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FINAL DRAFT

Which patients with chronic obstructive pulmonary disease benefit from the addition of an inhaled corticosteroid to their bronchodilator? A cluster analysis

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Article focus: This paper describes a cluster analysis of a pooled cohort of COPD patients receiving salmeterol (SAL) alone or in combination with fluticasone propionate (SFC) for 1 year. The analysis sought to identify clusters of patients who could benefit most from the addition of fluticasone propionate to their bronchodilator therapy based on the annual rates of moderate/severe exacerbations

Key messages: Three clusters were identified. Patients receiving diuretics, and those not receiving diuretics but with baseline bronchodilator reversibility of ≥12% exhibited a significantly greater reduction in exacerbations when treated with SFC vs. SAL. No difference was seen between treatments for non-reversible patients not receiving diuretics. These data suggest who might benefit most from inhaled corticosteroid therapy in additional to a bronchodilator among COPD patients.

Strengths and limitations of this study: Well characterised, pooled data from >1500 patients from a randomized controlled trial were used in the analysis, which was validated using half of the study population. Cluster analysis was limited to the patient characteristics collected in the randomized trial at baseline and generalized to those patients with COPD with a history of exacerbation.

ABSTRACT

Objectives: Cluster analysis to identify subsets of chronic obstructive pulmonary disease (COPD) patients who are more protected from exacerbations with the use of an inhaled cosrticosteroid/long-acting ß$_2$ agonist (ICS/LABA) combination, compared to the use of LABA monotherapy.

Design: Post hoc analysis of two 1-year studies of salmeterol/fluticasone propionate (SFC) and salmeterol (SAL) with a primary endpoint of moderate/severe exacerbations.

Setting: Centres in North America.

Participants: 1543 COPD patients were studied.

Interventions: SFC 50/250 µg or SAL 50 µg, twice daily.

Primary and secondary outcome measures: The analysis identified characteristics of COPD patients more responsive to SFC versus SAL with respect to annual rate of moderate/severe exacerbations.

Results: SFC significantly reduced the annual rate of moderate/severe exacerbations (rate ratio [RR]=0.701, p<0.001). Three patient clusters were identified using baseline characteristics. COPD patients receiving diuretics (RR=0.56, p<0.001) and patients not receiving diuretics but with FEV_1 reversibility ≥12% (RR=0.67, p<0.001) exhibited a significant reduction in the annual rate of moderate/severe exacerbations relative to SAL. A third cluster, consisting of patients not receiving diuretics and without FEV_1 reversibility, demonstrated no difference for SFC versus SAL. Patients receiving diuretics had a significantly higher prevalence of comorbid cardiovascular disease.

Conclusions: Cluster analysis identified three potential COPD patient clusters. Those receiving diuretics, and those not receiving diuretics but with FEV_1 reversibility

were significantly more likely to experience a reduction in COPD-associated exacerbations with SFC versus SAL.

Trial registration: clinicaltrials.gov NCT00115492, NCT00144911

INTRODUCTION

The *Global Strategy for the Diagnosis, Management and Prevention of COPD* (GOLD) was revised in 2011 to reflect that Forced Expiratory Volume in 1 second (FEV_1) alone is an insufficient marker of disease severity[1]. Importantly, therapy with an inhaled corticosteroid/ long-acting ß$_2$ agonist (ICS/LABA) combination, or a long-acting muscarinic antagonist, is recommended for patients at risk of two or more exacerbations per year, even in the presence of low airflow limitation. This recommendation reflects the established association between frequent exacerbations and a more rapid decline in lung function[2], and a greater impairment of health status.[3 4]

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease with pulmonary and extra-pulmonary manifestations.[5] Significant in-roads have recently been made in developing an understanding of this complexity and heterogeneity,[6] and how these features of the disease may contribute to the development of a tailored approach to therapeutic intervention based on the patient's individual COPD phenotype.[7] Han *et al* have advocated the following process for selection of a COPD phenotype: identify a candidate phenotype, determine its relevance to clinical outcomes, and then validate the phenotype with longitudinal data collection in carefully characterised patient groups.[7] An example of such a phenotype established through this process is that of the 'frequent exacerbator' identified in the ECLIPSE cohort. In that analysis the presence of two or more exacerbations in the prior year was shown to strongly predict the occurrence of an exacerbation in the coming year.[8]

Statistical techniques may assist in the identification of COPD phenotypes, with cluster analysis being the most commonly used approach.[9 10 11] Cluster analysis

uses algorithms to group patients, without an *a priori* hypothesis, in populations where those in the same group are more similar than they are to those in other groups.[12] This is in contrast to subgroup analysis, where populations are pre-defined and then statistical testing is applied to identify differences.

In the present study, cluster analysis was conducted using data pooled from two clinical trials[13 14] that studied differences in exacerbation rates in COPD patients randomly assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA (salmeterol/fluticasone propionate [SFC]). The objective of the cluster analysis was to identify patients who benefit most from the addition of ICS to bronchodilator therapy by maximising treatment differences within a cluster for mean annual rate of moderate/severe exacerbations for SFC compared to SAL.

METHODS

Clinical study design and subjects

The methodology for the two clinical trials has been previously published.[13 14] These were randomised, double-blind, parallel group studies comparing twice-daily SFC 50/250 µg or SAL 50 µg via DISKUS® (Seretide, Serevent, GlaxoSmithKline, Research Triangle Park, NC, USA) on the annual rate of moderate/severe exacerbations in patients with COPD.

Subjects were aged 40 years or older, with a clinical history of COPD, a pre-bronchodilator FEV_1 ≤50% of predicted, a pre-bronchodilator FEV_1/forced vital capacity (FVC) ratio of ≤70%, a cigarette smoking history of ≥10 pack-years, and a documented history of at least one moderate or severe COPD exacerbation in the year prior to screening. A moderate exacerbation was defined as requiring outpatient antibiotic and/or oral corticosteroid use, and a severe exacerbation was defined as requiring hospitalisation. Current and former smokers were included. Key exclusion criteria were a current diagnosis of asthma, other active chronic respiratory disorders apart from COPD, a moderate/severe exacerbation that had not resolved prior to visit 1, or concurrent use of anticholinergics, theophyllines, and leukotriene modifiers.

Cluster analysis methodology

The annual moderate/severe exacerbation rate was entered into a cluster analysis using an interaction tree algorithm[12] to maximise the identification of subgroups showing differences in their response to SFC and SAL treatment.

The cluster analysis aimed to find subgroups in the study subjects that had similar baseline characteristics and with maximum treatment differences for mean yearly moderate/severe exacerbation risk ratio (RR). Subjects (n=36) with protocol

violations or missing data required for the primary model were excluded (n=1543 analysed vs n=1579 enrolled). Subjects included in the cluster analysis were required to have baseline variables comprising FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% improvement and ≥200 mL), time on treatment, and geographical region. Missing values for the remaining baseline variables were imputed during cluster analysis as the median for continuous/ordinal variables, or the most frequent value for categorical variables. The baseline characteristics are listed in table 1.

Baseline characteristics were examined before inclusion in the model to ensure that there was no significant co-linearity that may influence the cluster analysis. Co-linearity was assessed by creating a correlation tree, and any two variables with an R^2 of ≥0.7 were examined. The variables considered most clinically relevant were retained. St. George's Respiratory Questionnaire (SGRQ) Impact and Activity scores were removed from cluster analysis since they highly correlated with the total score.

Modelling to define the tree: supervised analysis

Modified recursive partitioning techniques were used to perform the supervised subgroup analysis. The frequency of each variable was examined to identify sparse values prior to inclusion into the tree. As the minimal subgroup size (terminal node) was set at 100, all categorical variables were required to have at least 100 subjects in a response category in order to be considered for the recursive partitioning algorithm. Variables with several responses were collapsed into fewer categories as appropriate, such that all categories had at least 100 subjects (eg, exacerbations requiring hospitalisation in the past year) or eliminated from consideration during the

cluster analysis (race, anti-hemorrhagics, anti-hypertensives [eg, anti-adrenergics and smooth muscle agents], vasodilators, and vasoprotectives [eg, topical haemorrhoid treatments and anti-varicose therapy]).

The best split of the tree was determined by maximising the subgroups according to treatment interaction effect, and subgroup membership was then assigned to each patient based on the selected tree. Internal validation was performed by using a split sample, so that a random sample of 50% of the patients was selected to create the tree and the remaining half was used for the computation of rate ratios and confidence intervals (CIs) to test statistical significance.

Generalised linear models using a negative binomial function were used to compare the likelihood of having an exacerbation by examining treatment by subgroup interaction. The model was adjusted for study baseline FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change), time on treatment and geographical region (8 regions), which was considered a random effect. The algorithm used in the study maximises treatment differences (mean moderate/severe exacerbation rates for SFC vs SAL) among subgroups. The RR for each cluster was estimated using linear contrast, and rate ratios plus 95% CIs were used to estimate the differences in annual mean moderate/severe exacerbation rates for each cluster. The programme was completed in the R statistical package.[15]

Clusters were characterised based on the tree, using descriptive statistics. Descriptive statistics were used to present the baseline differences among clusters; proportions were used for categorical variables, and medians with interquartile ranges were used for continuous variables. The χ^2 test was used to examine the statistical differences among the subgroups for categorical variables and the non-

parametric Wilcoxon Rank sum test was performed to test the statistical differences among the subgroups for continuous variables.

RESULTS

Pooled demographics and efficacy

Baseline characteristics of the pooled population were well matched between those receiving SFC and those receiving SAL (table 2). The majority of patients reported a moderate, and not a severe, exacerbation in the 12 months prior to study. Thirty-seven per cent of patients had 2 or more moderate exacerbations and 2% had 2 or more severe exacerbations.

In the primary studies[13 14] the annual moderate/severe exacerbation rates were significantly lower with SFC (1.10 and 1.06) than with SAL (1.59 and 1.53). A treatment effect was observed in the pooled study population (1.10 vs 1.58 for SFC and SAL respectively, RR=0.701, p<0.001).

Cluster analysis results

Supervised cluster analysis identified four distinct clusters based on the use of diuretics and the extent of FEV_1 reversibility, expressed solely as a percentage of the pre-bronchodilator value. Reversibility was categorised initially into three levels; <11.5%, 11.5 to 28% and >28%. When maximising differences in response to therapy with SFC versus SAL (data not shown) we pruned the tree at the ≥12% reversibility threshold as 11.5% was close to the ≥12% component of the ERS/ATS threshold for reversibility, although it should be noted that this definition also requires a volume response of ≥200 mL.[16] The initial reversibility clusters were otherwise similar with respect to baseline characteristics (data not shown).

Three final COPD clusters were defined (figure 1) based on use of diuretics and the presence or absence of reversibility. The first cluster (cluster 1) identified subjects treated with diuretics (predominantly furosemide). Approximately half of the

diuretic use reported (n=282) was for hypertension, with the remaining use for unspecified oedema, coronary artery disease, and/or congestive heart failure. No other sub-populations were identified in this cluster. In patients not using diuretics at baseline two further clusters were defined based on the presence or absence of FEV_1 reversibility. Cluster 2 patients exhibited reversibility, defined as a post-bronchodilator change of ≥12%. Cluster 3 patients did not exhibit reversibility, ie, a post-bronchodilator change in FEV_1 of <12%. Compared to SAL, significant reductions in the rate of moderate/severe exacerbations were observed with SFC therapy in cluster 1 (44% reduction) and cluster 2 (33% reduction). Similar reductions were not observed in cluster 3 (table 3).

Baseline demographics that were significantly different across clusters are presented in table 4. Subjects in cluster 1 tended to be older, had a higher BMI, were more likely to be former smokers than current smokers, and had the greatest smoking pack-year history. Cluster 1 subjects also had a higher prevalence of treatment for comorbidities (eg, cardiovascular disease [CVD], hypertension, diabetes) than clusters 2 and 3. Subjects in cluster 3 had a higher % predicted FEV_1 compared with those in clusters 1 and 2, whereas those in cluster 2 had the lowest % predicted FEV_1. No difference was observed in the baseline incidence of moderate/severe exacerbations between the clusters.

DISCUSSION

This study identified three clusters; cluster 1: diuretic users with treatment for cardiovascular comorbidity; cluster 2: reversible, not taking diuretics; cluster 3: not reversible, not taking diuretics. Subjects in clusters 1 and 2 benefited more from receiving combination therapy with SFC, compared to SAL with a greater reduction in exacerbation. This exercise identified two groups that are more likely to respond to SFC. The largest benefit with SFC was observed in cluster 1.

A number of hypotheses can be put forward to explain the lower exacerbation rates with SFC relative to SAL among diuretic users. The use of diuretics may identify a group of patients with cardiovascular disease, such as those with hypertension or heart failure, who may be more responsive to the addition of an ICS to a LABA. There was a significantly higher use of cardiovascular (CVD) medications in cluster 1. ICS (FP) could exert a benefit on exacerbations in COPD patients with CVD if 1) CVD comorbidity reflects an increased inflammatory state related to COPD[17] and 2) if CVD is a driver for COPD exacerbation occurrence[18] and severity[19] as has been reported. It is therefore plausible to conjecture that subjects with CVD would exhibit higher levels of inflammation than those without CVD. Inflammation, as demonstrated by elevated C-reactive protein or fibrinogen, increases the risk of a COPD exacerbation[8 20] and this logic supports the value of the addition of an ICS (FP) in cluster 1.

It is also possible that cluster 1 (diuretic) was predisposed to an increased exacerbation risk as a consequence of heart failure. Heart failure can be aggravated by increased aortic stiffness, a marker of cardiovascular risk found in greater prevalence among COPD patients than in the general population.[21] Dransfield *et al*[22]

recently found that SFC lowered aortic pulse wave velocity (aPWV), a marker of aortic stiffness, in COPD patients with elevated aPWV.

Another possible explanation of the lower rate in moderate/severe exacerbations with SFC over SAL in cluster 1 reflects the direct activity of the concomitant diuretic therapy. Recent studies have examined the effectiveness of diuretics in the treatment of chronic respiratory diseases, in particular furosemide (which was the predominantly used diuretic in cluster 1). Mechanistically, furosemide inhibits inflammatory cytokines[23] and enhances the anti-inflammatory impact of ICS.[24] Clinically it has been shown to alleviate exertional dyspnoea in COPD,[25] and to protect against bronchoconstriction in asthma.[26 27 28]

In cluster 2 (reversibility ≥12%) a significant effect of SFC was also observed over SAL in terms of a lower rate of moderate/severe exacerbations. Subjects exhibiting reversibility have been shown to have greater improvement in lung function compared to those without reversibility.[29] Recent data suggest that an improvement in lung function of 100 mL relates to a reduction in exacerbation rate of 12%,[30] while a 12% increase in exacerbation rate has been reported for each 100 mL loss of lung function.[8] The effect of SFC in COPD[31] has been shown to provide a significantly greater effect on lung function in reversible versus irreversible subjects. This suggests a potential mechanism for the lower rate of moderate/severe exacerbations in cluster 2 patients receiving SFC.

None of the subjects in cluster 3 exhibited reversibility, and this together with the highest prevalence of current smokers (which is known to attenuate ICS effects in COPD)[32] may explain why no difference was observed between SFC and SAL in this cluster.

A number of recent studies have investigated COPD heterogeneity, and have identified independent factors such as dyspnoea, airway inflammation, and asthma-like features,[10 33] or subgroups associated with differential outcome.[34 35] However, only one[36] has validated the COPD subtypes identified against clinically meaningful outcomes. Garcia-Aymerich *et al*[36] identified three clusters of subjects, comprising those with severely impaired lung function, those with more mildly impaired lung function and, importantly, those with more mildly impaired lung function *and* evidence of cardiovascular disorders, obesity, diabetes and systemic inflammation. The clusters identified in the present study align to some extent with those already identified, such as increased reversibility and the presence of CVD. This suggests a convergence of COPD subtypes that warrants further examination.

Cluster analysis is limited due to its retrospective nature and the fact that it is limited to assessing only the categorical variables collected at baseline. In addition, the splitting into groups is automated by the computer-driven algorithm to maximise treatment differences, and is not necessarily robust, thus external validation is warranted. Another potential issue with the clusters isolated in this study is the split by reversibility. While it is apparent that COPD subjects can be more or less reversible, there is considerable within-patient variability both on single testing[29] and at testing on multiple occasions.[37] It is conceivable that had reversibility testing in the original studies been conducted within a different time frame (eg, 3 months later or earlier) a different population of subjects would have been defined as reversible, and it is not clear whether a treatment effect would have been observed. Despite the limitations with reversibility, there is evidence that subjects who are less reversible are likely to have a less robust response to treatment than those who are more reversible.[29 31] Alternatively, this less reversible cluster could simply be a measure of

those with less severe disease (less obstruction, smaller proportion of comorbidities). Subjects with less severe disease may not require the addition of an ICS to their bronchodilator therapy.

In conclusion, cluster analysis of subjects taking part in two exacerbation studies of SFC versus SAL identified three distinct groups of COPD subjects based on diuretic use and reversibility. These subjects varied in their response with subjects in two of the three groups experiencing a greater reduction in the annual rate of moderate/severe exacerbations with SFC versus SAL. Those in the remaining group received no additional benefit in terms of reduction in the annual moderate/severe exacerbation rate over that provided by SAL alone. This study highlights the future potential for a personalised medicine approach to the treatment of patients with COPD. It additionally suggests how this methodology can be used to generate potential hypothesis for future studies.

Tables:

Table 1 Baseline characteristics and other variables employed in the cluster analysis

Variable	
Demographics	Age (years)
	Gender
	Smoking status (current/former)
	Pack-years
	Body mass index (m/kg^2)
Lung function/QOL	FEV_1 % predicted
	FEV_1 % reversibility
	FEV_1/FVC ratio post-albuterol
	FVC % predicted
	SGRQ activity score
	SGRQ impacts score
	SGRQ symptom score
	SGRQ total score
COPD history	Duration of COPD (years)
	Chronic bronchitis (self-reported, yes/no)
	Emphysema (self-reported, yes/no)
	Exacerbations requiring hospitalisation (past 12 months)
	Exacerbations requiring OCS/antibiotic (past 12 months)
	Gold Stage indicator variables based on lung function (II, III/IV)
Medications (ATC classification)	Agents acting on the renin-angiotensin system (ACE inhibitors)
	Antianaemic preparations
	Antihaemorragics
	Antihistamines
	Antihypertensives
	Antithrombotics
	Antihaemorrhagics
	Anti-inflammatory and antirheumatic products
	Beta blockers
	Bone disease (including muscle pain) medications
	Calcium channel blockers
	Cardiac therapies
	Diabetes medications
	Diuretics
	Lipid modifying agents
	Psychoanaleptics
	Psycholeptics
	Vasodilators

ACE, angiotensin converting enzyme; ATC, Anatomical Therapeutic Chemical; COPD, Chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory Questionnaire.

Table 2 Demographic and baseline clinical characteristics of subjects participating in the primary clinical studies of SFC versus SAL (cluster analysis population)

Demographic characteristics	SFC 50/250 µg N=771	SAL N=772	TOTAL N=1543
Age, median years (IQR)	65 (59-72)	65 (59-71.5)	65 (59-72)
Gender, male/female ratio	54/46	54/46	54/46
Race, n (%)			
Caucasian	94	94	94
Non-Caucasian	6	6	6
Body mass index, mean m/kg^2 (IQR)	27 (23-31)	27 (23-30)	27 (23-31)
Smoking history, %			
Former	59	59	59
Current	41	41	41
Exacerbations requiring hospitalisation (past year) (%)			
0	78	76	77
1	20	22	21
≥2	3	2	2
Exacerbations requiring oral steroids/antibiotics (past year) (%)			
0	<1	1	1
1	65	60	63
2	20	24	22
≥3	14	14	15
FEV_1 % predicted (SD)	33.1 (25.1-41.8)	33.8 (24.9-41.9)	33.6 (25.0-41.9)
FEV_1 % reversibility (SD)	20.1 (9.1-33.4)	18.6 (8.5-30.5)	18.9 (8.9-31.7)
Reversibility stratum[1] [no/yes], %	58/42	61/39	60/40
SGRQ total, mean (SE)	46.60 (35.88-59.41)	48.67 (36.60-60.34)	47.5 (36.1-59.9)

[1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase; SD, Standard Deviation; SE, Standard Error; SFC, Salmeterol/Fluticasone Propionate; SAL, Salmeterol; IQR, Intraquartile range; FEV_1, forced expiratory volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT

Table 3 Pooled analysis of SFC effect on mean annual moderate/severe exacerbation rate by cluster

	Cluster 1: diuretic (N=454)		Cluster 2: reversible, no diuretic (N=756)		Cluster 3: not reversible, no diuretic (N=333)	
	SFC (n=231)	**SAL (n=223)**	**SFC (n=376)**	**SAL (n=380)**	**SFC (n=164)**	**SAL (n=169)**
Mean exacerbation rate	0.95	1.66	1.12	1.66	1.26	1.26
SFC vs SAL treatment ratio	0.56		0.67		1.00	
(95% CI)	(0.47–0.71)		(0.56–0.80)		(0.74–1.36)	
p Value	<0.001		<0.001		ns	

SFC, Salmeterol/Fluticasone Propionate; SAL, Salmeterol; CI, Confidence interval; ns, not significant; Rate estimates, ratio, CI and p-value are from a negative binomial regression model.

Table 4 Baseline characteristics of interest according to cluster group

Covariate	Cluster 1: diuretic (N=454)	Cluster 2: reversible, no diuretic (N=756)	Cluster 3: not reversible, no diuretic (N=333)	p Value
Age, median years (IQR)	67 (62-74)	64 (58-70)	65 (59-71)	<.0001
Body mass index, median m/kg^2 (IQR)	28 (25-34)	26 (23-30)	25 (22-29)	<.0001
Smoking status (%)				
Former	65	58	53	0.0024
Current	35	42	47	.
Smoking, mean pack-years (IQR)	52 (40-77)	50 (37-70)	48.5 (36-70)	0.0401
FEV_1 % predicted (SD)	33.9 (25.1-42.6)	31.3 (23.9-39.4)	37.7 (29.0-44.6)	<.0001
FEV_1 % reversibility (SD)	18.55 (7.40-31.70)	26.25 (18.60-38.20)	4.50 (-1.00-8.70)	<.0001
Reversibility stratum[1] [no/yes], %	60/40	41/59	100/0	<.0001
Exacerbations requiring hospitalisation (past year) (%)				
0	73.8	78.9	76.3	ns
1	23.6	19.5	19.8	
2	2.6	1.6	3.9	
Exacerbations requiring oral steroids/antibiotics (past year) (%)				
1	62.6	61.1	67.0	ns
2	20.0	25.0	19.2	
3	8.1	7.7	7.2	
Baseline medications (%)				
Diuretics	100	0.0	0.0	<.0001
Anti-thrombotics	50.7	32.0	40.2	<.0001
ACE inhibitors	50.0	26.7	30.6	<.0001
Lipid modifiers	49.3	28.7	33.6	<.0001
Calcium channel blockers	33.5	16.3	14.1	<.0001
Psycholeptics	32.6	21.4	24.0	<.0001
Antihistamines	30.4	22.4	23.7	0.0062
Beta blockers	24.0	10.8	12.9	<.0001
Cardiac therapy	23.1	8.6	7.2	<.0001
Diabetes	17.4	7.8	8.4	<.0001
Antianemics	13.2	5.8	3.6	<.0001
Antihypertensives	7.3	3.2	1.5	<.0001

FEV_1, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant; SD, Standard Deviation; [1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

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Author contribution and role of funding source

All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. All authors helped to develop the design and concept of this analysis, had full access to and interpreted the data, and critically reviewed the manuscript and revised it for important intellectual content. All authors vouch for the accuracy and completeness of the data and the data analysis. RD co-wrote the protocol, led outline and editing of manuscript. HL co-wrote protocol,

conducted analyses, and provided methodological expertise. DR was involved in planning the analyses, interpretation of results. DS was involved in planning of analyses, interpretation of results.

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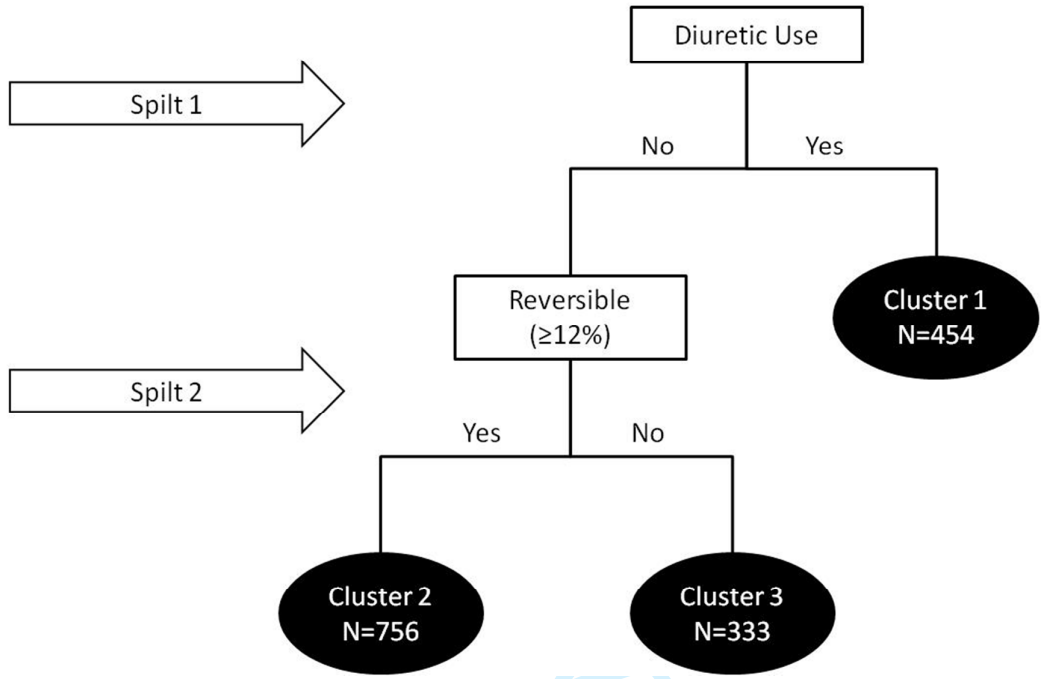
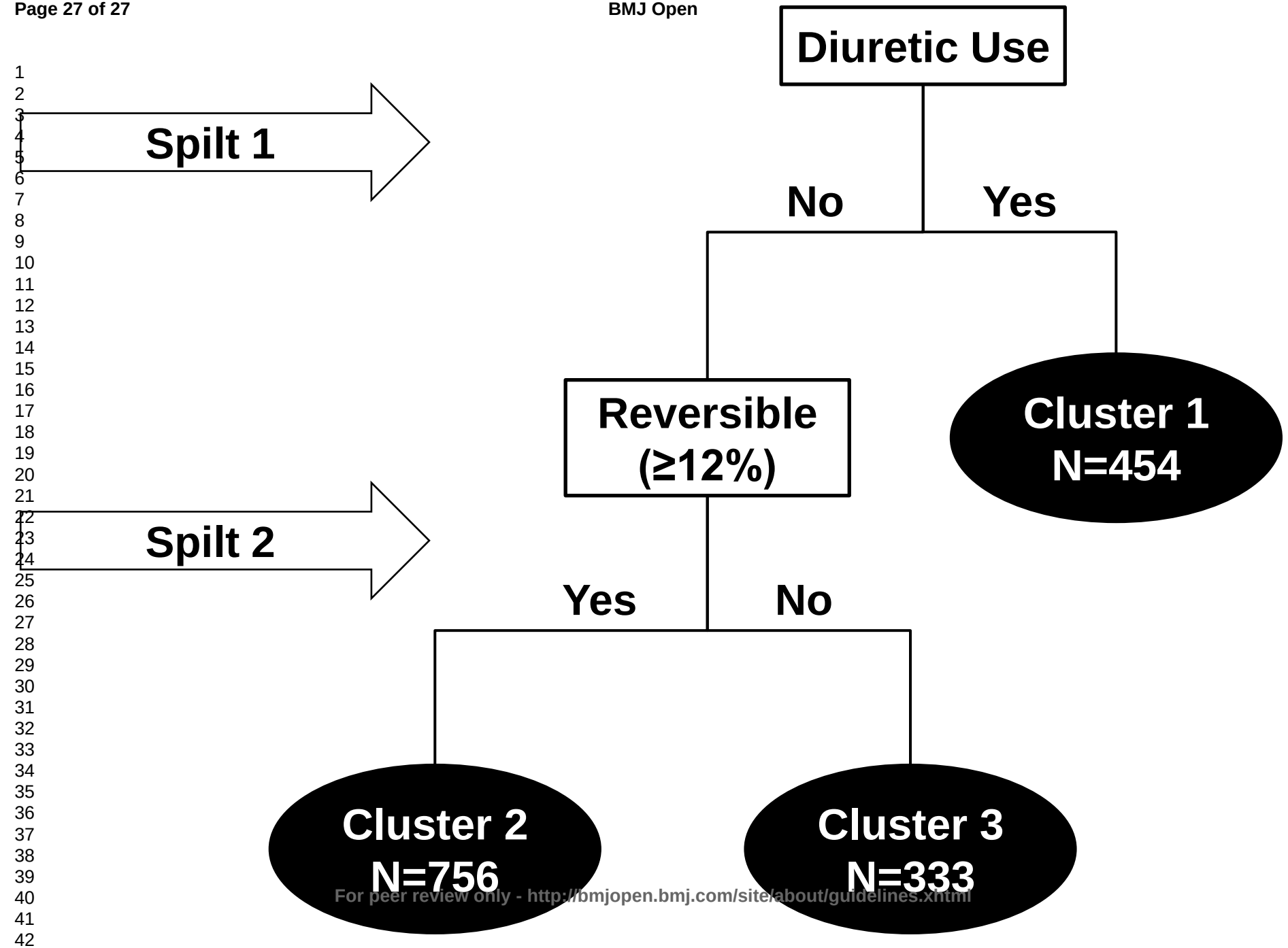
Figures

Figure 1 Interaction tree generated by supervised cluster analysis

Figure 1





Which patients with chronic obstructive pulmonary disease benefit from the addition of an inhaled corticosteroid to their bronchodilator? A cluster analysis

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Which patients with chronic obstructive pulmonary disease benefit from the addition of an inhaled corticosteroid to their bronchodilator? A cluster analysis

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ARTICLE SUMMARY

Article focus: This paper describes a cluster analysis of a pooled cohort of COPD patients receiving salmeterol (SAL) alone or in combination with fluticasone propionate (SFC) for 1 year. The analysis sought to identify clusters of patients who could benefit most from the addition of fluticasone propionate to their long-acting bronchodilator therapy based on the annual rates of moderate/severe exacerbations

Key messages: Three clusters were identified. Two clusters, patients receiving diuretics, and those not receiving diuretics but with baseline bronchodilator reversibility of ≥12% exhibited a significantly greater reduction in exacerbations when treated with SFC vs. SAL. No difference was seen between treatments in the third patient cluster - persons without bronchodilator reversibility and not receiving diuretics. These analyses highlight two strata of COPD patients who may be more likely to benefit from inhaled corticosteroid therapy combined with a long-acting ß2 agonist bronchodilator.

Strengths and limitations of this study: Pooled systematically collected data from >1500 well characterized patients from two randomized controlled trials were used in the analysis, which was validated using half of the study population. The conclusions are limited by the uncertainty of extrapolating results derived from participants enrolled in a randomized clinical trial in which exacerbation in the prior year was an entry requirement, to COPD patients in the general population.

ABSTRACT

Objective: To identify subsets of chronic obstructive pulmonary disease (COPD) patients who are more protected from exacerbations with the use of an inhaled cosrticosteroid/long-acting ß$_2$ agonist (ICS/LABA) combination, compared to the use of LABA monotherapy.

Design: Post hoc cluster analysis of patients from two randomized clinical trials of salmeterol/fluticasone propionate (SFC) and salmeterol (SAL) that had primary endpoints of moderate/severe exacerbation rates.

Setting: Centres in North America.

Participants: 1543 COPD patients were studied.

Interventions: SFC 50/250 µg or SAL 50 µg, twice daily.

Primary and secondary outcome measures: The analysis identified clusters of COPD patients more responsive to SFC versus SAL with respect to annual rate of moderate/severe exacerbations and compared their baseline clinical characteristics.

Results: Overall, SFC significantly reduced the annual rate of moderate/severe exacerbations as compared to SAL alone (rate ratio [RR]=0.701, p<0.001). Three patient clusters were identified: COPD patients receiving diuretics (RR=0.56, p<0.001); and patients not receiving diuretics but with FEV_1 reversibility ≥12% (RR=0.67, p<0.001) exhibited a substantial reduction in the annual rate of moderate/severe exacerbations relative to SAL. A third cluster, consisting of patients not receiving diuretics and without FEV_1 reversibility, demonstrated no difference for SFC versus SAL. Patients receiving diuretics had a significantly higher prevalence of comorbid cardiovascular disease.

Conclusions: COPD patients, receiving diuretics, and those not receiving diuretics but with FEV_1 reversibility >12% at baseline were significantly more likely to experience a reduction in COPD-associated exacerbations with SFC versus SAL alone.

Trial registration: NCT00115492, NCT00144911

INTRODUCTION

The *Global Strategy for the Diagnosis, Management and Prevention of COPD* (GOLD) was revised in 2011 to reflect that Forced Expiratory Volume in 1 second (FEV_1) alone is an insufficient measure of disease severity[1]. Importantly, the revised GOLD strategy document also recommends therapy with an inhaled corticosteroid/ long-acting $ß_2$ agonist (ICS/LABA) combination, or a long-acting muscarinic antagonist, for patients at risk of two or more exacerbations per year, even in the presence of mild airflow limitation. This recommendation reflects the established associations between frequent exacerbations, more rapid decline in lung function[2], and greater impairment of health status.[3 4]

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease with pulmonary and extra-pulmonary manifestations.[5] Significant in-roads have recently been made in understanding clinical subtypes and their pathophysiology,[6] and how these may contribute to the development of a customised approach to therapeutic intervention based on the patient's individual COPD phenotype.[7] Han *et al* have advocated the following process for selection of a COPD phenotype: identify a candidate phenotype, determine its relevance to clinical outcomes, and then validate the phenotype with longitudinal data collection in carefully characterised patient groups.[7] An example of such a phenotype established through this process is that of the 'frequent exacerbator' identified in the ECLIPSE cohort. In that analysis the presence of two or more exacerbations in the prior year was shown to strongly predict the occurrence of an exacerbation in the coming year.[8]

Statistical techniques may assist in the identification of COPD phenotypes, with cluster analysis being the most commonly used approach.[9 10 11 12] Cluster analysis uses algorithms to group a patient population, without an *a priori* hypothesis, into cohorts where those in the same group are more similar than they are to those in other groups. This is in contrast to subgroup analysis, where populations are pre-defined and then statistical testing is applied to identify differences. [13]

In the present study, cluster analysis was conducted using data pooled from two clinical trials that studied differences in exacerbation rates in COPD patients randomly

assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA (salmeterol/fluticasone propionate [SFC]). [14 15] The objective of this cluster analysis was to identify patients who benefit most from the addition of ICS to bronchodilator therapy in terms of the reduction of the mean annual rate of moderate/severe exacerbations for SFC compared to SAL.

METHODS

Clinical study design and subjects

The methodology for the two clinical trials has been previously published.[14 15] These were randomised, double-blind, parallel group studies comparing twice-daily SFC 50/250 µg (Seretide, Advair, GlaxoSmithKline, Research Triangle Park, NC, USA) or SAL 50 µg via DISKUS® (Seretide, Serevent, GlaxoSmithKline, Research Triangle Park, NC, USA) on the annual rate of moderate/severe exacerbations in patients with COPD.

Subjects in the United States and Canada were aged 40 years or older, with a clinical history of COPD, a pre-bronchodilator FEV_1 ≤50% of predicted, a pre-bronchodilator FEV_1/forced vital capacity (FVC) ratio of ≤70%, a cigarette smoking history of ≥10 pack-years, and a documented history of at least one moderate or severe COPD exacerbation in the year prior to screening. A moderate exacerbation was defined as requiring outpatient antibiotic and/or oral corticosteroid use, and a severe exacerbation was defined as requiring hospitalisation. Current and former smokers were included. Key exclusion criteria were a current diagnosis of asthma based on American Thoracic Society standards for diagnosis,[16] other active chronic respiratory disorders apart from COPD, a moderate/severe exacerbation that had not resolved prior to visit 1, or concurrent use of anticholinergics, theophyllines, and leukotriene modifiers, or history or current significant health conditions that could affect subject safety or effectiveness evaluation if the condition exacerbates during the study. Subjects with a history of or current clinically significant cardiac arrhythmias, uncontrolled/unstable congestive heart failure, uncontrolled hypertension, or unstable angina were excluded from the study. Subjects (n=36) with protocol violations or missing data required for the primary model were excluded (n=1543 analysed vs n=1579).

Cluster analysis methodology

The annual moderate/severe exacerbation rate was entered into a cluster analysis using an interaction tree algorithm to maximise the identification of subgroups showing differences in their response to SFC and SAL treatment. [13]

The cluster analysis aimed to find subgroups in the study subjects that had similar baseline characteristics and with maximum treatment differences for mean yearly moderate/severe exacerbation risk ratio (RR).

Subjects included in the cluster analysis were required to have the following baseline variables: FEV1 % predicted, FEV1 reversibility stratum (yes/no for ≥12% improvement and ≥200 mL), time on treatment, and geographical region. Reversibility following administration of 4 puffs of albuterol was determined prior to randomization to treatment, following completion of the 4-week FSC 250/50 run-in period. Missing values for the remaining baseline variables were imputed during cluster analysis as the median for continuous/ordinal variables, or the most frequent value for categorical variables. The baseline characteristics are listed in table 1. Baseline medications were classified by the Anatomical Therapeutic Chemical (ATC) Classification System, controlled by the World health organization. Medication are classified based on the organ or system they affect and/or their therapeutic and chemical characteristics.[17] When medications could have more than one ATC code, the second-level ATC code corresponding to the patient-supplied indication was evaluated to classify the medication (e.g, aspirin as a platelet inhibitor versus analgesic would be assigned to B01 Antithrombotic vs. N02 Analegesic).

Baseline characteristics were examined before inclusion in the model to ensure that there was no significant co-linearity that may influence the cluster analysis. Co-linearity was assessed by creating a correlation tree, and any two variables with an R^2 of ≥0.7 were examined. The variables considered most clinically relevant were retained. St. George's Respiratory Questionnaire (SGRQ) Impact and Activity scores were removed from cluster analysis since they highly correlated with the total score.

Modelling to define the tree: supervised analysis

Modified recursive partitioning techniques were used to perform the supervised subgroup analysis. The frequency of each variable was examined to identify sparse values

prior to inclusion into the tree. As the minimal subgroup size (terminal node) was set at 100, all categorical variables were required to have at least 100 subjects in a response category in order to be considered for the recursive partitioning algorithm. Variables with several responses were collapsed into fewer categories as appropriate, such that all categories had at least 100 subjects (eg, exacerbations requiring hospitalisation in the past year) or eliminated from consideration during the cluster analysis (race, anti-hemorrhagics, anti-hypertensives [eg, anti-adrenergics and smooth muscle agents], vasodilators, and vasoprotectives [eg, topical haemorrhoid treatments and anti-varicose therapy]).

The best split of the tree was determined by maximising the subgroups according to treatment interaction effect, and subgroup membership was then assigned to each patient based on the selected tree. Internal validation was performed by using a split sample, so that a random sample of 50% of the patients was selected to create the tree and the remaining half was used for the computation of rate ratios and confidence intervals (CIs) to test statistical significance.

Generalised linear models using a negative binomial function were used to compare the likelihood of having an exacerbation by examining treatment by subgroup interaction. The model was adjusted for study baseline FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change), time on treatment and geographical region (8 regions), which was considered a random effect. The algorithm used in the study maximises treatment differences (mean moderate/severe exacerbation rates for SFC vs SAL) among subgroups. The RR for each cluster was estimated using linear contrast, and rate ratios plus 95% CIs were used to estimate the differences in annual mean moderate/severe exacerbation rates for each cluster. The programme was completed in the R statistical package.[18]

Clusters were clinically characterised based on the tree. Descriptive statistics were used to present the baseline differences in clinical features among clusters; proportions were used for categorical variables, and medians with interquartile ranges were used for continuous variables. The χ^2 test was used to examine the statistical differences among the

subgroups for categorical variables and the non-parametric Wilcoxon Rank sum test was performed to test the statistical differences among the subgroups for continuous variables.

RESULTS

Pooled demographics and efficacy

Baseline characteristics of the pooled population were well matched between those receiving SFC and those receiving SAL (table 2). The majority of patients reported a moderate, and not a severe, exacerbation in the 12 months prior to study. Thirty-seven per cent of patients had 2 or more moderate exacerbations and 2% had 2 or more severe exacerbations.

In the primary studies[14 15] the annual combined moderate or severe exacerbation rates were significantly lower with SFC (1.10 and 1.06) than with SAL (1.59 and 1.53). The risk of a moderate or severe exacerbation among SFC users in the pooled study population was decreased by 30% as compared to those using SAL alone (RR=0.701, p<0.001).

Cluster analysis results

Supervised cluster analysis identified four distinct clusters based on the use of diuretics and the extent of FEV_1 reversibility, expressed solely as a percentage of the pre-bronchodilator value. Reversibility was categorised initially into three levels; <11.5%, 11.5 to 28% and >28%. When maximising differences in response to therapy with SFC versus SAL (data not shown) we pruned the tree at the ≥12% reversibility threshold as 11.5% was close to the ≥12% component of the ERS/ATS threshold for reversibility, although it should be noted that this definition also requires a volume response of ≥200 mL[19]. The initial reversibility clusters were otherwise similar with respect to baseline characteristics (data not shown). The final model used to generate the clusters had adjusted for baseline FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change), and region. Only baseline FEV_1% of predicted was statistically significant (p<0.01) in the final model.

Three final COPD clusters were defined (figure 1) based on use of diuretics and the presence or absence of ≥12% FEV_1 reversibility based on the model. The first cluster

(cluster 1) identified subjects treated with diuretics (predominantly furosemide). Approximately half of the diuretic use reported (n=282) was for hypertension, with the remaining use for unspecified oedema, coronary artery disease, and/or congestive heart failure. No other sub-populations were identified in this cluster. In patients not using diuretics at baseline two further clusters were defined based on the presence or absence of FEV_1 reversibility. Cluster 2 patients exhibited reversibility, defined as a post-bronchodilator change of ≥12%. Cluster 3 patients did not exhibit reversibility, ie, a post-bronchodilator change in FEV_1 of <12%. Compared to SAL, significant reductions in the rate of moderate/severe exacerbations were observed with SFC therapy in cluster 1 (44% reduction) and cluster 2 (33% reduction). Similar reductions were not observed in cluster 3 (figure 2)

Baseline demographics that were significantly different across clusters are presented in table 3. Subjects in cluster 1 tended to be older, had a higher BMI, were more likely to be former smokers than current smokers, and had the greatest smoking pack-year history. Cluster 1 subjects also had a higher prevalence of treatment for comorbidities (eg, cardiovascular disease [CVD], hypertension, diabetes) than clusters 2 and 3. Subjects in cluster 3 had a higher % predicted FEV_1 compared with those in clusters 1 and 2, whereas those in cluster 2 had the lowest % predicted FEV_1. No difference was observed in the baseline incidence of moderate/severe exacerbations between the clusters.

DISCUSSION

This study identified three clusters; cluster 1: diuretic users with treatment for cardiovascular comorbidity; cluster 2: reversible, not taking diuretics; cluster 3: not reversible, not taking diuretics. Subjects in clusters 1 and 2 benefited from receiving combination therapy with SFC, compared to SAL alone, with a greater reduction in exacerbations. This exercise identified two groups that are more likely to respond to SFC. The largest benefit with SFC was observed in cluster 1, but the difference in RR between clusters 1 and 2 did not quite reach statistical significance.

A number of hypotheses can be put forward to explain the lower exacerbation rates with SFC relative to SAL among diuretic users. The use of diuretics may identify a group of patients with, or at risk of, cardiovascular disease (CVD), such as those with hypertension or heart failure though with the limited data available from the source studies this cannot be confirmed. There was a significantly higher use of CVD medications in cluster 1 which suggests a preponderance of CVD diagnosis in this group; it may also suggest the presence of metabolic syndrome, as more patients in cluster 1 were in receipt of statins and ACE inhibitors than those in other clusters, furthermore the proportion of patients with diabetes was greater in cluster 1 than other clusters, as was the baseline BMI. Metabolic syndrome is more frequent among COPD than non-COPD patients, reflecting CVD and diabetes concurrent with airway obstruction.[20]

ICS (FP) could exert a benefit on exacerbations in COPD patients with CVD if 1) CVD comorbidity reflects an increased inflammatory state related to COPD[21] and 2) if CVD is a driver for COPD exacerbation occurrence[22] and severity,[23] as has been reported. It is therefore plausible to conjecture that subjects with CVD would exhibit higher levels of inflammation than those without CVD. Inflammation, as demonstrated by elevated C-reactive protein or fibrinogen, increases the risk of a COPD exacerbation[8 24] and this logic supports the value of the addition of an ICS (FP) in cluster 1.

The heterogeneity of COPD is well established[6] and it has recently been suggested, through use of a rigorous assessment of co-morbidity, that patients with CVD and metabolic

syndrome form discrete clusters of COPD patients[12] which are represented in our analysis in Cluster 1. Another assessment of co-morbidity in COPD has found that certain CVDs increase the risk of all-cause mortality in COPD;[25] Although the TORCH study failed to show a significant effect (p=0.052) of FP/SAL versus placebo for all-cause mortality[26] a subsequent analysis of CV-related mortality and AEs found a positive effect of FP/SAL versus SAL in terms of CV-related outcomes[27] which further implies a potential benefit of ICS in COPD patients with co-morbid CVD.

It is also possible that cluster 1 (diuretic) was predisposed to an increased exacerbation risk as a consequence of heart failure, especially as compared with cluster 2 and 3 more patients in cluster 1 were in receipt of anti-thrombotics, beta-blockers and cardiac therapy, all of which suggest a greater degree of heart failure in cluster 1 compared with the other clusters. Heart failure can be aggravated by increased aortic stiffness, a marker of cardiovascular risk found in greater prevalence among COPD patients than in the general population.[28] Dransfield *et al*[29] recently found that SFC lowered aortic pulse wave velocity (aPWV), a marker of aortic stiffness, in COPD patients with elevated aPWV.

Another possible explanation of the lower rate in moderate/severe exacerbations with SFC over SAL in cluster 1 reflects the direct activity of the concomitant diuretic therapy. Recent studies have examined the effectiveness of diuretics in the treatment of chronic respiratory diseases, in particular furosemide (which was the predominantly used diuretic in cluster 1). Mechanistically, furosemide inhibits inflammatory cytokines[30] and enhances the anti-inflammatory impact of ICS.[31] Clinically it has been shown to alleviate exertional dyspnoea in COPD,[32] and to protect against bronchoconstriction in asthma.[33 34 35]

The findings in cluster 1 relating to co-morbidity in COPD are of particular interest given recent publications on the prevalence and impact of co-morbidity in COPD[12 25 36] and the concept of multiple morbidities and their impact on clinical practice.[37] Certainly our findings and others[11 12] suggest that patients with multiple diseases may benefit from a different approach to management than those with a single disease, a concept which has recently been raised as a major issue in primary care.[38]

In cluster 2 (reversibility ≥12%) a significant effect of SFC was also observed over SAL in terms of a lower rate of moderate/severe exacerbations. There was also reversibility in Cluster 1 (median,18.6%). Subjects exhibiting reversibility have been shown to have greater improvement in lung function compared to those without reversibility which could explain the significant effect of SFC relative to SAL in terms of a lower rate of moderate/severe exacerbations.[39] Recent data suggest that an improvement in lung function of 100 mL relates to a reduction in exacerbation rate of 12%,[40] while a 12% increase in exacerbation rate has been reported for each 100 mL loss of lung function.[8] The effect of SFC in COPD[41] has been shown to provide a significantly greater effect on lung function in reversible versus irreversible subjects. This suggests a potential mechanism for the lower rate of moderate/severe exacerbations in cluster 1 and cluster 2 for patients receiving SFC. The rationale for this is that a greater improvement in lung function is typically associated with a greater effect on exacerbations (e.g. Jones *et al*[40]).

The role of reversibility as a distinguishing feature in COPD has recently been questioned. While it is apparent that COPD subjects can be more or less reversible, there is considerable within-patient variability both on single testing[39] and at testing on multiple occasions.[42] Subject reversibility will vary over time, such that over 1 year only 4% of patients were reversible on every occasion tested.[42] As such it has been shown that while the percentage of reversible patients is between 20-30% in any given population at any time (as was the case in these studies), the actual patients who are reversible may change. Despite the limitations with reversibility, there is evidence that subjects who are more reversible are likely to have a more robust bronchodilator response to treatment than those who are less reversible.[39 41] None of the subjects in cluster 3 exhibited reversibility by definition, and this together with the highest prevalence of current smokers (which is known to attenuate ICS effects in COPD)[43] may explain why no difference was observed between SFC and SAL in this cluster.

Although the studies excluded subjects with current asthma based on the investigator's judgment, subjects could have had a history of asthma but not a diagnosis of

active asthma. It has been suggested that Asthma and COPD form part of the same disease continuum[44] and though this is a controversial concept, the idea of an asthma-COPD overlap syndrome may give insight into the response to combination ICS/LABA.[45 46] Forty percent and 59% of patients in Clusters 1 and 2, respectively, were ≥12%reversible and had an increase of ≥200mL in FEV_1. However, the reversibility stratum (≥12% and ≥200mL) was adjusted for in the overall negative binomial model examining mean annual exacerbation rates and was found to be not statistically significant, suggesting that any impact of reversibility as determined by both ≥12% and ≥200mL was minimal.

A number of recent studies have investigated COPD heterogeneity, and have identified independent factors such as dyspnoea, airway inflammation, and asthma-like features,[10 47] or subgroups associated with differential outcome.[48 49] However, only one[11] has validated the COPD subtypes identified against clinically meaningful outcomes. Garcia-Aymerich *et al*[11] identified three clusters of subjects, comprising those with severely impaired lung function, those with more mildly impaired lung function and, importantly, those with more mildly impaired lung function *and* evidence of cardiovascular disorders, obesity, diabetes and systemic inflammation. The clusters identified in the present study align to some extent with those already identified, such as increased reversibility and the presence of CVD. This suggests a convergence of COPD subtypes that warrants further examination.

Cluster analysis is limited due to its retrospective nature and the fact that it is limited to assessing only the categorical variables collected at baseline. In addition, the splitting into groups is automated by the computer-driven algorithm to maximise treatment differences, and is not necessarily robust, thus external validation is warranted. Because this analysis includes only those patients with a history of exacerbation, it is also difficult to generalise to subjects with COPD who do not have a history of exacerbation.

In conclusion, cluster analysis of subjects taking part in two exacerbation studies of SFC versus SAL identified three distinct groups of COPD subjects based on diuretic use and reversibility. These subjects varied in their response with subjects in two of the three groups experiencing a greater reduction in the annual rate of moderate/severe exacerbations with

SFC versus SAL. Those in the remaining group received no additional benefit in terms of reduction in the annual moderate/severe exacerbation rate over that provided by SAL alone. This study highlights the future potential for a personalised medicine approach to the treatment of patients with COPD. It additionally suggests how this methodology can be used to generate potential hypothesis for future studies.

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Author contribution and role of funding source

All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. All authors helped to develop the design and concept of this analysis, had full access to and interpreted the data, and critically reviewed the manuscript and revised it for important intellectual content. All authors vouch for the accuracy and completeness of the data and the data analysis. RD co-wrote the protocol, led outline and editing of manuscript. HL co-wrote protocol, conducted analyses, and provided methodological expertise. DR was involved in planning the analyses, interpretation of

results. DS was involved in planning of analyses, interpretation of results. All authors have read and approved the final manuscript.

Licence Statement

Data sharing

No additional data available.

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Tables:

Table 1 Baseline characteristics and other variables employed in the cluster analysis

Variable	
Demographics	Age (years)
	Gender
	Smoking status (current/former)
	Pack-years
	Body mass index (m/kg^2)
Lung function/QOL	FEV_1 % predicted
	FEV_1 % reversibility
	FEV_1/FVC ratio post-albuterol
	FVC % predicted
	SGRQ activity score
	SGRQ impact score
	SGRQ symptom score
	SGRQ total score
COPD history	Duration of COPD (years)
	Chronic bronchitis (self-reported, yes/no)
	Emphysema (self-reported, yes/no)
	Exacerbations requiring hospitalisation (past 12 months)
	Exacerbations requiring OCS/antibiotic (past 12 months)
	Gold Stage indicator variables based on lung function (II, III/IV)
Medications (ATC classification)	Agents acting on the renin-angiotensin system (ACE inhibitors)
	Antianaemic preparations
	Antihaemorragics
	Antihistamines
	Antihypertensives
	Antithrombotics
	Antihaemorrhagics
	Anti-inflammatory and antirheumatic products
	Beta blockers
	Bone disease (including muscle pain) medications
	Calcium channel blockers
	Cardiac therapies
	Diabetes medications
	Diuretics
	Lipid modifying agents
	Psychoanaleptics
	Psycholeptics
	Vasodilators

ACE, angiotensin converting enzyme; ATC, Anatomical Therapeutic Chemical; COPD, Chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory Questionnaire.

Table 2 Demographic and baseline clinical characteristics of subjects participating in the primary clinical studies of SFC versus SAL (cluster analysis population)

Demographic characteristics	SFC 50/250 µg N=771	SAL N=772	TOTAL N=1543
Age, median years (IQR)	65 (59-72)	65 (59-71.5)	65 (59-72)
Gender, male/female ratio	54/46	54/46	54/46
Race, n (%)			
Caucasian	94	94	94
Non-Caucasian	6	6	6
Body mass index, mean m/kg^2 (IQR)	27 (23-31)	27 (23-30)	27 (23-31)
Smoking history, %			
Former	59	59	59
Current	41	41	41
Exacerbations requiring hospitalisation (past year) (%)			
0	78	76	77
1	20	22	21
≥2	3	2	2
Exacerbations requiring oral steroids/antibiotics (past year) (%)			
0	<1	1	1
1	65	60	63
2	20	24	22
≥3	14	14	15
FEV_1 % predicted (IQR)	33.1 (25.1-41.8)	33.8 (24.9-41.9)	33.6 (25.0-41.9)
FEV_1 % reversibility (IQR)	20.1 (9.1-33.4)	18.6 (8.5-30.5)	18.9 (8.9-31.7)
Reversibility stratum[1] [no/yes], %	58/42	61/39	60/40
SGRQ total, mean (IQR)	46.60 (35.88-59.41)	48.67 (36.60-60.34)	47.5 (36.1-59.9)

[1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase; SD, Standard Deviation; SE, Standard Error; SFC, Salmeterol/Fluticasone Propionate; SAL, Salmeterol; IQR, Intraquartile range; FEV_1, forced expiratory volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

Table 3 Baseline characteristics of interest according to cluster group

Covariate	Cluster 1: diuretic (N=454)	Cluster 2: reversible, no diuretic (N=756)	Cluster 3: not reversible, no diuretic (N=333)	p Value
Age, median years (IQR)	67 (62-74)	64 (58-70)	65 (59-71)	<.0001
Body mass index, median m/kg^2 (IQR)	28 (25-34)	26 (23-30)	25 (22-29)	<.0001
Smoking status (%)				
Former	65	58	53	0.0024
Current	35	42	47	.
Smoking, mean pack-years (IQR)	52 (40-77)	50 (37-70)	48.5 (36-70)	0.0401
FEV_1 % predicted (SD)	33.9 (25.1-42.6)	31.3 (23.9-39.4)	37.7 (29.0-44.6)	<.0001
FEV_1 % reversibility (SD)	18.55 (7.40-31.70)	26.25 (18.60-38.20)	4.50 (-1.00-8.70)	<.0001
Reversibility stratum[1] [no/yes], %	60/40	41/59	100/0	<.0001
Exacerbations requiring hospitalisation (past year) (%)				
0	73.8	78.9	76.3	ns
1	23.6	19.5	19.8	
2	2.6	1.6	3.9	
Exacerbations requiring oral steroids/antibiotics (past year) (%)				
1	62.6	61.1	67.0	ns
2	20.0	25.0	19.2	
3	8.1	7.7	7.2	
Baseline medications (%)				
Diuretics	100	0.0	0.0	<.0001
Anti-thrombotics	50.7	32.0	40.2	<.0001
ACE inhibitors	50.0	26.7	30.6	<.0001
Lipid modifiers	49.3	28.7	33.6	<.0001
Calcium channel blockers	33.5	16.3	14.1	<.0001
Psycholeptics	32.6	21.4	24.0	<.0001
Antihistamines	30.4	22.4	23.7	0.0062
Beta blockers	24.0	10.8	12.9	<.0001
Cardiac therapy	23.1	8.6	7.2	<.0001
Diabetes	17.4	7.8	8.4	<.0001
Antianemics	13.2	5.8	3.6	<.0001
Antihypertensives	7.3	3.2	1.5	<.0001

FEV_1, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant; SD, Standard Deviation; [1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

Figure Legends

Figure 1. Interaction tree generated by supervised cluster analysis

MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination

Figure 2. Pooled analysis of SFC effect on mean annual moderate/severe exacerbation rate by cluster

ns = not significant (p>0.05); SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination

Which patients with chronic obstructive pulmonary disease benefit from the addition of an inhaled corticosteroid to their bronchodilator? A cluster analysis

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Key Words: COPD, Exacerbation, Cluster Analysis, Long-acting beta-2 agonist, inhaled corticosteroid

Article focus: This paper describes a cluster analysis of a pooled cohort of COPD patients receiving salmeterol (SAL) alone or in combination with fluticasone propionate (SFC) for 1 year. The analysis sought to identify clusters of patients who could benefit most from the addition of fluticasone propionate to their long-acting bronchodilator therapy based on the annual rates of moderate/severe exacerbations

Key messages: Three clusters were identified. ~~Patients~~Two clusters, patients receiving diuretics, and those not receiving diuretics but with baseline bronchodilator reversibility of ≥12% exhibited a significantly greater reduction in exacerbations when treated with SFC vs. SAL. No difference was seen between treatments ~~for non-reversible patients~~in the third patient cluster - persons without bronchodilator reversibility and not receiving diuretics. These ~~data suggest~~analyses highlight two strata of COPD patients who ~~might~~ may be more likely to benefit ~~most~~ from inhaled corticosteroid therapy ~~in additional to~~ combined with a long-acting ß2 agonist bronchodilator ~~among COPD patients~~.

Strengths and limitations of this study: ~~Well characterised, pooled~~ Pooled systematically collected data from >1500 well characterized patients from ~~a~~two randomized controlled ~~trial~~trials were used in the analysis, which was validated using half of the study population. ~~Cluster analysis was~~ The conclusions are limited ~~to~~by the ~~patient characteristics collected in the randomized trial at baseline and generalized to those patients with COPD with a history~~uncertainty of extrapolating results derived from participants enrolled in a randomized clinical trial in which exacerbation in the prior year was an entry requirement, to COPD patients in the general population.

ABSTRACT

~~Objectives~~~~: Cluster analysis to~~**Objective**: To identify subsets of chronic obstructive pulmonary disease (COPD) patients who are more protected from exacerbations with the use of an inhaled cosrticosteroid/long-acting ß$_2$ agonist (ICS/LABA) combination, compared to the use of LABA monotherapy.

Design: Post hoc cluster analysis of patients from two ~~1-year studies~~randomized clinical trials of salmeterol/fluticasone propionate (SFC) and salmeterol (SAL) ~~with a~~that had primary ~~endpoint~~endpoints of moderate/severe ~~exacerbations~~exacerbation rates.

Setting: Centres in North America.

Participants: 1543 COPD patients were studied.

Interventions: SFC 50/250 µg or SAL 50 µg, twice daily.

Primary and secondary outcome measures: The analysis identified ~~characteristics~~clusters of COPD patients more responsive to SFC versus SAL with respect to annual rate of moderate/severe exacerbations~~.~~ and compared their baseline clinical characteristics.

Results: Overall, SFC significantly reduced the annual rate of moderate/severe exacerbations as compared to SAL alone (rate ratio [RR]=0.701, p<0.001). Three patient clusters were identified ~~using baseline characteristics.~~: COPD patients receiving diuretics (RR=0.56, p<0.001~~)~~); and patients not receiving diuretics but with FEV$_1$ reversibility ≥12% (RR=0.67, p<0.001) exhibited a ~~significant~~substantial reduction in the annual rate of moderate/severe exacerbations relative to SAL. A third cluster, consisting of patients not receiving diuretics and without FEV$_1$ reversibility, demonstrated no difference for SFC versus SAL. Patients receiving diuretics had a significantly higher prevalence of comorbid cardiovascular disease.

Conclusions: ~~Cluster analysis identified three potential~~ COPD ~~patient clusters. Those~~patients, receiving diuretics, and those not receiving diuretics but with FEV$_1$

reversibility >12% at baseline were significantly more likely to experience a reduction in COPD-associated exacerbations with SFC versus SAL alone.

Trial registration: ~~clinicaltrials.gov~~ NCT00115492, NCT00144911

INTRODUCTION

The *Global Strategy for the Diagnosis, Management and Prevention of COPD* (GOLD) was revised in 2011 to reflect that Forced Expiratory Volume in 1 second (FEV_1) alone is an insufficient ~~marker~~measure of disease severity[1]. Importantly, the revised GOLD strategy document also recommends therapy with an inhaled corticosteroid/ long-acting ß$_2$ agonist (ICS/LABA) combination, or a long-acting muscarinic antagonist, ~~is recommended~~ for patients at risk of two or more exacerbations per year, even in the presence of ~~low~~mild airflow limitation. This recommendation reflects the established ~~association~~associations between frequent exacerbations ~~and a~~, more rapid decline in lung function[2], and ~~a~~ greater impairment of health status.[3 4]

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease with pulmonary and extra-pulmonary manifestations.[5] Significant in-roads have recently been made in ~~developing an~~ understanding ~~of this complexity~~clinical subtypes and ~~heterogeneity~~their pathophysiology,[6] and how these ~~features of the disease~~ may contribute to the development of a ~~tailored~~customised approach to therapeutic intervention based on the patient's individual COPD phenotype.[7] Han *et al* have advocated the following process for selection of a COPD phenotype: identify a candidate phenotype, determine its relevance to clinical outcomes, and then validate the phenotype with longitudinal data collection in carefully characterised patient groups.[7] An example of such a phenotype established through this process is that of the 'frequent exacerbator' identified in the ECLIPSE cohort. In that analysis the presence of two or more exacerbations in the prior year was shown to strongly predict the occurrence of an exacerbation in the coming year.[8]

Statistical techniques may assist in the identification of COPD phenotypes, with cluster analysis being the most commonly used approach.[9 10 11 12] Cluster analysis uses algorithms to group ~~patients~~a patient population, without an *a priori* hypothesis, ~~in populations~~into cohorts where those in the same group are more similar than they are to

those in other groups.[12] This is in contrast to subgroup analysis, where populations are pre-defined and then statistical testing is applied to identify differences.[13]

In the present study, cluster analysis was conducted using data pooled from two clinical ~~trials~~[13,14]trials that studied differences in exacerbation rates in COPD patients randomly assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA (salmeterol/fluticasone propionate [SFC]).[14,15] The objective of ~~the~~this cluster analysis was to identify patients who benefit most from the addition of ICS to bronchodilator therapy ~~by maximising treatment differences within a cluster for~~in terms of the reduction of the mean annual rate of moderate/severe exacerbations for SFC compared to SAL.

METHODS

Clinical study design and subjects

The methodology for the two clinical trials has been previously published.[13 14 15] These were randomised, double-blind, parallel group studies comparing twice-daily SFC 50/250 µg (Seretide, Advair, GlaxoSmithKline, Research Triangle Park, NC, USA) or SAL 50 µg via DISKUS® (Seretide, Serevent, GlaxoSmithKline, Research Triangle Park, NC, USA) on the annual rate of moderate/severe exacerbations in patients with COPD.

Subjects in the United States and Canada were aged 40 years or older, with a clinical history of COPD, a pre-bronchodilator FEV_1 ≤50% of predicted, a pre-bronchodilator FEV_1/forced vital capacity (FVC) ratio of ≤70%, a cigarette smoking history of ≥10 pack-years, and a documented history of at least one moderate or severe COPD exacerbation in the year prior to screening. A moderate exacerbation was defined as requiring outpatient antibiotic and/or oral corticosteroid use, and a severe exacerbation was defined as requiring hospitalisation. Current and former smokers were included. Key exclusion criteria were a current diagnosis of asthma~~,~~ based on American Thoracic Society standards for diagnosis,[16] other active chronic respiratory disorders apart from COPD, a moderate/severe exacerbation that had not resolved prior to visit 1, or concurrent use of anticholinergics, theophyllines, and leukotriene modifiers~~.~~

~~**Cluster analysis methodology**~~

~~The annual moderate/severe exacerbation rate was entered into a cluster analysis using an interaction tree algorithm[12] to maximise the identification of subgroups showing differences in their response to SFC and SAL treatment.~~

~~The cluster analysis aimed to find subgroups in the study subjects that had similar baseline characteristics and with maximum treatment differences for mean yearly moderate/severe exacerbation risk ratio (RR).~~, or history or current significant health conditions that could affect subject safety or effectiveness evaluation if the condition

exacerbates during the study. Subjects with a history of or current clinically significant cardiac arrhythmias, uncontrolled/unstable congestive heart failure, uncontrolled hypertension, or unstable angina were excluded from the study. Subjects (n=36) with protocol violations or missing data required for the primary model were excluded (n=1543 analysed vs n=1579 ~~enrolled).~~).

Cluster analysis methodology

The annual moderate/severe exacerbation rate was entered into a cluster analysis using an interaction tree algorithm to maximise the identification of subgroups showing differences in their response to SFC and SAL treatment. [13]

The cluster analysis aimed to find subgroups in the study subjects that had similar baseline characteristics and with maximum treatment differences for mean yearly moderate/severe exacerbation risk ratio (RR).

Subjects included in the cluster analysis were required to have the following baseline variables ~~comprising~~: FEV1 % predicted, FEV1 reversibility stratum (yes/no for ≥12% improvement and ≥200 mL), time on treatment, and geographical region. Reversibility following administration of 4 puffs of albuterol was determined prior to randomization to treatment, following completion of the 4-week FSC 250/50 run-in period. Missing values for the remaining baseline variables were imputed during cluster analysis as the median for continuous/ordinal variables, or the most frequent value for categorical variables. The baseline characteristics are listed in table 1. Baseline medications were classified by the Anatomical Therapeutic Chemical (ATC) Classification System, controlled by the World health organization. Medication are classified based on the organ or system they affect and/or their therapeutic and chemical characteristics.[17] When medications could have more than one ATC code, the second-level ATC code corresponding to the patient-supplied indication was evaluated to classify the medication

(e.g, aspirin as a platelet inhibitor versus analgesic would be assigned to B01 Antithrombotic vs. N02 Analegesic).

Baseline characteristics were examined before inclusion in the model to ensure that there was no significant co-linearity that may influence the cluster analysis. Co-linearity was assessed by creating a correlation tree, and any two variables with an R^2 of ≥0.7 were examined. The variables considered most clinically relevant were retained. St. George's Respiratory Questionnaire (SGRQ) Impact and Activity scores were removed from cluster analysis since they highly correlated with the total score.

Modelling to define the tree: supervised analysis

Modified recursive partitioning techniques were used to perform the supervised subgroup analysis. The frequency of each variable was examined to identify sparse values prior to inclusion into the tree. As the minimal subgroup size (terminal node) was set at 100, all categorical variables were required to have at least 100 subjects in a response category in order to be considered for the recursive partitioning algorithm. Variables with several responses were collapsed into fewer categories as appropriate, such that all categories had at least 100 subjects (eg, exacerbations requiring hospitalisation in the past year) or eliminated from consideration during the cluster analysis (race, anti-hemorrhagics, anti-hypertensives [eg, anti-adrenergics and smooth muscle agents], vasodilators, and vasoprotectives [eg, topical haemorrhoid treatments and anti-varicose therapy]).

The best split of the tree was determined by maximising the subgroups according to treatment interaction effect, and subgroup membership was then assigned to each patient based on the selected tree. Internal validation was performed by using a split sample, so that a random sample of 50% of the patients was selected to create the tree and the remaining half was used for the computation of rate ratios and confidence intervals (CIs) to test statistical significance.

Generalised linear models using a negative binomial function were used to compare the likelihood of having an exacerbation by examining treatment by subgroup interaction.

The model was adjusted for study baseline FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change), time on treatment and geographical region (8 regions), which was considered a random effect. The algorithm used in the study maximises treatment differences (mean moderate/severe exacerbation rates for SFC vs SAL) among subgroups. The RR for each cluster was estimated using linear contrast, and rate ratios plus 95% CIs were used to estimate the differences in annual mean moderate/severe exacerbation rates for each cluster. The programme was completed in the R statistical package.[~~15~~18]

Clusters were clinically characterised based on the tree~~, using descriptive statistics.~~. Descriptive statistics were used to present the baseline differences in clinical features among clusters; proportions were used for categorical variables, and medians with interquartile ranges were used for continuous variables. The χ^2 test was used to examine the statistical differences among the subgroups for categorical variables and the non-parametric Wilcoxon Rank sum test was performed to test the statistical differences among the subgroups for continuous variables.

RESULTS

Pooled demographics and efficacy

Baseline characteristics of the pooled population were well matched between those receiving SFC and those receiving SAL (table 2). The majority of patients reported a moderate, and not a severe, exacerbation in the 12 months prior to study. Thirty-seven per cent of patients had 2 or more moderate exacerbations and 2% had 2 or more severe exacerbations.

In the primary ~~studies[13 14]~~studies[14 15] the annual combined moderate~~/~~ or severe exacerbation rates were significantly lower with SFC (1.10 and 1.06) than with SAL (1.59 and 1.53). ~~A treatment effect was observed~~The risk of a moderate or severe exacerbation among SFC users in the pooled study population ~~(1.10 vs 1.58 for SFC and~~was decreased by 30% as compared to those using SAL ~~respectively,~~ alone (RR=0.701, p<0.001).

Cluster analysis results

Supervised cluster analysis identified four distinct clusters based on the use of diuretics and the extent of FEV_1 reversibility, expressed solely as a percentage of the pre-bronchodilator value. Reversibility was categorised initially into three levels; <11.5%, 11.5 to 28% and >28%. When maximising differences in response to therapy with SFC versus SAL (data not shown) we pruned the tree at the ≥12% reversibility threshold as 11.5% was close to the ≥12% component of the ERS/ATS threshold for reversibility, although it should be noted that this definition also requires a volume response of ≥200 ~~mL.~~[16]mL[19]. The initial reversibility clusters were otherwise similar with respect to baseline characteristics (data not shown). The final model used to generate the clusters had adjusted for baseline FEV_1 % predicted, FEV_1 reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change), and region. Only baseline FEV_1% of predicted was statistically significant (p<0.01) in the final model.

Three final COPD clusters were defined (figure 1) based on use of diuretics and the presence or absence of ≥12% FEV_1 reversibility based on the model. The first cluster (cluster 1) identified subjects treated with diuretics (predominantly furosemide). Approximately half of the diuretic use reported (n=282) was for hypertension, with the remaining use for unspecified oedema, coronary artery disease, and/or congestive heart failure. No other sub-populations were identified in this cluster. In patients not using diuretics at baseline two further clusters were defined based on the presence or absence of FEV_1 reversibility. Cluster 2 patients exhibited reversibility, defined as a post-bronchodilator change of ≥12%. Cluster 3 patients did not exhibit reversibility, ie, a post-bronchodilator change in FEV_1 of <12%. Compared to SAL, significant reductions in the rate of moderate/severe exacerbations were observed with SFC therapy in cluster 1 (44% reduction) and cluster 2 (33% reduction). Similar reductions were not observed in cluster 3 (~~table 3).~~ figure 2)

Baseline demographics that were significantly different across clusters are presented in table ~~4~~3. Subjects in cluster 1 tended to be older, had a higher BMI, were more likely to be former smokers than current smokers, and had the greatest smoking pack-year history. Cluster 1 subjects also had a higher prevalence of treatment for comorbidities (eg, cardiovascular disease [CVD], hypertension, diabetes) than clusters 2 and 3. Subjects in cluster 3 had a higher % predicted FEV_1 compared with those in clusters 1 and 2, whereas those in cluster 2 had the lowest % predicted FEV_1. No difference was observed in the baseline incidence of moderate/severe exacerbations between the clusters.

DISCUSSION

This study identified three clusters; cluster 1: diuretic users with treatment for cardiovascular comorbidity; cluster 2: reversible, not taking diuretics; cluster 3: not reversible, not taking diuretics. Subjects in clusters 1 and 2 benefited ~~more~~ from receiving combination therapy with SFC, compared to SAL alone, with a greater reduction in ~~exacerbation~~exacerbations. This exercise identified two groups that are more likely to respond to SFC. The largest benefit with SFC was observed in cluster 1~~.~~, but the difference in RR between clusters 1 and 2 did not quite reach statistical significance.

A number of hypotheses can be put forward to explain the lower exacerbation rates with SFC relative to SAL among diuretic users. The use of diuretics may identify a group of patients with, or at risk of, cardiovascular disease~~,~~ (CVD), such as those with hypertension or heart failure~~, who may be more responsive to~~ though with the ~~addition of an ICS to a LABA.~~limited data available from the source studies this cannot be confirmed. There was a significantly higher use of ~~cardiovascular (~~CVD~~)~~ medications in cluster 1~~.~~ which suggests a preponderance of CVD diagnosis in this group; it may also suggest the presence of metabolic syndrome, as more patients in cluster 1 were in receipt of statins and ACE inhibitors than those in other clusters, furthermore the proportion of patients with diabetes was greater in cluster 1 than other clusters, as was the baseline BMI. Metabolic syndrome is more frequent among COPD than non-COPD patients, reflecting CVD and diabetes concurrent with airway obstruction.[20]

ICS (FP) could exert a benefit on exacerbations in COPD patients with CVD if 1) CVD comorbidity reflects an increased inflammatory state related to ~~COPD~~[17]COPD[21] and 2) if CVD is a driver for COPD exacerbation ~~occurrence~~[18]occurrence[22] and ~~severity~~[19]severity,[23] as has been reported. It is therefore plausible to conjecture that subjects with CVD would exhibit higher levels of inflammation than those without CVD. Inflammation, as demonstrated by elevated C-reactive protein or fibrinogen, increases the

risk of a COPD exacerbation[8 ~~20~~24] and this logic supports the value of the addition of an ICS (FP) in cluster 1.

The heterogeneity of COPD is well established[6] and it has recently been suggested, through use of a rigorous assessment of co-morbidity, that patients with CVD and metabolic syndrome form discrete clusters of COPD patients[12] which are represented in our analysis in Cluster 1. Another assessment of co-morbidity in COPD has found that certain CVDs increase the risk of all-cause mortality in COPD;[25] Although the TORCH study failed to show a significant effect (p=0.052) of FP/SAL versus placebo for all-cause mortality[26] a subsequent analysis of CV-related mortality and AEs found a positive effect of FP/SAL versus SAL in terms of CV-related outcomes[27] which further implies a potential benefit of ICS in COPD patients with co-morbid CVD.

It is also possible that cluster 1 (diuretic) was predisposed to an increased exacerbation risk as a consequence of heart failure~~.~~, especially as compared with cluster 2 and 3 more patients in cluster 1 were in receipt of anti-thrombotics, beta-blockers and cardiac therapy, all of which suggest a greater degree of heart failure in cluster 1 compared with the other clusters. Heart failure can be aggravated by increased aortic stiffness, a marker of cardiovascular risk found in greater prevalence among COPD patients than in the general population.[~~21~~28] Dransfield *et ~~al~~ al*[~~22~~29] recently found that SFC lowered aortic pulse wave velocity (aPWV), a marker of aortic stiffness, in COPD patients with elevated aPWV.

Another possible explanation of the lower rate in moderate/severe exacerbations with SFC over SAL in cluster 1 reflects the direct activity of the concomitant diuretic therapy. Recent studies have examined the effectiveness of diuretics in the treatment of chronic respiratory diseases, in particular furosemide (which was the predominantly used diuretic in cluster 1). Mechanistically, furosemide inhibits inflammatory ~~cytokines~~[23]cytokines[30] and enhances the anti-inflammatory impact of ICS.[~~24~~31] Clinically it has been shown to alleviate exertional dyspnoea in COPD,[~~25~~32] and to protect against bronchoconstriction in asthma.[~~26 27 28~~33 34 35]

The findings in cluster 1 relating to co-morbidity in COPD are of particular interest given recent publications on the prevalence and impact of co-morbidity in COPD[12 25 36] and the concept of multiple morbidities and their impact on clinical practice.[37] Certainly our findings and others[11 12] suggest that patients with multiple diseases may benefit from a different approach to management than those with a single disease, a concept which has recently been raised as a major issue in primary care.[38]

In cluster 2 (reversibility ≥12%) a significant effect of SFC was also observed over SAL in terms of a lower rate of moderate/severe exacerbations. There was also reversibility in Cluster 1 (median,18.6%). Subjects exhibiting reversibility have been shown to have greater improvement in lung function compared to those without reversibility~~.~~[29] which could explain the significant effect of SFC relative to SAL in terms of a lower rate of moderate/severe exacerbations.[39] Recent data suggest that an improvement in lung function of 100 mL relates to a reduction in exacerbation rate of 12%,[30~~40~~] while a 12% increase in exacerbation rate has been reported for each 100 mL loss of lung function.[8] The effect of SFC in ~~COPD~~[31]COPD[41] has been shown to provide a significantly greater effect on lung function in reversible versus irreversible subjects. This suggests a potential mechanism for the lower rate of moderate/severe exacerbations in cluster ~~2 patients receiving SFC.~~1 and cluster 2 for patients receiving SFC. The rationale for this is that a greater improvement in lung function is typically associated with a greater effect on exacerbations (e.g. Jones *et al*[40]).

The role of reversibility as a distinguishing feature in COPD has recently been questioned. While it is apparent that COPD subjects can be more or less reversible, there is considerable within-patient variability both on single testing[39] and at testing on multiple occasions.[42] Subject reversibility will vary over time, such that over 1 year only 4% of patients were reversible on every occasion tested.[42] As such it has been shown that while the percentage of reversible patients is between 20-30% in any given population at any time (as was the case in these studies), the actual patients who are reversible may change.

Despite the limitations with reversibility, there is evidence that subjects who are more reversible are likely to have a more robust bronchodilator response to treatment than those who are less reversible.[39 41] None of the subjects in cluster 3 exhibited reversibility by definition, and this together with the highest prevalence of current smokers (which is known to attenuate ICS effects in COPD)[32 43] may explain why no difference was observed between SFC and SAL in this cluster.

Although the studies excluded subjects with current asthma based on the investigator's judgment, subjects could have had a history of asthma but not a diagnosis of active asthma. It has been suggested that Asthma and COPD form part of the same disease continuum[44] and though this is a controversial concept, the idea of an asthma-COPD overlap syndrome may give insight into the response to combination ICS/LABA.[45 46] Forty percent and 59% of patients in Clusters 1 and 2, respectively, were ≥12%reversible and had an increase of ≥200mL in FEV_1. However, the reversibility stratum (≥12% and ≥200mL) was adjusted for in the overall negative binomial model examining mean annual exacerbation rates and was found to be not statistically significant, suggesting that any impact of reversibility as determined by both ≥12% and ≥200mL was minimal.

A number of recent studies have investigated COPD heterogeneity, and have identified independent factors such as dyspnoea, airway inflammation, and asthma-like features,[10 33 47] or subgroups associated with differential outcome.[34 35 48 49] However, only ~~one~~[36] one[11] has validated the COPD subtypes identified against clinically meaningful outcomes. Garcia-Aymerich *et ~~al~~*[36] *al*[11] identified three clusters of subjects, comprising those with severely impaired lung function, those with more mildly impaired lung function and, importantly, those with more mildly impaired lung function *and* evidence of cardiovascular disorders, obesity, diabetes and systemic inflammation. The clusters identified in the present study align to some extent with those already identified, such as increased reversibility and the presence of CVD. This suggests a convergence of COPD subtypes that warrants further examination.

Cluster analysis is limited due to its retrospective nature and the fact that it is limited to assessing only the categorical variables collected at baseline. In addition, the splitting into groups is automated by the computer-driven algorithm to maximise treatment differences, and is not necessarily robust, thus external validation is warranted. Because this analysis includes only those patients with a history of exacerbation, it is also difficult to generalise to subjects with COPD who do not have a history of exacerbation.

In conclusion, cluster analysis of subjects taking part in two exacerbation studies of SFC versus SAL identified three distinct groups of COPD subjects based on diuretic use and reversibility. These subjects varied in their response with subjects in two of the three groups experiencing a greater reduction in the annual rate of moderate/severe exacerbations with SFC versus SAL. Those in the remaining group received no additional benefit in terms of reduction in the annual moderate/severe exacerbation rate over that provided by SAL alone. This study highlights the future potential for a personalised medicine approach to the treatment of patients with COPD. It additionally suggests how this methodology can be used to generate potential hypothesis for future studies.

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All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. All authors helped to develop the design and concept of this analysis, had full access to and interpreted the data, and critically reviewed the manuscript and revised it for important intellectual content. All authors vouch for the accuracy and completeness of the data and the data analysis. RD co-wrote the protocol, led outline and editing of manuscript. HL co-wrote protocol, conducted analyses, and provided

methodological expertise. DR was involved in planning the analyses, interpretation of results. DS was involved in planning of analyses, interpretation of results.

Licence Statement

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Tables:

Table 1 Baseline characteristics and other variables employed in the cluster analysis

Variable	
Demographics	Age (years)
	Gender
	Smoking status (current/former)
	Pack-years
	Body mass index (m/kg^2)
Lung function/QOL	FEV_1 % predicted
	FEV_1 % reversibility
	FEV_1/FVC ratio post-albuterol
	FVC % predicted
	SGRQ activity score
	SGRQ impact score
	SGRQ symptom score
	SGRQ total score
COPD history	Duration of COPD (years)
	Chronic bronchitis (self-reported, yes/no)
	Emphysema (self-reported, yes/no)
	Exacerbations requiring hospitalisation (past 12 months)
	Exacerbations requiring OCS/antibiotic (past 12 months)
	Gold Stage indicator variables based on lung function (II, III/IV)
Medications (ATC classification)	Agents acting on the renin-angiotensin system (ACE inhibitors)
	Antianaemic preparations
	Antihaemorragics
	Antihistamines
	Antihypertensives
	Antithrombotics
	Antihaemorrhagics
	Anti-inflammatory and antirheumatic products
	Beta blockers
	Bone disease (including muscle pain) medications
	Calcium channel blockers
	Cardiac therapies
	Diabetes medications
	Diuretics
	Lipid modifying agents
	Psychoanaleptics
	Psycholeptics
	Vasodilators

ACE, angiotensin converting enzyme; ATC, Anatomical Therapeutic Chemical; COPD, Chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory Questionnaire.

Table 2 Demographic and baseline clinical characteristics of subjects participating in the primary clinical studies of SFC versus SAL (cluster analysis population)

Demographic characteristics	SFC 50/250 µg N=771	SAL N=772	TOTAL N=1543
Age, median years (IQR)	65 (59-72)	65 (59-71.5)	65 (59-72)
Gender, male/female ratio	54/46	54/46	54/46
Race, n (%)			
Caucasian	94	94	94
Non-Caucasian	6	6	6
Body mass index, mean m/kg^2 (IQR)	27 (23-31)	27 (23-30)	27 (23-31)
Smoking history, %			
Former	59	59	59
Current	41	41	41
Exacerbations requiring hospitalisation (past year) (%)			
0	78	76	77
1	20	22	21
≥2	3	2	2
Exacerbations requiring oral steroids/antibiotics (past year) (%)			
0	<1	1	1
1	65	60	63
2	20	24	22
≥3	14	14	15
FEV_1 % predicted (IQR)	33.1 (25.1-41.8)	33.8 (24.9-41.9)	33.6 (25.0-41.9)
FEV_1 % reversibility (IQR)	20.1 (9.1-33.4)	18.6 (8.5-30.5)	18.9 (8.9-31.7)
Reversibility stratum[1] [no/yes], %	58/42	61/39	60/40
SGRQ total, mean (IQR)	46.60 (35.88-59.41)	48.67 (36.60-60.34)	47.5 (36.1-59.9)

[1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase; SD, Standard Deviation; SE, Standard Error; SFC, Salmeterol/Fluticasone Propionate; SAL, Salmeterol; IQR, Intraquartile range; FEV_1, forced expiratory volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

Table 3 Baseline characteristics of interest according to cluster group

Covariate	Cluster 1: diuretic (N=454)	Cluster 2: reversible, no diuretic (N=756)	Cluster 3: not reversible, no diuretic (N=333)	p Value
Age, median years (IQR)	67 (62-74)	64 (58-70)	65 (59-71)	<.0001
Body mass index, median m/kg^2 (IQR)	28 (25-34)	26 (23-30)	25 (22-29)	<.0001
Smoking status (%)				
Former	65	58	53	0.0024
Current	35	42	47	.
Smoking, mean pack-years (IQR)	52 (40-77)	50 (37-70)	48.5 (36-70)	0.0401
FEV_1 % predicted (SD)	33.9 (25.1-42.6)	31.3 (23.9-39.4)	37.7 (29.0-44.6)	<.0001
FEV_1 % reversibility (SD)	18.55 (7.40-31.70)	26.25 (18.60-38.20)	4.50 (-1.00-8.70)	<.0001
Reversibility stratum[1] [no/yes], %	60/40	41/59	100/0	<.0001
Exacerbations requiring hospitalisation (past year) (%)				
0	73.8	78.9	76.3	ns
1	23.6	19.5	19.8	
2	2.6	1.6	3.9	
Exacerbations requiring oral steroids/antibiotics (past year) (%)				
1	62.6	61.1	67.0	ns
2	20.0	25.0	19.2	
3	8.1	7.7	7.2	
Baseline medications (%)				
Diuretics	100	0.0	0.0	<.0001
Anti-thrombotics	50.7	32.0	40.2	<.0001
ACE inhibitors	50.0	26.7	30.6	<.0001
Lipid modifiers	49.3	28.7	33.6	<.0001
Calcium channel blockers	33.5	16.3	14.1	<.0001
Psycholeptics	32.6	21.4	24.0	<.0001
Antihistamines	30.4	22.4	23.7	0.0062
Beta blockers	24.0	10.8	12.9	<.0001
Cardiac therapy	23.1	8.6	7.2	<.0001
Diabetes	17.4	7.8	8.4	<.0001
Antianemics	13.2	5.8	3.6	<.0001
Antihypertensives	7.3	3.2	1.5	<.0001

FEV_1, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant; SD, Standard Deviation; [1]Reversibility based on change in FEV_1 from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

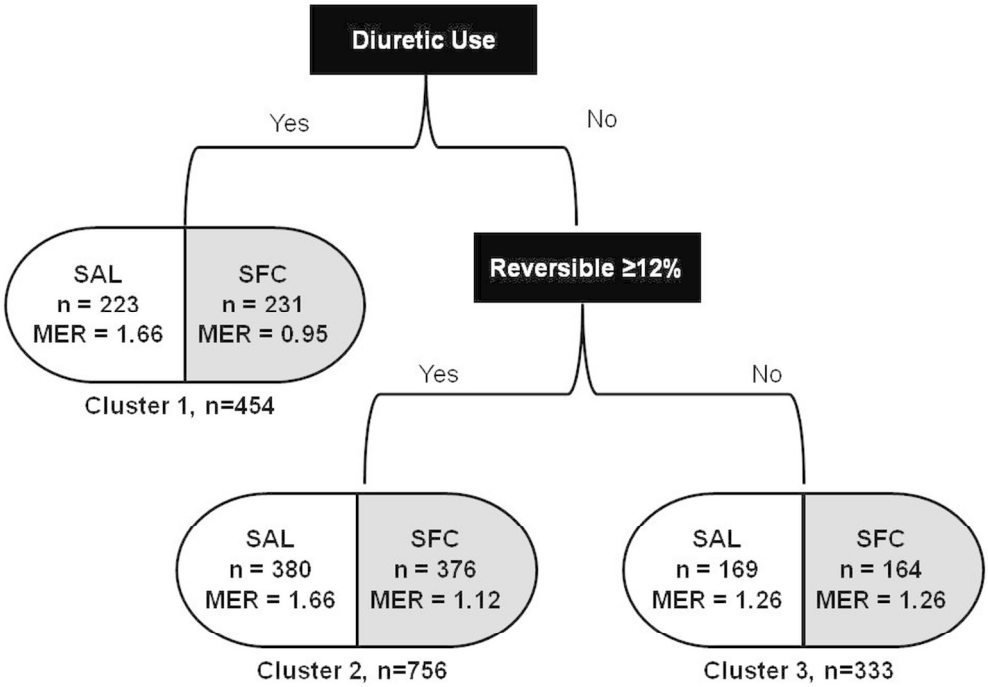
Figure Legends

Figure 1. Interaction tree generated by supervised cluster analysis

MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination

Figure 2. Pooled analysis of SFC effect on mean annual moderate/severe exacerbation rate by cluster

ns = not significant (p>0.05); SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination



MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination
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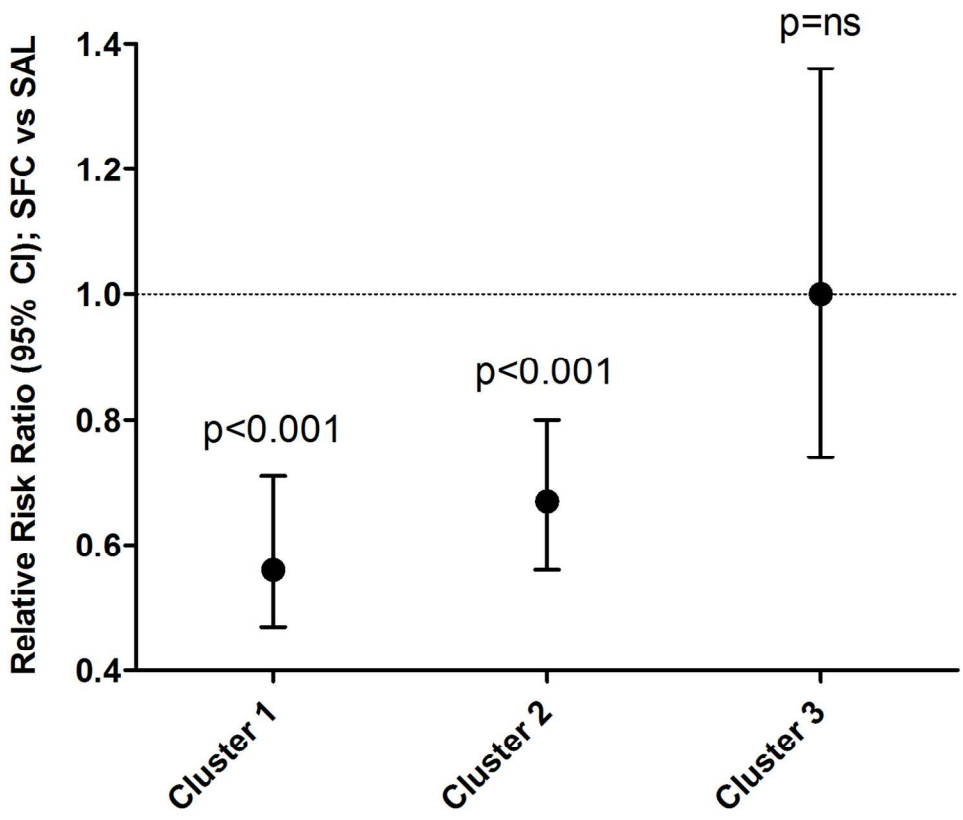

ns = not significant (p>0.05); SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination
128x111mm (300 x 300 DPI)