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Induced sputum and other outcome measures in chronic obstructive pulmonary disease: safety and repeatability

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Abstract The forced expiratory volume in 1 sec (FEV_1) is the most established outcome measure in chronic obstructive pulmonary disease (COPD). However, changes in FEV_1 in response to treatment are small in relation to the repeatability of the measurement and there is increasing interest in other measures including markers of lower airway inflammation in induced sputum, assessment of symptoms and health status using visual analogue scores, and questionnaires. Little is known about the repeatability of these measures or the safety of sputum induction in COPD. We have assessed the safety of sputum induction in 61 subjects with moderate and severe COPD who participated in a placebo-controlled cross-over study. The within-subject repeatability of sputum markers of airway inflammation, health status using the chronic respiratory disease questionnaire (CRQ) and symptom visual analogue scores (VAS) were estimated from the data obtained from before and after 2 weeks of treatment with placebo. Sputum induction was performed on 122 occasions and was successful resulting in a cytospin adequate to assess a differential cell count in 95% of inductions. The group mean (SEM) FEV_1 was 1·09 (0·05)[41·6 (2·9)% predicted] and the mean (SEM) fall in FEV_1 after sputum induction was 120 ml (6) and % fall 10·9% (0·55%). Seven inductions were stopped due to a fall in FEV_1 >20% and at a further 13 visits the full sputum induction protocol was not completed due to development of symptoms. The reproducibility of measurements, calculated by the intra-class correlation coefficient, was relatively high for all indices measured (0·4–0·95) with the exception of the proportion of lymphocytes (0·15) and epithelial cells (0·3). The ICC for symptom scores and the CRQ domains ranged between 0·87 and 0·96. In conclusion, sputum induction is safe and the cell and fluid phase mediators repeatable in the investigation of airway inflammation in patients with COPD. VAS symptom scores and the CRQ are reproducible outcome measures in COPD.

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Keywords COPD; outcome measures; health status; sputum induction.

INTRODUCTION

The forced expiratory volume in 1 sec (FEV_1) has long been established as the main outcome measure in assessing the progress or response to treatment in patients with chronic obstructive pulmonary disease (COPD). The FEV_1 has shortcomings as an outcome measure in COPD as most patients by definition have irreversible airflow obstruction and any possible response to treatment is small in relation to the repeatability of the measurement (1). Consequently, interest has increased in the use of other perhaps more responsive measures.

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COPD is associated with sputum (2) and bronchial biopsy (3) evidence of neutrophilic airway inflammation and the extent of this inflammation as reflected by the induced sputum neutrophil differential count inversely correlates with the FEV_1 and decline in FEV_1 (2,4). These observations are in keeping with a causal relationship between neutrophilic airway inflammation and disease progression in COPD, and suggest that assessment of markers of neutrophilic airway inflammation might be a meaningful outcome measure in COPD. In asthma sputum induction is widely used and it has been established as safe and sputum differential cell counts and supernatant concentrations are valid and repeatable (5–7). In COPD the repeatability of differential cell counts and safety (8,9) of sputum induction in patients with chronic obstructive pulmonary disease (COPD) is less clear. Sputum induction with hypertonic saline is a potential bronchoconstrictor and thus there have been

some concerns about its use in subjects who already have moderate or severe airflow obstruction.

Patients with COPD have significant morbidity (10). Disease-specific health status questionnaires have been developed, which have been shown to be valid, repeatable and responsive in patients with COPD (11–13). Symptom visual analogue scores have been used in a number of clinical settings particularly in the assessment of pain (14), but their possible role as an outcome measure in COPD is uncertain.

We have used data from a recent cross-over, placebo-controlled trial (15) to assess the safety of sputum induction and to investigate the repeatability of cell counts, fluid phase mediators, symptom scores and the chronic respiratory disease questionnaire measured 2 weeks apart in a group of patients with moderate and severe COPD.

METHODS

Subjects

Subjects had partaken in a randomized, placebo-controlled, cross-over trial of 2 weeks of prednisolone or placebo (15). Data from the placebo arm of this trial was used to assess safety of sputum induction and repeatability of outcome measures.

Subjects were recruited from respiratory clinics with symptoms of chronic airflow obstruction and a post-bronchodilator FEV_1 of $<70\%$ predicted and FEV_1 forced vital capacity (FVC) ratio of $<70\%$. There was no significant improvement in FEV_1 after 2·5 mg nebulized salbutamol ($<15\%$, or if $FEV_1 < 1{\cdot}2$ l <200 ml improvement). Subjects were excluded if they had a clinical diagnosis of asthma, a history of childhood respiratory problems, variability in symptoms not associated with infections, a history of acute wheeze, breathlessness or deterioration associated with allergens or an exacerbation within 6 weeks of trial entry. Subjects had not taken oral or inhaled corticosteroids for at least 1 month. The study was approved by the local research ethics committee and all subjects gave written informed consent.

Study design

Subjects attended for sputum induction before and 4–6 h after the last dose of placebo at the same time of day on each occasion more than 6 h after their last dose of bronchodilator and 24 h after the last dose of long-acting β_2-agonists.

Measurements

Details of the subject's smoking, treatment and childhood respiratory history was obtained. Spirometry was performed with a Compact Vitalograph spirometer (Vitalograph, Buckinghamshire, U.K.). Salbutamol was administered via a Flaem Nuova Type II nebulizer (Deva Medical, Runcorn, Cheshire, U.K.) with a median particle size 2 μm and the patient breathing tidally. FEV_1 was recorded as the better of two successive readings within 100 ml. Pulmonary function tests were performed using a PK Morgan Benchmark (Chatham, U.K.) and lung volumes were assessed using the helium dilution method.

Symptom scores were recorded using a 100 mm visual analogue scale (VAS) from no symptom to the worst symptom ever for dyspnoea, cough, sputum production and wheeze. Health status was assessed using the chronic respiratory disease questionnaire (CRQ), consisting of 20 questions measuring four domains: dyspnoea, fatigue, emotions and mastery. A seven-point Likert scale was used for each question and the total CRQ and each domain score was recorded out of seven (11).

Sputum was induced and processed as previously described (6,16). Briefly, sputum was induced using 3, 4 and 5% saline inhaled in sequence for 5 min each via a low output (0·9 ml min^{-1}, median particle size 5 μm) ultrasonic nebulizer (Medix, Harlow, U.K.), 30 min after nebulized salbutamol 2·5 mg. Sputum induction was not performed in subjects with an $FEV_1 < 0{\cdot}5$ l. The fall in FEV_1 during sputum induction was measured from the post-bronchodilator FEV_1. Expectorated sputum was stored on ice and analysed within 2 h of expectoration. A differential cell count was obtained by counting >400 non-squamous cells on Romanovski-stained cytospins.

Sputum elastase was measured in the cell free supernatant using a spectrofluorimetric assay (15). IL-8 was measured using a commercial enzyme linked immunosorbent assay (OptEIA Set, Pharmingen, U.K.) and eosinophilic cationic protein (ECP) was measured using a commercial fluroimmunoassay (Unicap, Pharmacia, Milton Keynes. U.K.). The limits of detection for the elastase, IL-8 and ECP were 1·5 μg g^{-1}, 30 pg g^{-1} and 18 ng g^{-1} of sputum respectively.

Analysis

Descriptive statistics were used for subject characteristics. Spirometry, per cent fall in FEV_1, symptom scores, total CRQ and CRQ domains were described as mean and standard error of the mean (SEM). Eosinophil count, total cell count and mediator concentrations were log normally distributed and were described as geometric mean (log SEM). Other sputum differential cell counts were reported as the mean (SEM). Repeatability was described as the within-subject standard deviation of the difference between duplicate

measurements and intra-class correlation coefficient of the measures.

RESULTS

Safety of sputum induction

Subject characteristics are shown in Table 1. Sputum induction was performed on 122 occasions and was successful resulting in a cytospin adequate to assess a differential cell count in 95% of inductions. The mean (SEM) fall in FEV_1 after sputum induction was 120 ml (6) and % fall 10·9% (0·55). There was no significant difference between the mean (SEM) % fall after the first 11·2% (0·7) or second 10·7% (0·8) induction (mean difference 0·45, 95% CI −1·4, 2·3; P=0·6) and the intra-class correlation coefficient was 0·32. Seven inductions were complicated by a fall in FEV_1 >20%, four of which were two subjects who had a significant fall in their FEV_1 following both inductions. On a further 13 occasions the full sputum induction protocol was not completed due to development of symptoms: dyspnoea in six and nausea or general discomfort in seven.

Table 1. Subject characteristics

Number of subjects	61
Age*	66 (13)
Male	37
Current smokers	15
Pack years*	30 (10)
FEV_1 (l)†	1·09 (0·05)
FEV_1 post-bronchodilator (l)†	1·14 (0·06)
FEV_1 (% predicted)†	41·6 (2·9)
FEV_1/FVC ratio (%)†	48·2 (3·1)
TLC (% predicted)†	95 (3·8)
RV/TLC (% predicted)†	122 (7·2)
KCO (% predicted)†	87 (7·9)

*Median (IQR), †Mean (SEM).

Repeatability of outcome measures

There were no significant differences between the differential cell counts, total cell count, symptom scores or CRQ total and domains before and after placebo. The repeatability of the measures are shown on Table 2.

DISCUSSION

We have shown that sputum induction using a low output nebulizer is well tolerated, safe and successful in patients with moderate and severe COPD. The reproducibility of the sputum cell counts and fluid phase mediators was relatively high for all indices measured with the exception of the proportion of lymphocytes and epithelial cells.

Table 2. Within-subject repeatability of outcome measures

	Visit 1	Visit 2	Within-subject SD	ICC
Neutrophil (%)†	74 (2·5)	69 (2·7)	17·8	0·57
Eosinophil (%)*	2·2 (0·09)	2·1 (0·13)	0·66	0·63
Macrophage (%)†	22 (2·5)	3 (2·4)	16·4	0·54
Lymphocyte (%)†	0·3 (0·05)	0·7 (0·25)	1·95	0·15
Epithelial cells (%)†	1·0 (0·16)	1·5 (0·35)	2·8	0·32
TCC $\times 10^6$ g^{-1} sputum*	2·7 (0·04)	2·6 (0·05)	0·31	0·54
ECP ng g^{-1}*	1149 (0·09)	1236 (0·09)	0·48	0·70
IL-8 ng g^{-1}*	112 (0·08)	97 (0·1)	0·62	0·71
Elastase μg ml^{-1}*	42·5 (0·11)	36·5 (0·11)	1·07	0·4
CRQ: Total†	4·03 (0·14)	4·08 (0·14)	0·18	0·95
CRQ: Dyspnoea†	3·52 (0·16)	3·44 (0·17)	0·33	0·91
CRQ: Fatigue†	3·72 (0·17)	3·77 (0·16)	0·33	0·90
CRQ: Emotion†	4·46 (0·17)	4·6 (0·17)	0·22	0·96
CRQ: Mastery†	48·61 (0·18)	4·59 (0·19)	0·26	0·94
VAS: Dyspnoea (mm)†	48·2 (3·2)	48·0 (3·2)	4·82	0·94
VAS: Wheeze (mm)†	32·1 (3·6)	31·8 (3·5)	8·62	0·87
VAS: Cough (mm)†	32·4 (4·6)	33·5 (3·4)	7·75	0·87
VAS: Sputum (mm)†	26·8 (3·0)	27·5 (3·1)	4·82	0·94

*Geometric mean (log SEM), †mean (SEM).

Our estimate of repeatability of cell counts is similar to that reported in normal and asthmatic subjects (6). The poor repeatability of the lymphocyte cell count has been noted before in asthma and probably reflects the difficulty in the accurate recognition of this cell type and their scarcity in induced sputum (17). To investigate this aspect of the inflammatory response bronchoalveolar lavage and biopsy would be more appropriate. Our findings show that the assessment of induced sputum markers of neutrophilic airway inflammation is feasible and repeatable in subjects with moderate and severe COPD.

We have previously established that sputum induction is safe in asthma (5). In this current study 94% of inductions were associated with a fall in FEV_1 <20% and the mean FEV_1 fall was 10·9%. This is broadly in line with the mean % fall we have observed in asthma (5) and similar to the 10·7% (8) and 11·7% (9) fall reported in two previous studies using a higher output nebulizer in COPD. The fall in FEV_1 was similar after both sputum inductions and was broadly repeatable. Although sputum induction can lead to a significant fall in lung function and symptoms in some patients these findings confirm that when performed carefully with frequent assessment of lung function, sputum induction is safe even in patients with moderate to severe airflow obstruction.

In keeping with previous reports we confirm that the CRQ is a reproducible outcome measure of health status in COPD (11,12). We have also established that symptom visual analogue scores are repeatable outcome measures. In the associated intervention study we found that symptom VAS were amongst the most responsive measures following prednisolone treatment (15). This, coupled with the ease of administration and excellent repeatability, suggests that they are particularly useful outcome measures.

Our study was not primarily designed to assess the repeatability of sputum markers of airway inflammation, health status and symptom scores and we can not exclude a possible bias due to the placebo effect. However, no placebo effect was observed on spirometry, health status or symptoms (15) and we consider it unlikely that the placebo medication would have influenced the effect of sputum induction on lung function or airway inflammation. Ideally further studies should assess repeatability of these measures in untreated stable patients. In the absence of such data our study does at least provide an estimate of the between and within subject variability.

In conclusion we have shown that sputum induction is safe and successful in subjects with moderate and severe COPD and that sputum cell counts, fluid phase mediators, CRQ scores and VAS symptom scores are repeatable outcome measures in COPD. Our findings will help other investigators plan and power future intervention studies.

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