruments, Leatherhead, UK) fitted with a 4-cm stainless steel parallel plate with a 250 µm gap and a target displacement of just $1x10^{-3}$ µm. Approximately 1 ml of sputum was placed between the parallel plates and care was taken to remove air bubbles. A dynamic oscillatory test was conducted from 1 Hz to 10 Hz at 20°C and the dynamic storage modulus (G') and the dynamic viscosity (η') were calculated. A minimum of two dynamic oscillatory tests were performed per aliquot and the mean was calculated to obtain a single G' and η' for each

aliquot. Tests which exhibited untypical curves due to air bubbles or shortage of sputum were excluded and repeated.

Rheological analysis was only performed on samples from the RBH patients.

Microbiology

Microbiological analysis of sputum samples at V1 was performed in the clinical microbiology laboratories of the respective hospitals, using selective culture media appropriate for a CF population.

Exhaled breath condensate

Exhaled breath condensate (EBC) was collected using a commercially available condensing machine (Ecoscreen; Jaeger Viasys, Hoechberg, Germany), as previously described [7]. Exhaled air is cooled but not frozen. Subjects provided a sample of EBC over a period of 5-10 min using tidal breathing and nose clips, until at least 3mL of condensate had been obtained.

pH was measured using a handheld pH meter (phBoy; Camlab, Cambridge, UK) with a two-point calibration performed at the start of each session; samples were assessed immediately following condensate collection.

Since nitrite is vulnerable to rapid degeneration, all samples were analysed for nitrite within 15 min of collection. Nitrite was measured on standard curves using the Griess reaction on triplicates of 200 μL condensate, at an absorbance wavelength of 540 nM (lower detection limit = 0.074 μM) [7]. Remaining sample was aliquotted and stored at -70^{o}C.

Ammonium was measured using a solid state ion selective electrode and 3345 ion meter (Jenway, Dunmow, UK) as previously described [8]. The ion probe was inverted and 130 μL of the sample was applied to this surface. A five-point standard curve of ammonium chloride

solution (1,000 parts per million (ppm), 100 ppm, 10 ppm, 1 ppm and 0.1 ppm; Sigma, UK) was generated and had a lower limit of detection at 5.5 mM (0.1 ppm); exponential extrapolation of data from the voltage recording was then performed.

4. Systemic markers of inflammation

Venous blood sampling

Venous blood was collected in standard clinical blood collection tubes (RBH: Becton Dickinson Vacutainers, Becton Dickinson, Oxford, UK. Edinburgh: Monovettes, Sarstedt AG, Numbrecht, Germany) and analyzed at the local clinical laboratories for full blood count and C-reactive protein (CRP). CRP was measured using an immunoturbidimetric assay on the Beckman LX20 analyzer (Beckman, High Wycombe, Buckinghamshire UK) (RBH) or an enzymatic sandwich immunoassay on a Vitros analyzer (Ortho Clinical Diagnostics, High Wycombe, Buckinghamshire, UK). Samples below the lower limit of detection (<1mg/ml for RBH samples, <3mg/ml for RHSC and WGH samples) have been given the value of 1mg/ml. Prior to separation, whole blood samples were stored on ice for up to 45 minutes. Samples were centrifuged at 1300g for 10 minutes at room temperature and serum separated into aliquots for storage and transport at -80^{o}C.

Serum inflammatory markers

Serum IL-8 assays were performed using a commercial kit (IL-8 Easia Kit, Biosource, Invitrogen, CA, USA), following manufacturer's instructions. Serum IL-1β, IL-6, IL-10 and TNFα were measured using Bio-plex cytokine assay reagents (Bio-Rad Laboratories, Hemel Hempstead, Hertfordshire UK) analyzed on a Luminex 100 analyzer (Luminex Corporation, Oosterhout, The Netherlands), following manufacturer's instructions. A minimum of 100 of each cytokine bead was detected per sample. Standard curves were fitted using a 4p-logistic

curve fit or a 5p-logistic curve fit using the Luminex 100 software. Acceptable curve fitting was judged by a regression coefficient of >0.95. With the exception of calprotectin, serum inflammatory marker assays were all conducted at RBH.

Lower limit of detection for serum assays are presented in Table E1.

Serum and sputum calprotectin

An in-house calprotectin ELISA was used, which has an intra-assay coefficient of variation of 5.6% (unpublished observations). Calprotectin monoclonal and polyclonal antibodies and calprotectin protein standard were kind gifts of Erling Sundrehagen, Oslo, Norway. Microtitre plates (Corning, Lowell, MA, USA) were coated with 100 µl mouse anti-calprotectin monoclonal (mouse anti-human) antibody overnight at 4°C at a concentration of 40µg/ml diluted in coating buffer (KPL Gaithersburg, MA, USA). Plates were then blocked with 1% BSA for 1 hour at 37°C and the plate washed three times with 0.05% Tween 20. 100 µl of sample was added to the plate in dilutions of 1/5000, 1/10000 and 1/50000 for sputum (0.05% DTT in PBS diluent); 1/500, 1/2500 and 1/5000 for serum (50% fetal calf serum in PBS diluent). Purified calprotectin standard was also added to the plate in the appropriate diluent for the assay being undertaken (i.e. DTT for sputum, fetal calf serum for serum) with a top standard of 100 ng/ml and limit of detection of 1.56 ng/ml. Samples were incubated at room temperature for 2 hrs and the plate washed three times as before. Anti-calprotectin (chicken anti-human) polyclonal antibody at 1 in 1000 was added and incubated for 2 hours and washed as before. 100 µl donkey anti-chicken antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch, Suffolk, UK) was added at a concentration of 1 in 250, incubated for 2 hrs and washed three times as before. 100 µl substrate to horseradish peroxidase (KPL Gaithersburg, MA, USA) was then added and plates were incubated for 20 minutes before reading on a microplate reader at 450 nm.

Concentrations of calprotectin were calculated from the standard curve. Calprotectin assays were performed at the WGH site.

Serum	
Assay	**Lower limit of detection**
IL-1β	17.6 pg/ml
IL-6	9.2 pg/ml
IL-8	10pg/ml
IL-10	13.6 pg/ml
TNFα	14.8pg/ml
CRP	1 mg/L
Calprotectin	1.6 ng/ml
Sputum	
Assay	**Lower limit of detection**
IL-1β	4.4 pg/ml
IL-6	2.3 pg/ml
IL-12	7.8 pg/ml
TNFα	3.7 pg/ml
RANTES	2.1 pg/ml
MMP-9	100 ng/ml
TIMP-1	3.1 ng/ml
Neutrophil elastase	100 U/L
IFN-γ	15.6pg/ml
Calprotectin	1.6 ng/ml

Table E1: Lower limits of detection for sputum and serum inflammatory marker assays. Abbreviations: IL – interleukin; TNF-α - tumor necrosis factor alpha; CRP - C-reactive protein; RANTES - Regulated upon Activation, Normal T-cell Expressed and Secreted; MMP- matrix metalloprotease; TIMP1 – tissue inhibitor of metalloproteinases. IFN-γ – interferon γ.

5. Computed Tomography (CT) assessment of lung structure

Chest CT images were acquired without contrast on 16 (WGH) and 64 (RHSC and RBH) channel multidetector scanners (Siemens Somatom Zoom, Siemens Medical Solutions, Erlangen, Germany). Siemens Sensation 64 CT scanner: 100kVp, 0.5 sec rotation time, 64x0.6mm collimation, 1.4 pitch, 1mm slice thickness, B70f very sharp kernel. Siemens Sensation 16 CT scanner: 100kVp, 0.5 sec rotation time, 24x1.0mm collimation, 1.4 pitch, 1mm slice thickness, B70f very sharp kernel. Identical CT protocols were used at all centres, comprising contiguous thin-sections through the entire volume of the lungs obtained during inspiration, and in addition, interspaced (1-mm sections at 10-mm increments) during end-expiration. In order to limit the effective radiation dose, 100kVp was used for all patients and mAs values were determined by patient weight: for patients weighing up to 30 kg – 1mAs per kg, for patients 30-50kg – 35mAs and patients above 50kg – 40 mAs.

All CT images were anonymised with respect to patient identity and the date of the CT and scored independently by two radiologists (MGM & PT) with a special interest in thoracic imaging. All the scoring was performed directly from workstations with access to image manipulation, including window settings. Images from the first and second visits (total of 72 scans) were scored in random order. The presence and severity of specific CT features were scored on a lobar basis using a revised semi-quantitative grading system based on that used by Roberts et al [12], and summarized in Table E2. The extent of bronchiectasis was quantified according to the percentage of each lobe involved (0 = none, 1 = <25% of lobe, 2 = 25-50%, 3 = 51-75% and 4 = 76-100%) and the severity of bronchial dilatation was defined according to the degree of dilatation compared to the size of the accompanying vessel (0 = absent, 1 = trivial dilatation, 2 = >1 but less than 2x diameter of vessel, 3 = 2-3x diameter of vessel and 4 = > 3x diameter of vessel). Similarly, a global assessment of bronchial wall

thickness in each lobe was made by comparison with the diameter of the adjacent vessel (0 = absent, 1 = trivial wall thickening, 2 = wall thickness up to 0.5x diameter of vessel, 3 = wall thickness >0.5x and up to diameter of vessel, 4 = wall thickness >1 and up to 2x diameter of vessel and 5 = wall thickness >2x diameter of adjacent vessel). Small mucus plugs depicted on CT as centrilobular nodules or a tree-in-bud pattern and large mucus plugs were categorized as being absent (0), mild (1), or extensive (2). Air trapping (scored only on the interspaced expiratory images), consolidation and ground glass opacification were quantified as a percentage of the lobe involved to the nearest 5%. Scores for each lobe were summed to give a total lung score for each CT feature and scores from both the observers were summed giving a range of scores from 12 to 84 for each CT feature. Final score is expressed as a percentage of the maximum possible score for that feature.

Inter-observer variation data for the different CT features, expressed as the weighted kappa for categorical variables and the single determination standard deviation for continuous variables, are presented in Table E3.

Feature	Score range	Maximum possible score
Extent of Bronchiectasis	0 = none 1 = <25% lobe involved 2 = 25-50% lobe involved 3 = 51-75% lobe involved 4 = 76-100% lobe involved	48
Severity of Bronchiectasis	0 = absent 1 = trivial dilatation 2 = >1 but <2x diameter of accompanying vessel 3 = 2-3x vessel diameter 4 = >3x vessel diameter	48
Airway wall thickening	0 = absent 1 = trivial wall thickness 2 = up to 0.5x diameter of adjacent vessel 3 = > 0.5 to 1x vessel diameter 4 = > 1 to 2x vessel diameter 5 = > 2x vessel diameter	60
Small mucus plugs	0 = absent 1 = mild 2 = extensive	24
Large mucus plugs		24
Air trapping	0-100%, scored to nearest 5%	1200
Consolidation		1200
Ground glass opacification		1200

Table E2: Summary of CT scoring protocol. Each lobe (of six) was scored independently and the maximum possible score represents the sum of all the lobe scores from two

radiologists (i.e. 12x the maximum single lobe score). CT score was then expressed as a percentage of the maximum possible score for that feature.

CT feature	Serial quantification
Extent of bronchiectasis	$\kappa_\omega = 0.88$
Severity of bronchiectasis	$\kappa_\omega = 0.87$
Bronchial wall thickness	$\kappa_\omega = 0.81$
Small mucus plugs	$\kappa_\omega = 0.88$
Large mucus plugs	$\kappa_\omega = 0.88$
Air trapping	3.50% *
Consolidation	0.57% *
Ground glass opacification	0.77% *

Table E3: Inter-observer agreement for CT features, quantified using the weighted kappa (κ_ω) for categorical variables and the single determination standard deviation for continuous variables (indicated by *).

Statistical analysis

Data were analyzed using Prism (GraphPad Software Inc, CA, USA) and SPSS (IBM corp, NY, USA). Normal distribution was assessed using the D'Agostino and Pearson omnibus normality test. Results are quoted as mean (SD) or median (interquartile range) unless otherwise stated. No attempt was made to substitute missing data.

Skewed data were log-transformed prior to analysis. Paired t-test was used for comparison of change in variables between paired visits and comparisons between multiple groups were performed using a one-way ANOVA and Tukey's HSD test. Biomarkers reported as below the lower limit of the assay have all been ascribed a value equal to the lower limit of detection (see Table E1).

Correlations between different assays were performed on assessments performed at V1, and included all those with valid assessments at that visit even if subsequent assessments were missing or excluded because of protocol violation. Correlations were assessed using the Pearson correlation coefficient (normally-distributed data) or Spearman rank correlation (skewed data). Change in assays was calculated as the V2 value minus V1. A p value of below 0.05 was considered as statistically significant

The multiple correlations presented in Tables E5–E11 are intended to assist generation of hypotheses about the pathophysiology of CF and response to therapy and are therefore presented in full, with no correction for multiple comparisons.

RESULTS

Patient demographics and clinical characteristics

Forty-six patients consented to the study. Two patients were subsequently excluded from all analyses for concomitant use of oral corticosteroids; cross-sectional data correlations from V1 were therefore been performed on 44 patients. A further six patients were excluded from longitudinal analyses because of an excessive time delay (>5 days) (n=2) or non-attendance (n=3) at V2, or because of commencing oral corticosteroids between assessments (n=1) (see Figure E2).

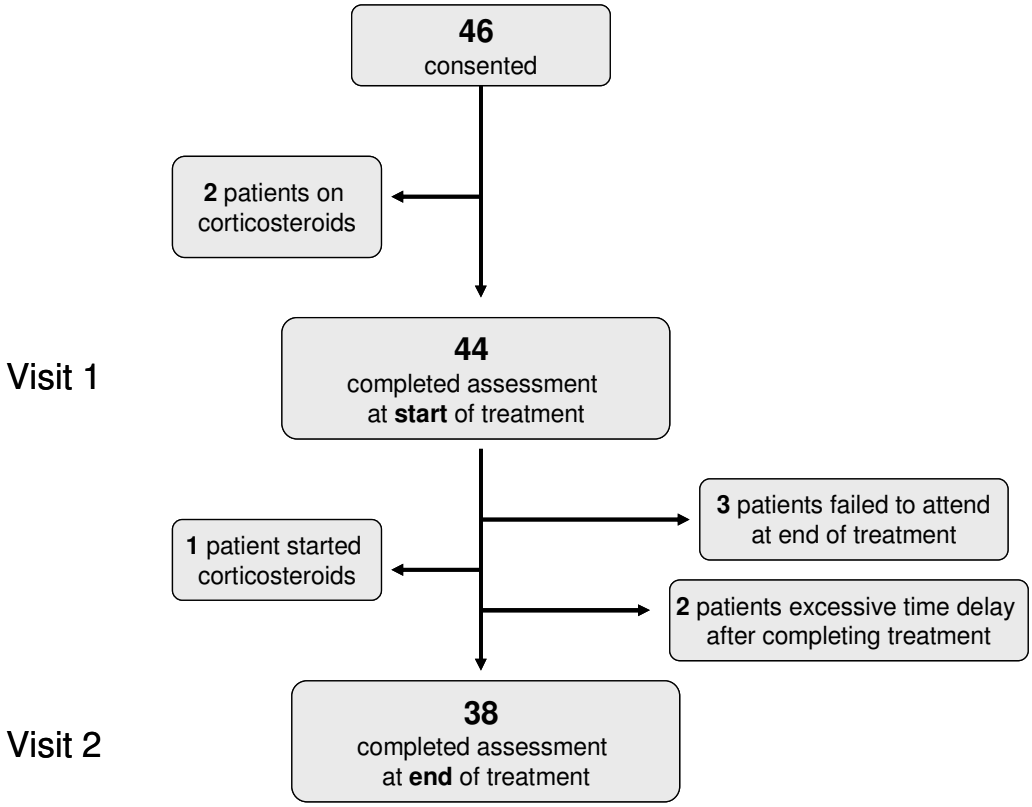

Figure E2: Number of patients recruited and assessed at each of the two study visits.

Demographics and presenting clinical features are summarised in Table E4 (Table 2 of the main manuscript). These patients had a median age of 23 years (range 11-44 years). Mean (SD) FEV_1 z score at start of treatment was -4.29 (1.03), or 52.1 (12.2) percent predicted.

27 (61%) were ΔF508 homozygotes, and 16 (36%) were ΔF508 heterozygotes. A single subject had no copies of the ΔF508 gene (genotype G551D/1717-1G→A).

The most common symptoms noted at the time of commencing antibiotics were increased cough (98%) and increased dyspnoea (93%).

Treatment

All patients were treated with a minimum of two combined intravenous antibiotics for a median [range] treatment duration of 14 [9 - 24] days. Treatment choice was at the discretion of the clinical team. The most common treatment regimen consisted of a combination of intravenous β-lactam antibiotic and an aminoglycoside (77%). The β-lactam antibiotics used were ceftazidime (31 cases, 71%), meropenem (nine, 21%), two cases each of temocillin and timentin and one case of aztreonam. The aminoglycosides prescribed were tobramycin (27, 61%), gentamicin (five, 11%) and a single case of amikacin. Colomicin was used as an alternative in seven cases (16%). Additional therapies consisted of chloramphenicol (two cases), Teicoplanin (one), levofloxacin (one) and flucloxacillin (two). The most common therapeutic regimen was intravenous tobramycin and ceftazidime (19, 43%).

Number of subjects	44
Sex (m/f)	24/20
Median [IQ range] age (yrs)	23 [18 – 28]
Characteristics of exacerbation: N (%)	
• Increased cough	43 (98)
• Increased dyspnoea	41 (93)
• Change in sputum	39 (89)
• Malaise	37 (84)
• Fall in FEV_1 >10%*	24 (55)
Mean (SD) FEV_1 at start of treatment: z score	-4.29 (1.03)
[% predicted]	52.1 (12.2)

Table E4: Demographics and symptoms at start of treatment

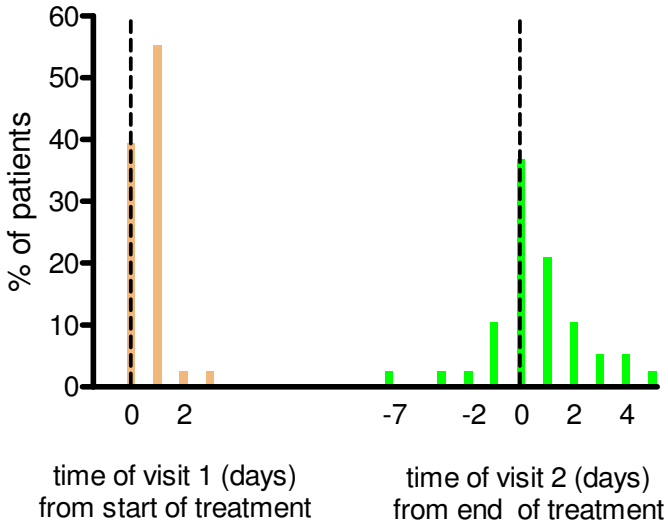

Figure E3: Timing of assessments compared to start and end of antibiotic treatment. A difference of 0 days indicates that assessment occurred on the same day that treatment was commenced (red bars) or completed (green).

Timing of assessments

Thirty-six (95%) of baseline assessments were performed within 24 hours of starting IV antibiotics (see Figure E3). One subject was assessed at 48hrs and one at 72hrs. The selection of a 72hr time window for assessment was a pragmatic one, to ensure that the majority of patients could be assessed including those commenced on IV antibiotics at the weekend. Post-hoc exclusion of the two subjects assessed at >=48rs, in order to assess the effect of this delay on magnitude of observed changes, did not alter the conclusions.

At V2, 22 (58%) of patients were assessed within 24 hours of completing treatment. A single subject was prescribed a third week of treatment after completing the V2 assessment – the subject was retained in the study because they had completed 2 weeks of IV antibiotics, and had improved symptomatically, albeit incompletely at the time of assessment.

Microbiology

At the initial assessment, all but one patient were able to spontaneously expectorate sputum for analysis. Following treatment, however, sputum induction was required in five (13%) patients. Three of these patients did not produce any sputum even after sputum induction.

26 patients (59%) were chronically colonized with *Pseudomonas aeruginosa*, whilst 11 subjects (25%) had never had infection with *Pseudomonas*. In addition, 10 subjects (23%) were chronically infected with organisms of the *Burkholderia cepacia* complex, 23 (50%) were chronically infected with *Staphylococcus aureus* (including four with methicillin-resistant strains) and eight (18%) were chronically infected with *Stenotrophomonas maltophilia*. In order to assess the effects of microbiology on assays, the patients were divided into three groups based upon the predominant infecting bacterial species. Twenty-three patients (55%) were classified as having chronic infection with *Pseudomonas aeruginosa*, nine (21%) with chronic *Burkholderia cepacia* complex infection and 10 (24%) with other infecting organisms. Difference between the three groups was assessed for all

assays at baseline (V1). Only three statistically significant differences were noted, these were: a mean increase of 4 breaths/minute in group 2 (*Burkholderia cepacia*) compared to group 1 (*Pseudomonas*) (p=0.015); a mean increase in log 24hr sputum weight of 0.5g in group 2 compared to group 3 (other) (p=0.029); and a mean increase in log sputum RANTES of 0.32 in group 2 compared with group 1 (p=0.032). None of these differences were considered to be clinically significant, and may have arisen by chance. No further attempt at post-hoc subgroup analysis on the basis of microbiology was attempted.

Additional results

2. Lung physiology

Spirometry

Median fall in FEV_1 at start of treatment, compared to best recorded FEV_1 in the preceding 6 months, was 12% (interquartile range 3.5% - 25.9% fall in FEV_1). Overall, 22 patients (55%) had experienced a fall in FEV_1>10% (L). Mean (SD) FEV_1 at the end of treatment was similar to patients' best recorded FEV_1 within the last 6 months for the group as a whole: 2.25 (0.76) L at end of treatment vs. 2.22 (0.86) L as recent best, p=0.8. However, the degree of change in FEV_1 with treatment was related to the severity of the fall in FEV_1 from recent best at start of IV antibiotics. Patients with an FEV_1 fall of <10% (absolute) at study entry (n=17) improved by a median of 7.2% to 6.4% above baseline. Where FEV_1 fall was >10% (absolute) at study entry, improvement was by a median of 31% (p=0.05 compared with those with no significant fall in FEV_1 at study entry), but remained 13% below baseline at the end of treatment (p=0.02).

Lung clearance index

Seventy triplicate washouts were analyzed as part of this study (38 V1 used in cross sectional analysis, 32 additional washouts at V2 for longitudinal analysis). Of these 210 washout repeats, 17 were excluded on the basis of reproducibility because FRC was >10% different from the other two washouts. In addition six triplicate sets of washout repeats were un-analysable due to technical error or inability of the patient to establish an interpretable and relaxed breathing pattern. Three additional individual washout repeats were also un-analysable due to technical or patient factors. Overall washout failure rate was therefore 18% of all washout repeats performed or 9% of those which could be analysed. This represents a worst case scenario for this technique since it involved patients unwell at the start of an exacerbation and includes technical issues which have since been resolved. Mean coefficient of variation of included washout repeats was 5.3% for LCI and 4.0% for FRC.

3. Pulmonary markers of inflammation

IFN-γ was only detectable in two samples (both V2) and IL-6 was detectable in only five V1 and six V2 samples (including two pairs of samples). For this reason, no further analysis has been conducted on sputum IFN-γ or IL-6.

4. Systemic markers of inflammation

IL-10 was detectable in only nine serum samples at V1 and in only a single pair of samples at both timepoints. IL-1β was only detectable in six samples at V1 and in two pairs at both timepoints. For this reason, no further analysis has been conducted on serum IL-10 or IL-1β.

Correlations between measurements

The following six tables represent cross sectional correlations between assays at V1. All data are presented, but divided by domain into five tables for ease of viewing. Numbers represent the Pearson r correlation coefficient (upper) and number of pairs of data (lower) for each correlation. Log transformed data indicated by +. Boxes shaded in pink, and correlation coefficients identified by *, have a P value <0.5. Boxes shaded in red, and correlation coefficients identified by **, have a P value <0.01. Boxes shaded in purple, and correlation coefficients identified by ***, have a P value <0.0001.

Table E5: Symptoms and clinical observations

	Symptom score	Weight	Heart rate	Respiratory rate	O_2 saturation	systolic BP	diastolic BP
Symptom score		-0.175 42	-0.209 43	0.227 41	0.254 43	0.322* 43	0.315* 43
Weight	-0.175 42		-0.256 43	-0.306 41	0.010 43	0.365* 43	0.339* 43
Heart rate	-0.209 43	-0.256 43		0.201 42	-0.284 44	-0.040 44	-0.051 44
Respiratory rate	0.227 41	-0.306 41	0.201 42		0.014 42	-0.045 42	-0.024 42
O_2 saturation	0.254 43	0.010 43	-0.284 44	0.014 42		0.183 44	0.194 44
systolic BP	0.322* 43	0.365* 43	-0.040 44	-0.045 42	0.183 44		0.746*** 44
diastolic BP	0.315* 43	0.339* 43	-0.051 44	-0.024 42	0.194 44	0.746*** 44	
FEV_1	0.301 41	0.569** 41	-0.377* 42	-0.038 40	0.101 42	0.392* 42	0.284 42
FEV_1 SDS	0.307 41	0.197 41	-0.295 42	0.133 40	0.142 42	0.170 42	0.202 42
FVC SDS	0.311* 41	0.132 41	-0.347* 42	0.131 40	-0.020 42	0.102 42	0.161 42
FEF_{25-75} SDS	0.038 19	0.179 19	-0.188 20	-0.197 18	0.284 20	0.167 20	0.198 20
LCI	-0.030 38	0.000 38	0.099 39	-0.223 37	-0.320* 39	0.187 39	0.076 39
FRC	0.080 38	0.379* 38	-0.202 39	-0.001 37	-0.050 39	0.343* 39	0.135 39

Extent bronchiectasis	0.018 33	-0.146 33	-0.038 34	-0.095 33	0.062 34	-0.112 34	-0.031 34
Severity bronchiectasis	0.187 33	-0.182 33	-0.136 34	-0.377* 33	0.246 34	-0.305 34	-0.124 34
Wall thickness	-0.144 33	-0.078 33	0.092 34	-0.373* 33	0.114 34	-0.257 34	-0.080 34
Air trapping	-0.109 32	0.110 32	0.124 33	0.003 32	-0.618*** 33	-0.047 33	-0.034 33
Small mucus plugs	-0.202 33	-0.165 33	0.201 34	0.003 33	-0.304 34	0.073 34	-0.087 34
Large mucus plugs	-0.161 33	-0.084 33	0.185 34	-0.252 33	0.041 34	-0.133 34	-0.118 34
Consolidated lung	-0.238 33	0.034 33	0.073 34	-0.125 33	-0.062 34	-0.317 34	-0.209 34
Ground glass	-0.199 33	0.190 33	0.088 34	-0.264 33	-0.271 34	-0.251 34	-0.156 34
White cell count	-0.192 41	0.028 41	0.108 42	0.155 40	-0.132 42	-0.062 42	-0.222 42
CRP⁺	-0.554** 41	0.104 41	0.258 42	-0.055 40	-0.298 42	-0.258 42	-0.361* 42
Serum IL-6⁺	-0.399* 35	0.108 35	0.284 36	0.239 34	-0.067 36	-0.240 36	-0.478** 36
Serum calprotectin⁺	-0.329* 38	0.195 38	0.088 39	-0.090 37	-0.190 39	-0.082 39	-0.319* 39
Serum IL-8⁺	0.140 36	0.073 36	-0.176 37	0.204 35	0.076 37	0.075 37	0.006 37
Serum TNFα	0.151 35	0.141 35	-0.215 36	0.212 34	0.310 36	0.034 36	0.000 36
Sputum 24 hr weight⁺	-0.091 22	0.375 22	0.023 22	-0.029 22	0.277 22	-0.082 22	-0.050 22
Sputum % dry weight	-0.070 29	0.434* 29	0.251 30	0.258 28	-0.189 30	0.123 30	0.111 30
Sputum total cell count⁺	-0.274 32	0.165 31	0.086 32	-0.413* 32	-0.385* 32	-0.290 32	-0.162 32
Sputum IL-12	0.286 35	-0.177 35	0.198 36	0.240 34	0.150 36	0.150 36	0.091 36
Sputum MMP9⁺	-0.053 39	0.056 39	0.073 40	-0.216 38	-0.093 40	0.093 40	-0.081 40
Sputum calprotectin⁺	-0.117 39	0.099 39	0.121 40	-0.023 38	-0.269 40	-0.060 40	-0.018 40
Sputum IL-8	0.110 38	0.209 38	-0.201 39	0.138 37	-0.002 39	0.280 39	0.248 39
Sputum TNFα⁺	-0.206 37	0.313 37	0.152 38	0.010 36	0.044 38	-0.21 38	-0.150 38
Sputum neutrophil elastase	-0.050 40	0.317* 40	-0.037 41	0.101 39	-0.103 41	0.244 41	0.215 41
Sputum MPO⁺	-0.143 38	0.268 38	0.019 39	-0.087 37	-0.294 39	0.110 39	0.124 39
Sputum	0.536***	-0.003	-0.261	0.253	0.216	0.278	0.203

RANTES^{+}	37	37	38	36	38	38	38
Sputum TIMP1^{+}	0.064 37	0.135 37	-0.170 38	0.304 36	0.264 38	0.280 38	0.185 38
Sputum IL-1β^{+}	0.253 35	-0.181 35	0.179 36	0.145 34	0.124 36	0.134 36	0.080 36
EBC pH	-0.124 42	0.015 42	-0.046 43	-0.095 41	-0.068 43	-0.097 43	-0.266 43
EBC nitrite	-0.027 39	-0.161 39	0.034 40	0.158 38	-0.036 40	-0.134 40	0.064 40
EBC NH_4^{+}	-0.141 41	0.243 41	-0.077 42	-0.212 41	-0.193 42	-0.111 42	-0.242 42
Sputum DNA	-0.177 29	0.074 29	-0.247 30	-0.270 28	0.035 30	0.045 30	-0.140 30
Sputum viscosity	-0.292 26	0.131 26	0.335 27	0.147 26	-0.344 27	0.140 27	-0.076 27
Sputum elasticity	-0.303 26	0.134 26	0.336 27	0.114 26	-0.345 27	0.202 27	-0.017 27

Table E6: Lung function and physiology

	FEV_1	FEV_1 SDS	FVC SDS	FEF_{25-75} SDS	LCI	FRC
Symptom score	0.301 41	0.307 41	0.311* 41	0.038 19	-0.030 38	0.080 38
Weight	0.569** 41	0.197 41	0.132 41	0.179 19	0.000 38	0.379* 38
Heart rate	-0.377* 42	-0.295 42	-0.347* 42	-0.188 20	0.099 39	-0.202 39
Respiratory rate	-0.038 40	0.133 40	0.131 40	-0.197 18	-0.223 37	-0.001 37
O_2 saturation	0.101 42	0.142 42	-0.020 42	0.284 20	-0.320* 39	-0.050 39
systolic BP	0.392* 42	0.170 42	0.102 42	0.167 20	0.187 39	0.343* 39
diastolic BP	0.284 42	0.202 42	0.161 42	0.198 20	0.076 39	0.135 39
FEV_1		0.721*** 42	0.649*** 42	0.432 20	-0.182 37	0.524** 37
FEV_1 SDS	0.721*** 42		0.829*** 42	0.828*** 20	-0.523** 37	0.058 37
FVC SDS	0.649*** 42	0.829*** 42		0.462* 20	-0.160 37	0.158 37
FEF_{25-75} SDS	0.432 20	0.828*** 20	0.462* 20		-0.684** 19	-0.149 19
LCI	-0.182 37	-0.523** 37	-0.160 37	-0.684** 19		0.191 39
FRC	0.524** 37	0.058 37	0.158 37	-0.149 19	0.191 39	
Extent bronchiectasis	-0.121 32	-0.286 32	-0.171 32	-0.792** 12	0.268 30	0.091 30
Severity bronchiectasis	-0.241 32	-0.264 32	-0.305 32	-0.298 12	0.050 30	-0.201 30
Wall thickness	-0.328 32	-0.512** 32	-0.481** 32	-0.556 12	0.151 30	-0.229 30
Air trapping	-0.061 31	-0.306 31	-0.050 31	-0.387 11	0.485** 29	0.103 29
Small mucus plugs	-0.213 32	-0.351* 32	-0.150 32	-0.574 12	0.347 30	0.094 30
Large mucus plugs	-0.157 32	-0.413* 32	-0.442* 32	-0.598* 12	0.258 30	0.015 30
Consolidated lung	-0.163 32	-0.154 32	-0.287 32	0.249 12	-0.092 30	-0.237 30
Ground glass	-0.133	-0.168	-0.234	-0.469	-0.082	-0.348

	32	32	32	12	30	30
White cell count	-0.137 40	-0.260 40	-0.275 40	-0.175 20	0.280 37	-0.088 37
CRP⁺	-0.165 40	-0.248 40	-0.291 40	-0.165 20	-0.087 37	-0.152 37
Serum IL-6⁺	-0.168 34	-0.245 34	-0.225 34	-0.257 17	-0.173 32	-0.065 32
Serum calprotectin⁺	-0.183 37	-0.392* 37	-0.399* 37	-0.301 19	0.338* 35	0.029 35
Serum IL-8⁺	0.423* 35	0.296 35	0.245 35	-0.024 19	-0.239 33	0.279 33
Serum TNFα	0.074 34	0.122 34	-0.033 34	0.069 17	-0.358* 32	0.035 32
Sputum 24 hr weight⁺	-0.010 20	-0.450* 20	-0.217 20	0.a 0	0.462* 19	0.447 19
Sputum % dry weight	0.103 28	-0.227 28	-0.160 28	-0.136 9	0.245 26	0.554** 26
Sputum total cell count⁺	-0.162 30	-0.262 30	-0.130 30	-0.228 10	0.305 28	-0.114 28
Sputum IL-12	0.168 34	0.125 34	0.015 34	0.162 17	-0.316 33	-0.067 33
Sputum MMP9⁺	0.197 38	0.247 38	0.155 38	0.270 18	-0.057 36	0.240 36
Sputum calprotectin⁺	-0.011 38	-0.172 38	-0.134 38	-0.251 18	0.065 37	0.099 37
Sputum IL-8	0.078 37	0.095 37	0.022 37	-0.114 18	0.085 36	0.145 36
Sputum TNFα⁺	0.108 36	-0.060 36	0.061 36	-0.431 16	-0.165 35	0.367* 35
Sputum neutrophil elastase	0.033 39	-0.129 39	-0.116 39	-0.341 19	0.113 37	0.138 37
Sputum MPO⁺	-0.103 37	-0.193 37	-0.105 37	-0.145 18	0.285 36	0.100 36
Sputum RANTES⁺	0.105 36	0.045 36	0.175 36	-0.339 16	0.054 35	0.163 35
Sputum TIMP1⁺	0.191 36	0.310 36	0.149 36	-0.069 16	-0.388* 35	0.147 35
Sputum IL-1β⁺	0.169 34	0.124 34	0.043 34	0.126 17	-0.247 33	-0.140 33
EBC pH	-0.088 41	-0.051 41	0.027 41	-0.152 20	0.123 38	-0.061 38
EBC nitrite	0.046 39	0.146 39	0.260 39	-0.108 19	0.078 36	-0.069 36
EBC NH_4^+	0.055	0.067	0.021	-0.179	0.299	0.082

	40	40	40	19	37	37
Sputum DNA	0.239 28	0.134 28	0.203 28	0.381 9	-0.029 26	0.074 26
Sputum viscosity	-0.056 25	-0.266 25	-0.220 25	-0.228 7	0.307 23	0.602** 23
Sputum elasticity	-0.044 25	-0.243 25	-0.211 25	-0.186 7	0.297 23	0.596** 23

Table E7: Lung structure

	Extent bronchiect	Severity bronchiect.	Wall thickness	Air trapping	Small mucus plugs	Large mucus plugs	Consol-idated lung	Ground glass
Symptom score	0.018 33	0.187 33	-0.144 33	-0.109 32	-0.202 33	-0.161 33	-0.238 33	-0.199 33
Weight	-0.146 33	-0.182 33	-0.078 33	0.110 32	-0.165 33	-0.084 33	0.034 33	0.190 33
Heart rate	-0.038 34	-0.136 34	0.092 34	0.124 33	0.201 34	0.185 34	0.073 34	0.088 34
Respiratory rate	-0.095 33	-0.377* 33	-0.373* 33	0.003 32	0.003 33	-0.252 33	-0.125 33	-0.264 33
O_2 saturation	0.062 34	0.246 34	0.114 34	-0.618*** 33	-0.304 34	0.041 34	-0.062 34	-0.271 34
systolic BP	-0.112 34	-0.305 34	-0.257 34	-0.047 33	0.073 34	-0.133 34	-0.317 34	-0.251 34
diastolic BP	-0.031 34	-0.124 34	-0.080 34	-0.034 33	-0.087 34	-0.118 34	-0.209 34	-0.156 34
FEV_1	-0.121 32	-0.241 32	-0.328 32	-0.061 31	-0.213 32	-0.157 32	-0.163 32	-0.133 32
FEV_1 SDS	-0.286 32	-0.264 32	-0.512** 32	-0.306 31	-0.351* 32	-0.413* 32	-0.154 32	-0.168 32
FVC SDS	-0.171 32	-0.305 32	-0.481** 32	-0.050 31	-0.150 32	-0.442* 32	-0.287 32	-0.234 32
FEF_{25-75} SDS	-0.792** 12	-0.298 12	-0.556 12	-0.387 11	-0.574 12	-0.598* 12	0.249 12	-0.469 12
LCI	0.268 30	0.050 30	0.151 30	0.485** 29	0.347 30	0.258 30	-0.092 30	-0.082 30
FRC	0.091 30	-0.201 30	-0.229 30	0.103 29	0.094 30	0.015 30	-0.237 30	-0.348 30
Extent bronchiectasis		0.588** 34	0.517** 34	0.030 33	0.356* 34	0.638*** 34	0.075 34	0.228 34
Severity bronchiectasis	0.588** 34		0.737*** 34	-0.288 33	-0.106 34	0.502** 34	0.077 34	0.193 34
Wall thickness	0.517** 34	0.737*** 34		0.028 33	0.162 34	0.724*** 34	0.300 34	0.301 34
Air trapping	0.030 33	-0.288 33	0.028 33		0.330 33	0.080 33	0.025 33	0.151 33
Small mucus plugs	0.356* 34	-0.106 34	0.162 34	0.330 33		0.341* 34	0.000 34	0.018 34
Large mucus plugs	0.638*** 34	0.502** 34	0.724*** 34	0.080 33	0.341* 34		0.187 34	0.150 34
Consolidated lung	0.075 34	0.077 34	0.300 34	0.025 33	0.000 34	0.187 34		0.489** 34
Ground glass	0.228 34	0.193 34	0.301 34	0.151 33	0.018 34	0.150 34	0.489** 34	

White cell count	0.193 32	0.031 32	0.137 32	0.410* 31	0.094 32	0.282 32	-0.009 32	-0.026 32
CRP⁺	0.215 32	-0.117 32	0.288 32	0.317 31	0.496** 32	0.315 32	0.355* 32	0.485** 32
Serum IL-6⁺	-0.173 29	-0.379* 29	-0.126 29	0.350 28	0.306 29	0.029 29	-0.133 29	0.073 29
Serum calprotectin⁺	0.288 30	0.055 30	0.227 30	0.316 29	0.295 30	0.299 30	0.308 30	0.459* 30
Serum IL-8⁺	0.051 28	-0.137 28	-0.203 28	-0.034 27	0.015 28	-0.086 28	0.124 28	0.093 28
Serum TNFα	-0.197 29	-0.063 29	-0.253 29	0.014 28	-0.312 29	-0.046 29	-0.211 29	-0.245 29
Sputum 24 hr weight⁺	0.467* 21	0.312 21	0.445* 21	0.073 21	0.100 21	0.317 21	0.052 21	-0.067 21
Sputum % dry weight	0.020 27	-0.258 27	-0.132 27	0.440* 26	0.237 27	0.101 27	-0.005 27	-0.112 27
Sputum total cell count⁺	-0.009 28	0.085 28	0.271 28	0.277 27	0.349 28	0.409* 28	0.093 28	0.308 28
Sputum IL-12	-0.057 28	-0.008 28	-0.113 28	-0.328 27	-0.149 28	-0.097 28	-0.054 28	0.055 28
Sputum MMP9⁺	-0.108 33	-0.141 33	-0.051 33	0.133 32	-0.003 33	0.130 33	-0.061 33	0.064 33
Sputum calprotectin⁺	0.322 32	0.032 32	0.246 32	0.490** 31	0.279 32	0.273 32	-0.022 32	0.260 32
Sputum IL-8	0.115 31	-0.091 31	-0.137 31	0.135 30	0.025 31	-0.033 31	-0.104 31	0.009 31
Sputum TNFα⁺	0.238 32	-0.050 32	0.109 32	0.157 31	0.237 32	0.132 32	-0.098 32	0.029 32
Sputum neutrophil elastase	0.151 33	-0.143 33	-0.125 33	0.166 32	0.303 33	-0.026 33	-0.229 33	-0.053 33
Sputum MPO⁺	0.096 31	-0.057 31	0.145 31	0.367* 30	0.240 31	0.239 31	-0.127 31	0.183 31
Sputum RANTES⁺	0.199 32	0.045 32	-0.016 32	-0.220 31	-0.016 32	-0.095 32	0.020 32	0.049 32
Sputum TIMP1⁺	0.016 32	-0.003 32	-0.170 32	-0.581** 31	-0.302 32	-0.119 32	-0.132 32	-0.088 32
Sputum IL-1β⁺	-0.123 28	0.021 28	-0.051 28	-0.326 27	-0.199 28	-0.149 28	-0.017 28	0.057 28
EBC pH	0.218 33	0.237 33	0.185 33	0.048 32	0.200 33	0.293 33	-0.020 33	0.124 33
EBC nitrite	-0.212 30	-0.243 30	-0.106 30	0.007 29	0.090 30	-0.255 30	0.180 30	0.035 30
EBC NH_4^+	0.036 33	0.234 33	-0.013 33	-0.015 32	-0.205 33	0.096 33	-0.113 33	0.026 33

Sputum DNA	-0.078 27	0.045 27	0.062 27	-0.128 26	-0.053 27	-0.116 27	-0.241 27	-0.231 27
Sputum viscosity	-0.042 26	-0.331 26	-0.161 26	0.458* 25	0.272 26	0.043 26	-0.090 26	-0.108 26
Sputum elasticity	-0.020 26	-0.311 26	-0.133 26	0.441* 25	0.288 26	0.057 26	-0.104 26	-0.113 26

Table E8: Serum inflammatory markers

	White cell count	**CRP⁺**	**Serum IL-6⁺**	**Serum calprotectin⁺**	**Serum IL-8⁺**	**Serum TNFα**
Symptom score	-0.192 41	-0.554** 41	-0.399* 35	-0.329* 38	0.140 36	0.151 35
Weight	0.028 41	0.104 41	0.108 35	0.195 38	0.073 36	0.141 35
Heart rate	0.108 42	0.258 42	0.284 36	0.088 39	-0.176 37	-0.215 36
Respiratory rate	0.155 40	-0.055 40	0.239 34	-0.090 37	0.204 35	0.212 34
O_2 saturation	-0.132 42	-0.298 42	-0.067 36	-0.190 39	0.076 37	0.310 36
systolic BP	-0.062 42	-0.258 42	-0.240 36	-0.082 39	0.075 37	0.034 36
diastolic BP	-0.222 42	-0.361* 42	-0.478** 36	-0.319* 39	0.006 37	0.000 36
FEV_1	-0.137 40	-0.165 40	-0.168 34	-0.183 37	0.423* 35	0.074 34
FEV_1 SDS	-0.260 40	-0.248 40	-0.245 34	-0.392* 37	0.296 35	0.122 34
FVC SDS	-0.275 40	-0.291 40	-0.225 34	-0.399* 37	0.245 35	-0.033 34
FEF_{25-75} SDS	-0.175 20	-0.165 20	-0.257 17	-0.301 19	-0.024 19	0.069 17
LCI	0.280 37	-0.087 37	-0.173 32	0.338* 35	-0.239 33	-0.358* 32
FRC	-0.088 37	-0.152 37	-0.065 32	0.029 35	0.279 33	0.035 32
Extent bronchiectasis	0.193 32	0.215 32	-0.173 29	0.288 30	0.051 28	-0.197 29
Severity bronchiectasis	0.031 32	-0.117 32	-0.379* 29	0.055 30	-0.137 28	-0.063 29
Wall thickness	0.137 32	0.288 32	-0.126 29	0.227 30	-0.203 28	-0.253 29
Air trapping	0.410* 31	0.317 31	0.350 28	0.316 29	-0.034 27	0.014 28

Small mucus plugs	0.094 32	0.496** 32	0.306 29	0.295 30	0.015 28	-0.312 29
Large mucus plugs	0.282 32	0.315 32	0.029 29	0.299 30	-0.086 28	-0.046 29
Consolidated lung	-0.009 32	0.355* 32	-0.133 29	0.308 30	0.124 28	-0.211 29
Ground glass	-0.026 32	0.485** 32	0.073 29	0.459* 30	0.093 28	-0.245 29
White cell count		0.223 42	0.239 36	0.596*** 38	-0.418* 36	0.079 36
CRP⁺	0.223 42		0.517** 36	0.665*** 38	-0.087 36	-0.220 36
Serum IL-6⁺	0.239 36	0.517** 36		0.410* 36	-0.047 34	0.366* 36
Serum calprotectin⁺	0.596*** 38	0.665*** 38	0.410* 36		-0.134 37	0.043 36
Serum IL-8⁺	-0.418* 36	-0.087 36	-0.047 34	-0.134 37		0.118 34
Serum TNFα	0.079 36	-0.220 36	0.366* 36	0.043 36	0.118 34	
Sputum 24 hr weight⁺	0.264 20	0.057 20	-0.006 18	0.373 19	-0.186 18	-0.126 18
Sputum % dry weight	0.245 28	-0.022 28	0.432* 25	0.252 27	-0.173 25	0.316 25
Sputum total cell count⁺	-0.037 30	0.215 30	0.106 25	0.278 28	-0.256 27	-0.192 25
Sputum IL-12	-0.202 36	0.047 36	-0.095 34	-0.112 36	0.138 34	-0.046 34
Sputum MMP9⁺	0.085 38	0.067 38	0.007 35	0.203 36	-.077 34	0.022 35
Sputum calprotectin⁺	0.223 39	0.291 39	0.272 34	0.192 36	-0.224 34	-0.068 34
Sputum IL-8	0.188 38	-0.090 38	-0.163 34	0.013 36	-0.123 34	0.025 34
Sputum TNFα⁺	0.096 36	0.237 36	0.350* 33	0.200 34	0.019 32	-0.254 33
Sputum neutrophil elastase	0.265 40	0.153 40	0.296 35	0.126 37	-0.164 35	0.061 35
sputum MPO⁺	0.260 38	0.196 38	0.086 34	0.203 36	-0.301 34	-0.234 34
Sputum RANTES⁺	-0.334* 36	-0.336* 36	-0.226 33	-0.193 34	0.276 32	0.083 33
	-0.217	-0.116	-0.072	-0.052	0.224	0.226

Sputum TIMP1^{+}	36	36	33	34	32	33
Sputum IL-1β^{+}	-0.199 36	0.046 36	-0.154 34	-0.144 36	0.080 34	-0.188 34
EBC pH	0.380* 41	0.330* 41	0.346* 35	0.429** 38	-0.384* 37	0.063 35
EBC nitrite	-0.182 38	-0.039 38	-0.147 32	-0.236 35	0.130 34	-0.193 32
EBC NH_4^+	0.200 40	-0.115 40	-0.014 34	0.153 37	-0.193 36	0.010 34
Sputum DNA	0.139 28	0.054 28	-0.072 25	-0.038 27	-0.331 25	-0.175 25
Sputum viscosity	0.164 25	0.130 25	0.548** 22	0.415* 24	-0.013 22	0.292 22
Sputum elasticity	0.158 25	0.132 25	0.482* 22	0.381 24	-0.037 22	0.257 22

Table E9: Sputum and sputum inflammatory markers

	24 hr weight [+]	Sptm % dry weight	Sptm total cell count[+]	IL-12	MMP9[+]	Calpro-tectin[+]	IL-8	Sputum TNFα[+]	NE	MPO[+]	RANTES[+]	TIMP1[+]	IL-1β[+]
Symptom score	-0.091 22	-0.070 29	-0.274 32	0.286 35	-0.053 39	-0.117 39	0.110 38	-0.206 37	-0.050 40	-0.143 38	0.536** 37	0.064 37	0.253 35
Weight	0.375 22	0.434* 29	0.165 31	-0.177 35	0.056 39	0.099 39	0.209 38	0.313 37	0.317* 40	0.268 38	-0.003 37	0.135 37	-0.181 35
Heart rate	0.023 22	0.251 30	0.086 32	0.198 36	0.073 40	0.121 40	-0.201 39	0.152 38	-0.037 41	0.019 39	-0.261 38	-0.170 38	0.179 36
Respira-tory rate	-0.029 22	0.258 28	-0.413* 32	0.240 34	-0.216 38	-0.023 38	0.138 37	0.010 36	0.101 39	-0.087 37	0.253 36	0.304 36	0.145 34
O_2 saturation	0.277 22	-0.189 30	-0.385* 32	0.150 36	-0.093 40	-0.269 40	-0.002 39	0.044 38	-0.103 41	-0.294 39	0.216 38	0.264 38	0.124 36
systolic BP	-0.082 22	0.123 30	-0.290 32	0.150 36	0.093 40	-0.060 40	0.280 39	-0.021 38	0.244 41	0.110 39	0.278 38	0.280 38	0.134 36
diastolic BP	-0.050 22	0.111 30	-0.162 32	0.091 36	-0.81 40	-0.018 40	0.248 39	-0.150 38	0.215 41	0.124 39	0.203 38	0.185 38	0.080 36
FEV_1	-0.010 20	0.103 28	-0.162 30	0.168 34	0.197 38	-0.011 38	0.078 37	0.108 36	0.033 39	-0.103 37	0.105 36	0.191 36	0.169 34
FEV_1 SDS	-0.450* 20	-0.227 28	-0.262 30	0.125 34	0.245 38	-0.172 38	0.095 37	-0.060 36	-0.129 39	-0.193 37	0.045 36	0.310 36	0.124 34
FVC SDS	-0.217 20	-0.160 28	-0.130 30	0.015 34	0.155 38	-0.134 38	0.022 37	0.061 36	-0.116 39	-0.105 37	0.175 36	0.149 36	0.043 34
FEF_{25-75} SDS	0.a 0	-0.136 9	-0.228 10	0.162 17	0.270 18	-0.251 18	-0.114 18	-0.431 16	-0.341 19	-0.145 18	-0.339 16	-0.069 16	0.126 17
LCI	0.462* 19	0.245 26	0.305 28	-0.316 33	-0.057 36	0.065 37	0.085 36	0.165 35	0.113 37	0.285 36	0.054 35	-0.388* 35	-0.247 33
FRC	0.447 19	0.554** 26	-0.114 28	-0.067 33	0.240 36	0.099 37	0.145 36	0.367* 35	0.138 37	0.100 36	0.163 35	0.147 35	-0.140 33
Extent bronchiect asis	0.467* 21	0.020 27	-0.009 28	-0.057 28	-0.108 33	0.322 32	0.115 31	0.238 32	0.151 33	0.096 31	0.199 32	0.016 32	-0.123 28
Severity bronchiect asis	0.312 21	-0.258 27	0.085 28	-0.008 28	-0.141 33	0.032 32	-0.091 31	-0.050 32	-0.143 33	-0.057 31	0.045 32	-0.003 32	0.021 28
Wall thickness	0.445* 21	-0.132 27	0.271 28	-0.113 28	-0.051 33	0.246 32	-0.137 31	0.109 32	-0.125 33	0.145 31	-0.016 32	-0.170 32	-0.051 28
Air trapping	0.073 21	0.440* 26	0.277 27	-0.328 27	0.133 32	0.490** 31	0.135 30	0.157 31	0.166 32	0.367* 30	-0.220 31	-0.581** 31	-0.326 27
Small mucus plugs	0.100 21	0.237 27	0.349 28	-0.149 28	0.003 33	0.279 32	0.025 31	0.237 32	0.303 33	0.240 31	-0.016 32	-0.302 32	-0.199 28
Large mucus plugs	0.317 21	0.101 27	0.409* 28	-0.097 28	0.130 33	0.273 32	-0.033 31	0.132 32	-0.026 33	0.239 31	-0.095 32	-0.119 32	-0.149 28

Consolid lung	0.052 21	-0.005 27	0.093 28	-0.054 28	-0.061 33	-0.022 32	-0.104 31	-0.098 32	-0.229 33	-0.127 31	0.020 32	-0.132 32	-0.017 28
Ground glass	-0.067 21	-0.112 27	0.308 28	0.055 28	0.064 33	0.260 32	0.009 31	0.029 32	-0.053 33	0.183 31	0.049 32	-0.088 32	0.057 28
White cell count	0.264 20	0.245 28	-0.037 30	-0.202 36	0.085 38	0.223 39	0.188 38	0.096 36	0.265 40	0.260 38	-0.334* 36	-0.217 36	-0.199 36
CRP⁺	0.057 20	-0.022 28	0.215 30	0.047 36	0.067 38	0.291 39	-0.090 38	0.237 36	0.153 40	0.196 38	-0.336* 36	-0.116 36	0.046 36
Serum IL-6⁺	-0.006 18	0.432* 25	0.106 25	-0.095 34	0.007 35	0.272 34	-0.163 34	0.350* 33	0.296 35	0.086 34	-0.226 33	-0.072 33	-0.154 34
Serum calpro-tectin⁺	0.373 19	0.252 27	0.278 28	-0.112 36	0.203 36	0.192 36	0.013 36	0.200 34	0.126 37	0.203 36	-0.193 34	-0.052 34	-0.144 36
Serum IL-8⁺	-0.186 18	-0.173 25	-0.256 27	0.138 34	-0.077 34	-0.224 34	-0.123 34	0.019 32	-0.164 35	-0.301 34	0.276 32	0.224 32	0.080 34
Serum TNFα	-0.126 18	0.316 25	-0.192 25	-0.046 34	0.022 35	-0.068 34	0.025 34	-0.254 33	0.061 35	-0.234 34	0.083 33	0.226 33	-0.188 34
Sputum 24 hr weight⁺		0.438 20	-0.039 22	-0.301 18	0.009 21	0.289 21	0.244 20	0.557** 21	0.329 21	0.244 20	0.341 21	-0.193 21	-0.332 18
Sputum % dry weight	0.438 20		-0.007 24	-0.545** 24	0.198 28	0.572** 27	0.471* 26	0.307 26	0.394* 29	0.412* 26	0.146 26	0.079 26	-0.601** 24
Sputum total cell count⁺	-0.039 22	-0.007 24		-0.174 26	0.213 29	0.146 30	-0.164 29	0.034 28	-0.124 31	0.403* 29	-0.405* 28	-0.480** 28	-0.197 26
Sputum IL-12	-0.301 18	-0.545** 24	-0.174 26		-0.206 33	-0.100 36	-0.218 36	-0.340 33	-0.092 35	-0.328 36	-0.087 33	0.147 33	0.956*** 36
Sputum MMP9⁺	0.009 21	0.198 28	0.213 29	-0.206 33		0.557** 37	0.424** 36	0.226 38	-0.252 38	0.403* 36	-0.290 38	0.164 38	-0.238 33
Sputum calpro-tectin⁺	0.289 21	0.572** 27	0.146 30	-0.100 36	0.579** 37		0.536** 39	0.266 38	0.611*** 39	0.629*** 39	-0.142 37	-0.105 37	-0.165 36
Sputum IL-8	0.244 20	0.471* 26	-0.164 29	-0.218 36	0.424** 36	0.536** 39		0.058 36	0.752*** 38	0.582** 39	0.082 36	0.185 36	-0.261 36
Sputum TNFα⁺	0.557** 21	0.307 26	0.034 28	-0.340 33	0.226 38	0.266 37	0.058 36		0.070 36	0.286 36	0.118 38	-0.236 38	-0.363* 33
Sputum neutrophil elastase	0.329 21	0.394* 29	-0.124 31	-0.092 35	-0.252 38	0.611*** 39	0.752*** 38	0.070 36		0.646*** 38	-0.010 36	0.018 36	-0.166 35
Sputum MPO⁺	0.244 20	0.412* 26	0.403* 29	-0.328 36	0.403* 36	0.629*** 39	0.582** 39	0.286 36	0.646*** 38		-0.077 36	-0.129 36	-0.381* 36
Sputum RANTES⁺	0.341 21	0.146 26	-0.405* 28	-0.087 33	-0.290 38	-0.142 37	0.082 36	0.118 38	-0.010 36	-0.077 36		0.279 38	-0.152 33
Sputum TIMP1⁺	-0.193 21	0.079 26	-0.480** 28	0.147 33	0.164 38	-0.105 37	0.185 36	-0.236 38	0.018 36	-0.129 36	0.279 38		0.115 33
Sputum IL-1β⁺	-0.332 18	-0.601** 24	-0.197 26	0.956*** 36	-0.238 33	-0.165 36	-0.261 36	-0.363* 33	-0.166 35	-0.381* 36	-0.152 33	0.115 33	
EBC pH	-0.080 22	-0.030 29	0.248 32	-0.211 35	0.099 39	0.082 39	-0.136 38	0.145 37	0.062 40	0.164 38	-0.077 37	-0.049 37	-0.220 35

EBC nitrite	-0.267 20	-0.271 26	0.147 29	0.089 33	-0.385* 36	-0.315 37	-0.117 36	-0.094 35	-0.148 37	-0.225 36	0.008 35	-0.334 35	0.115 33
EBC NH_4^+	-0.063 22	0.273 28	0.165 32	-0.282 34	0.263 38	0.167 38	0.395* 37	0.086 36	0.041 39	0.186 37	-0.228 36	0.064 36	-0.234 34
Sputum DNA	0.249 20	-0.291 30	0.272 24	0.037 24	0.048 28	-0.192 27	-0.204 26	-0.069 26	-0.061 29	-0.150 26	-0.317 26	0.116 26	0.134 24
Sputum viscosity	0.213 19	0.825*** 27	-0.099 23	-0.439* 22	0.508** 25	0.433* 25	0.382 24	0.134 24	0.307 26	0.226 24	-0.139 24	0.136 24	-0.492* 22
Sputum elasticity	0.190 19	0.790*** 27	-0.140 23	-0.402 22	0.493* 25	0.428* 25	0.413* 24	0.093 24	0.348 26	0.220 24	-0.160 24	0.179 24	-0.437* 22

Table E10: EBC and sputum rheology

	EBC pH	EBC nitrite	EBC NH_4^+	Sputum DNA	Sputum viscosity	Sputum elasticity
Symptom score	-0.124 42	-0.027 39	-0.141 41	-0.161 29	-0.292 26	-0.303 26
Weight	0.015 42	-0.161 39	0.243 41	0.032 29	0.131 26	0.134 26
Heart rate	-0.046 43	0.034 40	-0.077 42	-0.252 30	0.335 27	0.336 27
Respiratory rate	-0.095 41	0.158 38	-0.212 41	-0.275 28	0.147 26	0.114 26
O_2 saturation	-0.068 43	-0.036 40	-0.193 42	0.036 30	-0.344 27	-0.345 27
systolic BP	-0.097 43	-0.134 40	-0.111 42	0.021 30	0.140 27	0.202 27
diastolic BP	-0.266 43	0.064 40	-0.242 42	-0.165 30	-0.076 27	-0.017 27
FEV_1	-0.088 41	0.046 39	0.055 40	0.233 28	-0.056 25	-0.044 25
FEV_1 SDS	-0.051 41	0.146 39	0.067 40	0.150 28	-0.266 25	-0.243 25
FVC SDS	0.027 41	0.260 39	0.021 40	0.222 28	-0.220 25	-0.211 25
FEF_{25-75} SDS	-0.152 20	-0.108 19	-0.179 19	0.375 9	-0.228 7	-0.186 7
LCI	0.123 38	0.078 36	0.299 37	-0.020 26	0.307 23	0.297 23
FRC	-0.061 38	-0.069 36	0.082 37	0.080 26	0.602** 23	0.596** 23
Extent bronchiectasis	0.218 33	-0.212 30	0.036 33	-0.064 27	-0.042 26	-0.020 26
Severity bronchiectasis	0.237 33	-0.243 30	0.234 33	0.061 27	-0.331 26	-0.311 26
Wall thickness	0.185 33	-0.106 30	-0.013 33	0.080 27	-0.161 26	-0.133 26
Air trapping	0.048 32	0.007 29	-0.015 32	-0.106 26	0.458* 25	0.441* 25
Small mucus plugs	0.200 33	0.090 30	-0.205 33	-0.069 27	0.272 26	0.288 26
Large mucus plugs	0.293 33	-0.255 30	0.096 33	-0.096 27	0.043 26	0.057 26
Consolidated lung	-0.020 33	0.180 30	-0.113 33	-0.229 27	-0.090 26	-0.104 26
Ground glass	0.124 33	0.035 30	0.026 33	-0.234 27	-0.108 26	-0.113 26

White cell count	0.380* 41	-0.182 38	0.200 40	0.124 28	0.164 25	0.158 25
CRP⁺	0.330* 41	-0.039 38	-0.115 40	0.039 28	0.130 25	0.132 25
Serum IL-6⁺	0.346* 35	-0.147 32	-0.014 34	-0.112 25	0.548** 22	0.482* 22
Serum calprotectin⁺	0.429** 38	-0.236 35	0.153 37	-0.040 27	0.415* 24	0.381 24
Serum IL-8⁺	-0.384* 37	0.130 34	-0.193 36	-0.303 25	-0.013 22	-0.037 22
Serum TNFα	0.063 35	-0.193 32	0.010 34	-0.176 25	0.292 22	0.257 22
Sputum 24 hr weight⁺	-0.080 22	-0.267 20	-0.063 22	0.258 20	0.213 19	0.190 19
Sputum % dry weight	-0.030 29	-0.271 26	0.273 28	-0.311 30	0.825*** 27	0.790*** 27
Sputum total cell count⁺	0.248 32	0.147 29	0.165 32	0.294 24	-0.099 23	-0.140 23
Sputum IL-12	-0.211 35	0.089 33	-0.282 34	0.034 24	-0.439* 22	-0.402 22
Sputum MMP9⁺	0.099 39	-0.385* 36	0.263 38	0.111 28	0.508** 25	0.493* 25
Sputum calprotectin	0.082 39	-0.315 37	0.167 38	-0.191 27	0.433* 25	0.428* 25
Sputum IL-8	-0.136 38	-0.117 36	0.395* 37	-0.205 26	0.382 24	0.413* 24
Sputum TNFα⁺	0.145 37	-0.094 35	0.086 36	-0.059 26	0.134 24	0.093 24
Sputum neutrophil elastase	0.062 40	-0.148 37	0.041 39	-0.160 29	0.307 26	0.348 26
Sputum MPO⁺	0.164 38	-0.225 36	0.186 37	-0.149 26	0.226 24	0.220 24
Sputum RANTES⁺	-0.077 37	0.008 35	-0.228 36	-0.316 26	-0.139 24	-0.160 24
Sputum TIMP1⁺	-0.049 37	-0.334 35	0.064 36	0.114 26	0.136 24	0.179 24
Sputum IL-1β⁺	-0.220 35	0.115 33	-0.234 34	0.131 24	-0.492* 22	-0.437* 22
EBC pH		-0.106 40	0.199 42	0.173 29	-0.147 26	-0.148 26
EBC nitrite	-0.106 40		-0.162 39	-0.049 26	-0.152 23	-0.133 23
EBC NH_4^+	0.199 42	-0.162 39		0.110 28	0.291 26	0.304 26

Sputum DNA	0.204 29	-0.047 26	0.062 28		-0.183 27	-0.145 27
Sputum viscosity	-0.147 26	-0.152 23	0.291 26	-0.192 27		0.990*** 27
Sputum elasticity	-0.148 26	-0.133 23	0.304 26	-0.157 27	0.990*** 27	

Correlation between change in assays

In order to explore whether assays that showed significant change with treatment reflected the same or different aspects of CF pathophysiology, the following mileage chart of correlations was prepared. Change in assays with statistically significant change between V1 and V2 was compared to change in all other assays. The data are split into two tables to make viewing easier. Correlations are either Pearson r correlation coefficients for parametric data, or Spearman rank correlation coefficients for skewed data (indicated by $^{+}$ next to assay name). Log transformation of non-parametric data was not possible because many of the assays contained both negative and positive change, reflecting the two-tailed nature of response in the assays. Numbers in the table represent correlation coefficient (upper) and number of pairs of data (lower) for each correlation. Boxes shaded in pink, and correlation coefficients identified by *, have a P value <0.5. Boxes shaded in red, and correlation coefficients identified by **, have a P value <0.01. Boxes shaded in purple, and correlation coefficients identified by ***, have a P value <0.0001.

Table E11: Correlation mileage chart of change in assays with significant change against change in all other assays.

	Symptom score	Weight	Heart rate	Resp. rate^{+}	Diastolic BP	FEV_1 SDS^{+}	FVC SDS^{+}	FEF SDS^{+}	LCI	Airway wall thickness^{+}	Air trapping	Small mucus plugs^{+}	Large mucus plugs	Lung consol dn^{+}
Symptom score		0.448* 32	-0.416* 37	-0.055 34	-0.162 37	0.374* 31	0.498* 22	0.046 14	-0.294 31	-0.437* 30	-0.206 29	-0.236 30	-0.400* 30	-0.108 30
Weight	0.448* 32		-0.318 33	-0.190 31	-0.170 33	0.254 27	0.380 18	-0.191 11	-0.155 27	-0.187 28	-0.304 27	-0.320 28	-0.370 28	-0.138 28
Heart rate	-0.416* 37	-0.318 33		0.197 35	0.187 38	-0.465** 32	-0.553** 23	-0.511 15	0.320 32	0.070 31	0.039 30	0.209 31	0.177 31	-0.026 31
Respiratory rate^{+}	-0.055 34	-0.190 31	0.197 35	10.000 35	-0.149 35	-0.310 29	-0.126 20	-0.413 12	0.197 29	0.293 29	0.261 28	-0.052 29	0.090 29	-0.052 29
O_2 saturation	0.059 37	0.317 33	-0.417** 38	0.121 35	0.237 38	0.226 32	0.407 23	0.285 15	-0.094 32	-0.017 31	-0.437* 30	0.016 31	-0.076 31	-0.251 31
systolic BP	-0.226 37	-0.071 33	0.183 38	0.140 35	0.400* 38	-0.179 32	-0.179 23	-0.283 15	0.370* 32	0.114 31	-0.199 30	0.118 31	-0.096 31	0.219 31
diastolic BP	-0.162 37	-0.170 33	0.187 38	-0.149 35		-0.151 32	-0.163 23	0.165 15	0.401* 32	-0.091 31	-0.108 30	0.026 31	-0.095 31	-0.065 31
FEV_1 SDS^{+}	0.374* 31	0.254 27	-0.465** 32	-0.310 29	-0.151 32		0.887*** 23	0.764** 15	-0.488** 28	-0.141 25	-0.363 24	-0.376 25	-0.410* 25	0.065 25
FVC SDS^{+}	0.498* 22	0.380 18	-0.553** 23	-0.126 20	-0.163 23	0.887*** 23		0.429 15	-0.550* 20	-0.526* 17	-0.419 16	-0.395 17	-0.617** 17	0.037 17
FEF_{25-75} SDS^{+}	0.046 14	-0.191 11	-0.511 15	-0.413 12	0.165 15	0.764** 15	0.429 15		-0.429 14	0.093 10	0.067 9	0.260 10	0.094 10	0.166 10
LCI	-0.294 31	-0.155 27	0.320 32	0.197 29	0.401* 32	-0.488** 28	-0.550* 20	-0.429 14		0.193 27	0.307 26	0.318 27	0.427* 27	0.204 27
FRC	-0.016 31	-0.118 27	-0.028 32	-0.498** 29	0.031 32	0.254 28	0.281 20	0.534* 14	-0.488** 32	-0.223 27	-0.018 26	-0.181 27	-0.009 27	0.039 27
Extent bronchiectasis	-0.076 30	-0.401* 28	-0.134 31	-0.112 29	0.024 31	-0.186 25	-0.403 17	0.328 10	0.196 27	0.225 31	0.238 30	0.413* 31	0.371* 31	-0.037 31

Severity bronchiectasis	-0.134 30	-0.143 28	-0.251 31	-0.418* 29	0.211 31	0.099 25	0.122 17	0.732* 10	-0.109 27	0.249 31	-0.057 30	0.397* 31	0.343 31	-0.157 31
Wall thickness⁺	-0.437* 30	-0.187 28	0.070 31	0.293 29	-0.091 31	-0.141 25	-0.526* 17	0.093 10	0.193 27		0.218 30	0.495** 31	0.632** 31	0.312 31
Air trapping	-0.206 29	-0.304 27	0.039 30	0.261 28	-0.108 30	-0.363 24	-0.419 16	0.067 9	0.307 26	0.218 30		0.017 30	0.141 30	0.262 30
Small mucus plugs⁺	-0.236 30	-0.320 28	0.209 31	-0.052 29	0.026 31	-0.376 25	-0.395 17	0.260 10	0.318 27	0.495** 31	0.017 30		0.590** 31	0.127 31
Large mucus plugs	-0.400* 30	-0.370 28	0.177 31	0.090 29	-0.095 31	-0.410* 25	-0.617** 17	0.094 10	0.427* 27	0.632** 31	0.141 30	0.590** 31		0.173 31
Consolidated lung⁺	-0.108 30	-0.138 28	-0.026 31	-0.052 29	-0.065 31	0.065 25	0.037 17	0.166 10	0.204 27	0.312 31	0.262 30	0.127 31	0.173 31	
Ground glass⁺	0.190 30	0.257 28	-0.134 31	-0.013 29	-0.357* 31	0.065 25	-0.160 17	-0.227 10	-0.064 27	0.330 31	-0.137 30	0.134 31	0.298 31	0.392* 31
White cell count	0.156 32	-0.274 28	-0.105 33	0.145 30	-0.001 33	0.090 28	0.186 20	-0.040 14	-0.417* 28	-0.034 28	-0.038 27	-0.220 28	-0.111 28	0.187 28
CRP	-0.473** 33	-0.342 29	0.245 34	0.036 31	-0.172 34	-0.239 28	-0.457* 19	0.209 14	0.080 28	0.202 27	0.164 26	0.189 27	0.221 27	0.007 27
Serum IL-6	-0.308 32	-0.121 28	0.473** 33	0.205 30	-0.119 33	-0.395* 27	-0.400 19	-0.495 14	0.061 28	-0.019 26	0.415* 25	-0.307 26	-0.307 26	-0.217 26
Serum calprotectin	-0.449* 30	-0.425* 26	0.131 31	-0.026 28	-0.075 31	-0.232 26	-0.324 18	0.011 14	0.283 27	0.121 25	0.356 24	0.108 25	0.080 25	0.271 25
Serum IL-8	0.151 28	0.118 24	-0.199 29	0.202 26	-0.208 29	-0.165 24	-0.176 17	0.114 14	-0.196 25	-0.081 23	0.223 22	-0.282 23	-0.214 23	-0.015 23
Serum TNFα	-0.275 32	-0.067 28	-0.118 33	-0.099 30	0.268 33	-0.122 27	-0.372 19	-0.275 14	0.189 28	0.375 26	0.400* 25	-0.053 26	0.138 26	-0.130 26
Sputum 24 hr weight	-0.576* 15	-0.761** 15	0.740** 15	0.161 15	0.444 15	-0.467 9	0. 1	0. 0	0.381 12	-0.151 14	-0.306 14	0.137 14	0.092 14	-0.058 14
Sputum % dry weight	-0.133 15	0.078 15	0.184 15	-0.061 15	0.518* 15	0.117 9	0.100 5	0. 1	0.567 11	0.192 14	-0.097 13	0.309 14	0.119 14	-0.162 14

Sputum total cell count	-0.087 23	-0.197 23	0.494* 23	0.161 23	0.357 23	-0.315 17	-0.601* 12	-0.257 6	-0.013 18	0.303 21	-0.187 20	0.329 21	0.012 21	-0.092 21
Sputum IL-12	-0.076 31	-0.107 27	-0.089 32	0.274 29	-0.500** 32	0.058 26	0.056 19	0.046 14	0.025 27	-0.042 25	-0.077 24	0.128 25	0.211 25	-0.243 25
Sputum MMP9	-0.219 31	0.275 27	0.153 32	0.000 29	0.029 32	-0.276 26	-0.020 22	-0.332 15	0.145 27	-0.061 26	0.298 25	-0.167 26	-0.099 26	0.052 26
Sputum calprotectin	-0.073 31	0.131 27	0.281 32	0.088 29	0.124 32	-0.258 27	-0.309 22	-0.407 15	0.297 27	0.203 26	0.312 25	0.040 26	0.125 26	-0.075 26
Sputum IL-8	-0.251 30	0.437* 26	0.129 31	-0.182 28	0.254 31	-0.137 26	-0.168 21	-0.007 15	0.348 26	0.095 25	0.120 24	0.033 25	0.143 25	0.013 25
Sputum neutrophil elastase	-0.247 32	0.191 28	0.027 33	0.170 30	0.289 33	-0.358 27	-0.251 22	-0.257 15	0.267 28	0.313 27	0.249 26	0.023 27	0.041 27	-0.113 27
sputum MPO	-0.102 30	0.359 26	0.268 31	-0.067 28	0.122 31	-0.390* 26	-0.600** 21	-0.489 15	0.295 26	0.146 25	0.105 24	-0.048 25	-0.012 25	-0.208 25
Sputum RANTES	0.266 25	0.023 22	-0.175 26	-0.082 23	0.217 26	0.238 21	0.390 17	-0.073 14	-0.034 23	-0.424 21	-0.385 20	-0.372 21	-0.572** 21	-0.207 21
Sputum TIMP1^{+}	0.186 27	0.148 23	-0.302 28	-0.085 25	0.103 28	0.201 24	0.120 22	0.130 15	0.048 24	-0.160 22	-0.357 21	-0.231 22	-0.365 22	0.268 22
Sputum IL-1β	-0.230 30	0.146 26	0.299 31	0.060 28	0.225 31	-0.270 25	-0.200 21	-0.402 14	0.244 27	0.133 25	0.391 24	-0.072 25	0.167 25	0.147 25
EBC pH^{+}	0.065 36	0.175 32	0.026 37	-0.119 34	-0.251 37	0.300 31	0.316 22	0.043 15	0.006 31	0.093 30	0.090 29	-0.007 30	-0.088 30	-0.141 30
EBC nitrite^{+}	-0.005 34	0.205 30	0.160 35	-0.013 32	0.090 35	-0.180 29	-0.122 21	0.018 15	0.213 29	-0.009 28	-0.150 27	0.066 28	0.147 28	-0.122 28
EBC NH_4	0.348* 35	0.098 31	-0.202 36	-0.264 34	-0.112 36	0.038 30	0.135 21	-0.011 14	-0.185 30	-0.153 30	0.079 29	-0.085 30	-0.095 30	0.275 30
Sputum DNA	-0.159 15	0.06 15	-0.229 15	-0.458 15	-0.007 15	0.217 9	0.600 5	0. 1	-0.322 11	-0.056 14	0.117 13	-0.091 14	-0.198 14	-0.355 14
Sputum viscosity^{+}	-0.068 14	0.252 14	-0.042 14	-0.296 14	0.104 14	-0.203 9	0.000 5	0. 1	0.306 10	0.091 13	0.062 12	0.140 13	-0.106 13	-0.003 13

Sputum elasticity⁺	-0.097 14	0.176 14	-0.121 14	-0.259 14	0.062 14	-0.117 9	0.000 5	0. 1	0.224 10	0.243 13	0.007 12	0.210 13	0.070 13	0.100 13

	WBC	**CRP**	**Serum IL-6**	**Serum calprotectin**	**Sputum 24hr weight**	**Sputum total cell count**	**Sputum MMP9**	**Sputum TIMP1⁺**	**Sputum IL-1β**	**EBC pH⁺**
Symptom score	0.156 32	-0.473** 33	-0.308 32	-0.449* 30	-0.576* 15	-0.087 23	-0.219 31	0.115 27	-0.230 30	0.065 36
Weight	-0.274 28	-0.342 29	-0.121 28	-0.425* 26	-0.761** 15	-0.197 23	0.275 27	0.096 23	0.146 26	0.175 32
Heart rate	-0.105 33	0.245 34	0.473** 33	0.131 31	0.740** 15	0.494* 23	0.153 32	-0.354 28	0.299 31	0.026 37
Respiratory rate⁺	0.145 30	0.036 31	0.205 30	-0.026 28	0.161 15	0.161 23	0.000 29	-0.085 25	0.060 28	-0.119 34
O_2 saturation	-0.180 33	-0.230 34	-0.207 33	-0.315 31	0.162 15	-0.184 23	0.024 32	0.185 28	0.075 31	-0.055 37
Systolic BP	-0.108 33	-0.050 34	-0.013 33	0.007 31	0.399 15	0.046 23	0.081 32	0.246 28	-0.028 31	-0.202 37
Diastolic BP	-0.001 33	-0.172 34	-0.119 33	-0.075 31	0.444 15	0.357 23	0.029 32	0.176 28	0.225 31	-0.251 37
FEV_1 SDS⁺	0.090 28	-0.239 28	-0.395* 27	-0.232 26	-0.467 9	-0.315 17	-0.276 26	0.201 24	-0.270 25	0.300 31
FVC SDS⁺	0.186 20	-0.457* 19	-0.400 19	-0.324 18	0. 1	-0.601* 12	-0.020 22	0.120 22	-0.200 21	0.316 22
FEF_{25-75} SDS⁺	-0.040 14	0.209 14	-0.495 14	0.011 14	0. 0	-0.257 6	-0.332 15	0.130 15	-0.402 14	0.043 15
LCI	-0.417* 28	0.080 28	0.061 28	0.283 27	0.381 12	-0.013 18	0.145 27	0.121 24	0.244 27	0.006 31
FRC	0.302 28	-0.249 28	-0.251 28	-0.198 27	0.324 12	-0.103 18	-0.050 27	-0.227 24	-0.086 27	0.024 31

Extent bronchiectasis	-0.198 28	0.336 27	-0.022 26	0.306 25	0.025 14	-0.059 21	-0.146 26	-0.006 22	-0.006 25	-0.019 30
Severity bronchiectasis	-0.054 28	0.161 27	-0.248 26	0.018 25	0.169 14	0.073 21	-0.043 26	-0.201 22	0.057 25	-0.020 30
Wall thickness^{+}	-0.034 28	0.202 27	-0.019 26	0.121 25	-0.151 14	0.303 21	-0.061 26	-0.160 22	0.133 25	0.093 30
Air trapping	-0.038 27	0.164 26	0.415* 25	0.356 24	-0.306 14	-0.187 20	0.298 25	-0.365 21	0.391 24	0.090 29
Small mucus plugs^{+}	-0.220 28	0.189 27	-0.307 26	0.108 25	0.137 14	0.329 21	-0.167 26	-0.231 22	-0.072 25	-0.007 30
Large mucus plugs	-0.111 28	0.221 27	-0.307 26	0.080 25	0.092 14	0.012 21	-0.099 26	-0.234 22	0.167 25	-0.088 30
Consolidated lung^{+}	0.187 28	0.007 27	-0.217 26	0.271 25	-0.058 14	-0.092 21	0.052 26	0.268 22	0.147 25	-0.141 30
Ground glass^{+}	0.143 28	-0.109 27	-0.392* 26	-0.044 25	-0.645* 14	0.171 21	-0.090 26	0.226 22	-0.111 25	-0.149 30
White cell count		-0.104 30	-0.269 29	-0.047 28	-0.084 13	0.272 18	-0.120 27	-0.047 24	-0.218 26	-0.041 32
CRP	-0.104 30		0.535** 32	0.761*** 30	0.124 14	0.004 19	0.012 29	-0.350 25	0.026 28	0.005 33
Serum IL-6	-0.269 29	0.535** 32		0.535** 31	-0.149 13	0.017 19	0.281 29	-0.198 25	0.338 29	0.136 32
Serum calprotectin	-0.047 28	0.761*** 30	0.535** 31		0.032 12	-0.151 17	0.150 27	0.002 23	0.203 27	-0.232 30
Serum IL-8	-0.261 26	-0.104 28	0.352 29	0.034 29	-0.499 11	-0.019 16	0.214 25	-0.003 21	0.086 25	-0.418* 29
Serum TNFα	-0.083 29	-0.017 32	0.081 33	0.150 31	-0.009 13	-0.230 19	0.186 29	-0.184 25	0.232 29	-0.073 32
Sputum 24 hr weight	-0.084 13	0.124 14	-0.149 13	0.032 12		0.057 12	-0.082 11	-0.750 7	0.007 11	0.027 15

Sputum % dry weight	-0.504 11	0.107 13	0.135 13	0.265 11	0.268 11	0.170 15	0.141 15	-0.218 11	0.501 15	0.265 15
Sputum total cell count	0.272 18	0.004 19	0.017 19	-0.151 17	0.057 12		0.169 21	-0.140 17	0.417 20	-0.243 23
Sputum IL-12	-0.479** 28	0.555** 31	0.204 32	0.185 30	-0.064 13	-0.226 19	-0.144 29	0.058 25	-0.272 29	0.074 31
Sputum MMP9	-0.120 27	0.012 29	0.281 29	0.150 27	-0.082 11	0.169 21	1 32	-0.288 28	0.546** 31	-0.045 31
Sputum calprotectin	-0.378* 28	0.234 29	0.387* 29	0.260 27	-0.082 11	0.382 21	0.446* 30	-0.625** 26	0.748*** 29	-0.013 31
Sputum IL-8	-0.472* 27	0.075 29	0.300 29	0.166 27	-0.461 10	0.096 20	0.673*** 29	-0.082 25	0.660*** 28	0.061 30
Sputum neutrophil elastase	-0.048 29	0.242 30	0.262 30	0.247 28	-0.398 12	0.418 22	0.558** 31	-0.276 27	0.552** 30	-0.267 32
Sputum MPO	-0.406* 27	0.161 29	0.370* 29	0.224 27	-0.718* 10	0.405 20	0.424* 29	-0.222 25	0.648*** 28	-0.142 30
Sputum RANTES	0.214 24	-0.171 25	-0.161 25	-0.117 24	-0.120 9	-0.166 16	-0.233 26	0.623** 22	-0.589** 25	0.073 25
Sputum TIMP1^{+}	-0.006 24	-0.449* 25	-0.184 25	-0.091 23	-0.847* 7	-0.203 17	-0.179 28		-0.415* 27	0.057 27
Sputum IL-1β	-0.218 26	0.026 28	0.338 29	0.203 27	0.007 11	0.417 20	0.546** 31	-0.300 27		0.138 30
EBC pH^{+}	-0.041 32	0.005 33	0.136 32	-0.232 30	0.027 15	-0.243 23	-0.045 31	0.057 27	0.138 30	
EBC nitrite^{+}	-0.250 30	-0.271 31	-0.158 30	-0.328 28	-0.341 14	0.131 21	0.013 29	0.213 25	-0.024 28	-0.044 35
EBC NH_4	0.006 31	-0.089 32	0.064 31	0.032 29	-0.503 15	-0.101 23	-0.101 30	0.157 26	-0.044 29	0.098 36
Sputum DNA	-0.116 11	-0.010 13	-0.066 13	0.436 11	-0.271 11	0.163 15	0.505 15	0.318 11	0.91 15	-0.545* 15

Sputum viscosity[+]	-0.423 10	0.222 12	0.289 12	0.546 10	-0.250 10	-0.376 14	-0.070 14	0.414 11	-0.401 14	0.176 14
Sputum elasticity[+]	-0.333 10	0.315 12	0.245 12	0.624 10	-0.394 10	-0.367 14	-0.200 14	0.582 11	-0.330 14	0.159 14

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