



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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-001838
Article Type:	Research
Date Submitted by the Author:	19-Jul-2012
Complete List of Authors:	DiSantostefano, Rachael; GlaxoSmithKline, Li, Hao; GlaxoSmithKline, Rubin, David; GlaxoSmithKline, Stempel, David; GlaxoSmithKline,
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Respiratory medicine
Keywords:	Chronic airways disease < THORACIC MEDICINE, Exacerbation, Long acting beta2 agonist, inhaled corticosteroid

SCHOLARONE™
Manuscripts

FINAL DRAFT

1
2
3 1 **Which patients with chronic obstructive pulmonary disease benefit**
4 **from the addition of an inhaled corticosteroid to their**
5 **bronchodilator? A cluster analysis**
6
7
8
9
10 4

11
12 5 Rachael L DiSantostefano,¹ Hao Li,¹ David Rubin,¹ David Stempel¹
13

14 ¹*GlaxoSmithKline, Research Triangle Park, Durham, North Carolina, USA*
15
16
17 7

18
19
20 8 **Correspondence to**

21
22 9 R L DiSantostefano

23
24 10 GlaxoSmithKline,

25
26 11 5 Moore Drive

27
28 12 Research Triangle Park,

29
30 13 Durham, North Carolina,

31
32 14 27709, USA

33
34 15 email; rachael.l.disantostefano@gsk.com

35
36 16 Tel: 00 1 919 483 9237

37
38 17 Fax: 00 1 919 549 7459

39
40 18 **Current Word Count:** 2739

41
42 19 **Key Words:** COPD, Exacerbation, Cluster Analysis, Long-acting beta-2 agonist,
43
44 20 inhaled corticosteroid
45
46
47
48
49
50
51 21

FINAL DRAFT

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

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

33 **Strengths and limitations of this study:** Well characterised, pooled data from
34 >1500 patients from a randomized controlled trial were used in the analysis, which
35 was validated using half of the study population. Cluster analysis was limited to the
36 patient characteristics collected in the randomized trial at baseline and generalized
37 to those patients with COPD with a history of exacerbation.

38

FINAL DRAFT

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 corticosteroid/long-acting β_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 FEV₁ reversibility $\geq 12\%$ (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 FEV₁ 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 FEV₁ reversibility

FINAL DRAFT

1
2
3 64 were significantly more likely to experience a reduction in COPD-associated
4
5 65 exacerbations with SFC versus SAL.
6

7 66 **Trial registration:** clinicaltrials.gov NCT00115492, NCT00144911
8

9
10 67

11
12 68
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

FINAL DRAFT

1
2
3 69 **INTRODUCTION**

4
5 70 The *Global Strategy for the Diagnosis, Management and Prevention of COPD*
6
7 71 (GOLD) was revised in 2011 to reflect that Forced Expiratory Volume in 1 second
8
9 72 (FEV₁) alone is an insufficient marker of disease severity¹. Importantly, therapy with
10
11 73 an inhaled corticosteroid/ long-acting β_2 agonist (ICS/LABA) combination, or a long-
12
13 74 acting muscarinic antagonist, is recommended for patients at risk of two or more
14
15 75 exacerbations per year, even in the presence of low airflow limitation. This
16
17 76 recommendation reflects the established association between frequent
18
19 77 exacerbations and a more rapid decline in lung function², and a greater impairment
20
21 78 of health status.^{3 4}

22
23
24
25 79 Chronic obstructive pulmonary disease (COPD) is a complex and
26
27 80 heterogeneous disease with pulmonary and extra-pulmonary manifestations.⁵
28
29 81 Significant in-roads have recently been made in developing an understanding of this
30
31 82 complexity and heterogeneity,⁶ and how these features of the disease may
32
33 83 contribute to the development of a tailored approach to therapeutic intervention
34
35 84 based on the patient's individual COPD phenotype.⁷ Han *et al* have advocated the
36
37 85 following process for selection of a COPD phenotype: identify a candidate
38
39 86 phenotype, determine its relevance to clinical outcomes, and then validate the
40
41 87 phenotype with longitudinal data collection in carefully characterised patient groups.⁷
42
43 88 An example of such a phenotype established through this process is that of the
44
45 89 'frequent exacerbator' identified in the ECLIPSE cohort. In that analysis the presence
46
47 90 of two or more exacerbations in the prior year was shown to strongly predict the
48
49 91 occurrence of an exacerbation in the coming year.⁸

50
51
52 92 Statistical techniques may assist in the identification of COPD phenotypes,
53
54 93 with cluster analysis being the most commonly used approach.^{9 10 11} Cluster analysis
55
56
57
58
59
60

FINAL DRAFT

1
2
3 94 uses algorithms to group patients, without an *a priori* hypothesis, in populations
4
5 95 where those in the same group are more similar than they are to those in other
6
7 96 groups.¹² This is in contrast to subgroup analysis, where populations are pre-defined
8
9
10 97 and then statistical testing is applied to identify differences.

11
12 98 In the present study, cluster analysis was conducted using data pooled from
13
14 99 two clinical trials^{13 14} that studied differences in exacerbation rates in COPD patients
15
16 100 randomly assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA
17
18 101 (salmeterol/fluticasone propionate [SFC]). The objective of the cluster analysis was
19
20
21 102 to identify patients who benefit most from the addition of ICS to bronchodilator
22
23 103 therapy by maximising treatment differences within a cluster for mean annual rate of
24
25 104 moderate/severe exacerbations for SFC compared to SAL.
26
27
28 105

FINAL DRAFT

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 FEV₁ ≤50% of predicted, a pre-bronchodilator FEV₁/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 algorithm¹² 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

FINAL DRAFT

1
2
3 131 violations or missing data required for the primary model were excluded (n=1543
4
5 132 analysed vs n=1579 enrolled). Subjects included in the cluster analysis were
6
7 133 required to have baseline variables comprising FEV₁ % predicted, FEV₁ reversibility
8
9 134 stratum (yes/no for ≥12% improvement and ≥200 mL), time on treatment, and
10
11 135 geographical region. Missing values for the remaining baseline variables were
12
13 136 imputed during cluster analysis as the median for continuous/ordinal variables, or the
14
15 137 most frequent value for categorical variables. The baseline characteristics are listed
16
17 138 in table 1.

19
20
21 139 Baseline characteristics were examined before inclusion in the model to
22
23 140 ensure that there was no significant co-linearity that may influence the cluster
24
25 141 analysis. Co-linearity was assessed by creating a correlation tree, and any two
26
27 142 variables with an R² of ≥0.7 were examined. The variables considered most clinically
28
29 143 relevant were retained. St. George's Respiratory Questionnaire (SGRQ) Impact and
30
31 144 Activity scores were removed from cluster analysis since they highly correlated with
32
33 145 the total score.
34
35
36
37

38 147 **Modelling to define the tree: supervised analysis**

39
40
41 148 Modified recursive partitioning techniques were used to perform the supervised
42
43 149 subgroup analysis. The frequency of each variable was examined to identify sparse
44
45 150 values prior to inclusion into the tree. As the minimal subgroup size (terminal node)
46
47 151 was set at 100, all categorical variables were required to have at least 100 subjects
48
49 152 in a response category in order to be considered for the recursive partitioning
50
51 153 algorithm. Variables with several responses were collapsed into fewer categories as
52
53 154 appropriate, such that all categories had at least 100 subjects (eg, exacerbations
54
55 155 requiring hospitalisation in the past year) or eliminated from consideration during the
56
57
58
59
60

FINAL DRAFT

1
2
3 156 cluster analysis (race, anti-hemorrhagics, anti-hypertensives [eg, anti-adrenergics
4
5 157 and smooth muscle agents], vasodilators, and vasoprotectives [eg, topical
6
7 158 haemorrhoid treatments and anti-varicose therapy]).

9
10 159 The best split of the tree was determined by maximising the subgroups
11
12 160 according to treatment interaction effect, and subgroup membership was then
13
14 161 assigned to each patient based on the selected tree. Internal validation was
15
16 162 performed by using a split sample, so that a random sample of 50% of the patients
17
18 163 was selected to create the tree and the remaining half was used for the computation
19
20
21 164 of rate ratios and confidence intervals (CIs) to test statistical significance.

22
23 165 Generalised linear models using a negative binomial function were used to
24
25 166 compare the likelihood of having an exacerbation by examining treatment by
26
27 167 subgroup interaction. The model was adjusted for study baseline FEV₁ % predicted,
28
29 168 FEV₁ reversibility stratum (yes/no for ≥12% and 200 mL post-bronchodilator change),
30
31 169 time on treatment and geographical region (8 regions), which was considered a
32
33
34 170 random effect. The algorithm used in the study maximises treatment differences
35
36 171 (mean moderate/severe exacerbation rates for SFC vs SAL) among subgroups. The
37
38 172 RR for each cluster was estimated using linear contrast, and rate ratios plus 95% CIs
39
40 173 were used to estimate the differences in annual mean moderate/severe exacerbation
41
42
43 174 rates for each cluster. The programme was completed in the R statistical package.¹⁵

44
45 175 Clusters were characterised based on the tree, using descriptive statistics.
46
47 176 Descriptive statistics were used to present the baseline differences among clusters;
48
49 177 proportions were used for categorical variables, and medians with interquartile
50
51
52 178 ranges were used for continuous variables. The χ^2 test was used to examine the
53
54 179 statistical differences among the subgroups for categorical variables and the non-

FINAL DRAFT

1
2
3 180 parametric Wilcoxon Rank sum test was performed to test the statistical differences
4
5 181 among the subgroups for continuous variables.
6
7 182
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

FINAL DRAFT

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 FEV₁ 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

FINAL DRAFT

1
2
3 208 diuretic use reported (n=282) was for hypertension, with the remaining use for
4
5 209 unspecified oedema, coronary artery disease, and/or congestive heart failure. No
6
7 210 other sub-populations were identified in this cluster. In patients not using diuretics at
8
9 211 baseline two further clusters were defined based on the presence or absence of
10
11 212 FEV₁ reversibility. Cluster 2 patients exhibited reversibility, defined as a post-
12
13 213 bronchodilator change of $\geq 12\%$. Cluster 3 patients did not exhibit reversibility, ie, a
14
15 214 post-bronchodilator change in FEV₁ of $< 12\%$. Compared to SAL, significant
16
17 215 reductions in the rate of moderate/severe exacerbations were observed with SFC
18
19 216 therapy in cluster 1 (44% reduction) and cluster 2 (33% reduction). Similar
20
21 217 reductions were not observed in cluster 3 (table 3).

22
23
24
25 218 Baseline demographics that were significantly different across clusters are
26
27 219 presented in table 4. Subjects in cluster 1 tended to be older, had a higher BMI, were
28
29 220 more likely to be former smokers than current smokers, and had the greatest
30
31 221 smoking pack-year history. Cluster 1 subjects also had a higher prevalence of
32
33 222 treatment for comorbidities (eg, cardiovascular disease [CVD], hypertension,
34
35 223 diabetes) than clusters 2 and 3. Subjects in cluster 3 had a higher % predicted FEV₁
36
37 224 compared with those in clusters 1 and 2, whereas those in cluster 2 had the lowest
38
39 225 % predicted FEV₁. No difference was observed in the baseline incidence of
40
41 226 moderate/severe exacerbations between the clusters.
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT

1
2
3 228 **DISCUSSION**
4

5 229 This study identified three clusters; cluster 1: diuretic users with treatment for
6
7 230 cardiovascular comorbidity; cluster 2: reversible, not taking diuretics; cluster 3: not
8
9 231 reversible, not taking diuretics. Subjects in clusters 1 and 2 benefited more from
10
11 232 receiving combination therapy with SFC, compared to SAL with a greater reduction
12
13
14 233 in exacerbation. This exercise identified two groups that are more likely to respond to
15
16 234 SFC. The largest benefit with SFC was observed in cluster 1.
17

18 235 A number of hypotheses can be put forward to explain the lower exacerbation
19
20 236 rates with SFC relative to SAL among diuretic users. The use of diuretics may
21
22 237 identify a group of patients with cardiovascular disease, such as those with
23
24 238 hypertension or heart failure, who may be more responsive to the addition of an ICS
25
26 239 to a LABA. There was a significantly higher use of cardiovascular (CVD) medications
27
28 240 in cluster 1. ICS (FP) could exert a benefit on exacerbations in COPD patients with
29
30 241 CVD if 1) CVD comorbidity reflects an increased inflammatory state related to
31
32 242 COPD¹⁷ and 2) if CVD is a driver for COPD exacerbation occurrence¹⁸ and severity¹⁹
33
34 243 as has been reported. It is therefore plausible to conjecture that subjects with CVD
35
36 244 would exhibit higher levels of inflammation than those without CVD. Inflammation, as
37
38 245 demonstrated by elevated C-reactive protein or fibrinogen, increases the risk of a
39
40 246 COPD exacerbation^{8 20} and this logic supports the value of the addition of an ICS
41
42 247 (FP) in cluster 1.
43
44
45
46

47 248 It is also possible that cluster 1 (diuretic) was predisposed to an increased
48
49 249 exacerbation risk as a consequence of heart failure. Heart failure can be aggravated
50
51 250 by increased aortic stiffness, a marker of cardiovascular risk found in greater
52
53 251 prevalence among COPD patients than in the general population.²¹ Dransfield *et al*²²
54
55
56
57
58
59
60

FINAL DRAFT

1
2
3 252 recently found that SFC lowered aortic pulse wave velocity (aPWV), a marker of
4
5 253 aortic stiffness, in COPD patients with elevated aPWV.
6

7 254 Another possible explanation of the lower rate in moderate/severe
8
9 255 exacerbations with SFC over SAL in cluster 1 reflects the direct activity of the
10
11 256 concomitant diuretic therapy. Recent studies have examined the effectiveness of
12
13 257 diuretics in the treatment of chronic respiratory diseases, in particular furosemide
14
15 258 (which was the predominantly used diuretic in cluster 1). Mechanistically, furosemide
16
17 259 inhibits inflammatory cytokines²³ and enhances the anti-inflammatory impact of
18
19 260 ICS.²⁴ Clinically it has been shown to alleviate exertional dyspnoea in COPD,²⁵ and
20
21 261 to protect against bronchoconstriction in asthma.^{26 27 28}
22
23
24

25 262 In cluster 2 (reversibility $\geq 12\%$) a significant effect of SFC was also observed
26
27 263 over SAL in terms of a lower rate of moderate/severe exacerbations. Subjects
28
29 264 exhibiting reversibility have been shown to have greater improvement in lung
30
31 265 function compared to those without reversibility.²⁹ Recent data suggest that an
32
33 266 improvement in lung function of 100 mL relates to a reduction in exacerbation rate of
34
35 267 12%,³⁰ while a 12% increase in exacerbation rate has been reported for each 100
36
37 268 mL loss of lung function.⁸ The effect of SFC in COPD³¹ has been shown to provide a
38
39 269 significantly greater effect on lung function in reversible versus irreversible subjects.
40
41 270 This suggests a potential mechanism for the lower rate of moderate/severe
42
43 271 exacerbations in cluster 2 patients receiving SFC.
44
45

46
47 272 None of the subjects in cluster 3 exhibited reversibility, and this together with
48
49 273 the highest prevalence of current smokers (which is known to attenuate ICS effects
50
51 274 in COPD)³² may explain why no difference was observed between SFC and SAL in
52
53 275 this cluster.
54
55
56
57
58
59
60

FINAL DRAFT

1
2
3 276 A number of recent studies have investigated COPD heterogeneity, and have
4
5 277 identified independent factors such as dyspnoea, airway inflammation, and asthma-
6
7 278 like features,^{10 33} or subgroups associated with differential outcome.^{34 35} However,
8
9 279 only one³⁶ has validated the COPD subtypes identified against clinically meaningful
10
11 280 outcomes. Garcia-Aymerich *et al*³⁶ identified three clusters of subjects, comprising
12
13 281 those with severely impaired lung function, those with more mildly impaired lung
14
15 282 function and, importantly, those with more mildly impaired lung function *and*
16
17 283 evidence of cardiovascular disorders, obesity, diabetes and systemic inflammation.
18
19 284 The clusters identified in the present study align to some extent with those already
20
21 285 identified, such as increased reversibility and the presence of CVD. This suggests a
22
23 286 convergence of COPD subtypes that warrants further examination.
24
25
26

27 287 Cluster analysis is limited due to its retrospective nature and the fact that it is
28
29 288 limited to assessing only the categorical variables collected at baseline. In addition,
30
31 289 the splitting into groups is automated by the computer-driven algorithm to maximise
32
33 290 treatment differences, and is not necessarily robust, thus external validation is
34
35 291 warranted. Another potential issue with the clusters isolated in this study is the split
36
37 292 by reversibility. While it is apparent that COPD subjects can be more or less
38
39 293 reversible, there is considerable within-patient variability both on single testing²⁹ and
40
41 294 at testing on multiple occasions.³⁷ It is conceivable that had reversibility testing in the
42
43 295 original studies been conducted within a different time frame (eg, 3 months later or
44
45 296 earlier) a different population of subjects would have been defined as reversible, and
46
47 297 it is not clear whether a treatment effect would have been observed. Despite the
48
49 298 limitations with reversibility, there is evidence that subjects who are less reversible
50
51 299 are likely to have a less robust response to treatment than those who are more
52
53 300 reversible.^{29 31} Alternatively, this less reversible cluster could simply be a measure of
54
55
56
57
58
59
60

FINAL DRAFT

1
2
3 301 those with less severe disease (less obstruction, smaller proportion of comorbidities).
4

5 302 Subjects with less severe disease may not require the addition of an ICS to their
6

7 303 bronchodilator therapy.
8

9
10 304 In conclusion, cluster analysis of subjects taking part in two exacerbation

11 305 studies of SFC versus SAL identified three distinct groups of COPD subjects based

12 306 on diuretic use and reversibility. These subjects varied in their response with

13 307 subjects in two of the three groups experiencing a greater reduction in the annual

14 308 rate of moderate/severe exacerbations with SFC versus SAL. Those in the remaining

15 309 group received no additional benefit in terms of reduction in the annual

16 310 moderate/severe exacerbation rate over that provided by SAL alone. This study

17 311 highlights the future potential for a personalised medicine approach to the treatment

18 312 of patients with COPD. It additionally suggests how this methodology can be used to

19 313 generate potential hypothesis for future studies.
20
21
22
23
24
25
26
27
28
29
30
31
32 314
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT

315 Tables:

316 **Table 1** Baseline characteristics and other variables employed in the cluster
 317 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
COPD history	SGRQ total score
	Duration of COPD (years)
	Chronic bronchitis (self-reported, yes/no)
	Emphysema (self-reported, yes/no)
	Exacerbations requiring hospitalisation (past 12 months)
Medications (ATC classification)	Exacerbations requiring OCS/antibiotic (past 12 months)
	Gold Stage indicator variables based on lung function (II, III/IV)
	Agents acting on the renin-angiotensin system (ACE inhibitors)
	Antianaemic preparations
	Antihaemorrhagics
	Antihistamines
	Antihypertensives
	Anti-thrombotics
	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	

318 ACE, angiotensin converting enzyme; ATC, Anatomical Therapeutic Chemical; COPD, Chronic
 319 obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 second; FVC, forced vital
 320 capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory
 321 Questionnaire.

322

323

324

FINAL DRAFT

325 **Table 2** Demographic and baseline clinical characteristics of subjects
 326 participating in the primary clinical studies of SFC versus SAL (cluster analysis
 327 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)

328 ¹Reversibility based on change in FEV₁ from baseline following 4 puffs (360 µg) albuterol, defined as
 329 a ≥12% and ≥200 mL increase; SD, Standard Deviation; SE, Standard Error; SFC,
 330 Salmeterol/Fluticasone Propionate; SAL, Salmeterol; IQR, Intraquartile range; FEV₁, forced expiratory
 331 volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT

334 **Table 3** Pooled analysis of SFC effect on mean annual moderate/severe
 335 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 (95% CI)	0.56 (0.47–0.71)		0.67 (0.56–0.80)		1.00 (0.74–1.36)	
p Value	<0.001		<0.001		ns	

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

338

FINAL DRAFT

339 **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

340 FEV₁, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant;
 341 SD, Standard Deviation; ¹Reversibility based on change in FEV₁ from baseline following 4 puffs (360
 342 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

343

FINAL DRAFT

Acknowledgements

Editorial support in the form of development of the manuscript first draft under the guidance of the corresponding author and co-authors, editorial suggestions to draft versions of this paper in collaboration with the corresponding author and co-authors, assembling tables and figures, collating author comments, copyediting, fact checking, referencing, and graphic services was provided by Geoff Weller, PhD at Gardiner-Caldwell Communications and was funded by GlaxoSmithKline. Amanda Emmett, an employee of GlaxoSmithKline, provided consultation regarding the clinical trials data and statistical review of the results and subsequent manuscript. Ms. Emmett was the primary statistician on the two COPD exacerbation randomised control trials.

Statement of interest

All co-authors are employees and shareholders of GlaxoSmithKline.

Funding

Funded by GlaxoSmithKline.

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,

FINAL DRAFT

1
2
3 369 conducted analyses, and provided methodological expertise. DR was involved in
4
5 370 planning the analyses, interpretation of results. DS was involved in planning of
6
7 371 analyses, interpretation of results.
8
9

372

373 **Licence Statement**

374 The Corresponding Author has the right to grant on behalf of all authors and does
375 grant on behalf of all authors, an exclusive licence (or non-exclusive for government
376 employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees
377 to permit this article (if accepted) to be published in BMJ Open and any other
378 BMJ PGL products to exploit all subsidiary rights, as set out in our licence (
379 [http://group.bmj.com/products/journals/instructions-for-](http://group.bmj.com/products/journals/instructions-for-authors/wholly_owned_licence.pdf)
380 [authors/wholly_owned_licence.pdf](http://group.bmj.com/products/journals/instructions-for-authors/wholly_owned_licence.pdf)) and the Corresponding Author accepts and
381 understands that any supply made under these terms is made by BMJ PGL to the
382 Corresponding Author.

383

FINAL DRAFT

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global Strategy for the Diagnosis, Management and Prevention of COPD*, 2011. Available from: <http://www.goldcopd.org/>.
2. Celli BR, Thomas NE, Anderson JA, *et al*. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med* 2008 Aug 15;178(4):332-8.
3. Spencer S, Calverley PM, Burge PS, Jones PW. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J* 2004 May;23(5):698-702.
4. Jones PW, Tabberer M, Chen WH. Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores. *BMC Pulm Med* 2011 Aug 11;11:42.
5. Agusti AG. COPD, a multicomponent disease: implications for management. *Respir Med* 2005 Jun;99(6):670-82.
6. Agusti A, Calverley P, Celli B, *et al*. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res* 2010;11:122.
7. Han MK, Agusti A, Calverley PM, *et al*. Chronic obstructive pulmonary disease phenotypes: the future of COPD. *Am J Respir Crit Care Med*. 2010 Sep 1;182(5):598-604.
8. Hurst JR, Vestbo J, Anzueto A. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *New Engl J Med* 2010;363:1128–38.
9. Cho MH, Washko GR, Hoffmann TJ, *et al*. Cluster analysis in severe emphysema subjects using phenotype and genotype data: an exploratory investigation. *Respir Res* 2010;11:30.
10. Roy K, Smith J, Kolsum U, *et al*. COPD phenotype description using principal components analysis. *Respir Res* 2009;10:41
11. Garcia-Aymerich J, Gomez F, Benet M, *et al*. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (COPD subtypes). *Thorax* 2011;66:430–37.
12. Su X, Tsai C-L, Wang H, Nickerson DM, Li B. Tree-based subgroup analysis via recursive partitioning. *J Machine Learning Res* 2009;10:141–58.
13. Anzueto A, Feldman G, Chinsky K, *et al*. Effect of fluticasone propionate/salmeterol (250/50) on COPD exacerbations and impact on patient outcomes. *J COPD* 2009;6:320–29.

FINAL DRAFT

- 1
2
3 432 14. Ferguson GT, Anzueto A, Fei R, *et al.* Effect of fluticasone
4 433 propionate/salmeterol (250/50 mcg) or salmeterol (50 mcg) on COPD
5 434 exacerbations. *Respir Med* 2008;102:1099–108.
6 435
7 436 15. Everitt B. *An R and S-PLUS® companion to multivariate analysis.* London,
8 437 Springer-Verlag, 2005.
9 438
10 439 16. Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung
11 440 function tests. *Eur Respir J* 2005; 26: 948–968
12 441
13 442 17. Man SF, Leipsic JA, Man JP, Sin DD. Is atherosclerotic heart disease in
14 443 COPD a distinct phenotype? *Chest.* 2011 Sep;140(3):569-71
15 444
16 445 18. Niewoehner DE, Lokhnygina Y, Rice K, *et al.* Risk indexes for exacerbations
17 446 and hospitalizations due to COPD. *Chest* 2007 Jan;131(1):20-8.
18 447
19 448 19. Chang CL, Robinson SC, Mills GD, *et al.* Biochemical markers of cardiac
20 449 dysfunction predict mortality in acute exacerbations of COPD. *Thorax.* 2011
21 450 Sep;66(9):764-8.
22 451
23 452 20. Groenewegen KH, Postma DS, Hop WC, *et al.* Increased systemic
24 453 inflammation is a risk factor for COPD exacerbations. *Chest* 2008
25 454 Feb;133(2):350-7.
26 455
27 456 21. Sabit R, Bolton CE, Edwards PH, *et al.* Arterial stiffness and osteoporosis in
28 457 chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2007 Jun
29 458 15;175(12):1259-65.
30 459
31 460 22. Dransfield MT, Cockcroft JR, Townsend RR, *et al.* Effect of fluticasone
32 461 propionate/salmeterol on arterial stiffness in patients with COPD. *Respir Med*
33 462 2011 Sep;105(9):1322-30.
34 463
35 464 23. Yuengsrigul A, Chin TW, Nussbaum E. Immunosuppressive and cytotoxic
36 465 effects of furosemide on human peripheral blood mononuclear cells. *Ann*
37 466 *Allergy Asthma Immunol* 1999;83:559–66.
38 467
39 468 24. Prandota J. Furosemide: progress in understanding its diuretic, anti-
40 469 inflammatory, and bronchodilating mechanism of action, and use in the
41 470 treatment of respiratory tract diseases. *Am J Ther* 2002;9:317–28.
42 471
43 472 25. Jensen D, Amjadi K, Harris-McAllister V, Webb KA, O'Donnell DE.
44 473 Mechanism of dyspnoea relief and improved exercise after furosemide
45 474 inhalation in COPD. *Thorax* 2008;63:606–13.
46 475
47 476 26. Roger A, Botey J, Esevri JL, *et al.* Prevention of exercise-induced asthma in
48 477 children using low doses of inhaled furosemide. *J Invest Allergol Clin Immunol*
49 478 1993;3:300–3.
50 479
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT

- 1
2
3 480 27. Novembre E, Frongia G, Lombardi E, *et al.* The preventative effect and
4 481 duration of action of two doses of inhaled furosemide on exercise-induced
5 482 asthma in children. *J Allergy Clin Immunol* 1995;96:906–9.
6 483
7 484 28. Melo RE, Sole D, Naspitz CK. Comparative efficacy of inhaled furosemide and
8 485 disodium cromoglycate in the treatment of exercise-induced asthma in
9 486 children. *J Allergy Clin Immunol* 1997;99:204–9.
10 487
11 488 29. Hanania N, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in
12 489 COPD. *Chest* 2011;140:1055–63.
13 490
14 491 30. Jones PW, Donohue JF, Nedelman J, *et al.* Correlating changes in lung
15 492 function with patient outcomes in chronic obstructive pulmonary disease: a
16 493 pooled analysis. *Respir Res.* 2011 Dec 29;12:161.
17 494
18 495 31. Bleecker ER, Emmett A, Crater G, *et al.* Lung function and symptom
19 496 improvement with fluticasone propionate/salmeterol and ipratropium
20 497 bromide/albuterol in COPD: response by beta-agonist reversibility. *Pulm*
21 498 *Pharmacol Ther* 2008 Aug;21(4):682-8.
22 499
23 500 32. Soriano JB, Sin DD, Zhang X, *et al.* A pooled analysis of FEV1 decline in
24 501 COPD patients randomized to inhaled corticosteroids or placebo. *Chest* 2007
25 502 Mar;131(3):682-9.
26 503
27 504 33. Bafadhel M, McKenna S, Terry S, *et al.* Acute exacerbations of chronic
28 505 obstructive pulmonary disease: identification of biologic clusters and their
29 506 biomarkers. *Am J Respir Crit Care Med* 2011;184:662–71.
30 507
31 508 34. Burgel P-R, Paillasseur J-L, Caillaud D, *et al.* Clinical COPD phenotypes: a
32 509 novel approach using principal component and cluster analyses. *Eur Respir J*
33 510 2010;36:531–39.
34 511
35 512 35. Weatherall M, Travers J, Shirtcliffe PM, *et al.* Distinct clinical phenotypes of
36 513 airways disease defined by cluster analysis. *Eur Respir J* 2009;34:812–18.
37 514
38 515 36. Garcia-Aymerich J, Gómez FP, Benet M, *et al.* Identification and prospective
39 516 validation of clinically relevant chronic obstructive pulmonary disease (COPD)
40 517 subtypes. *Thorax.* 2011 May;66(5):430-7.
41 518
42 519 37. Albert P, Agusti A, Edwards L, *et al.* Bronchodilator responsiveness as a
43 520 phenotypic characteristic of established chronic obstructive pulmonary
44 521 disease. *Thorax* 2012 Jun 13. [Epub ahead of print].
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

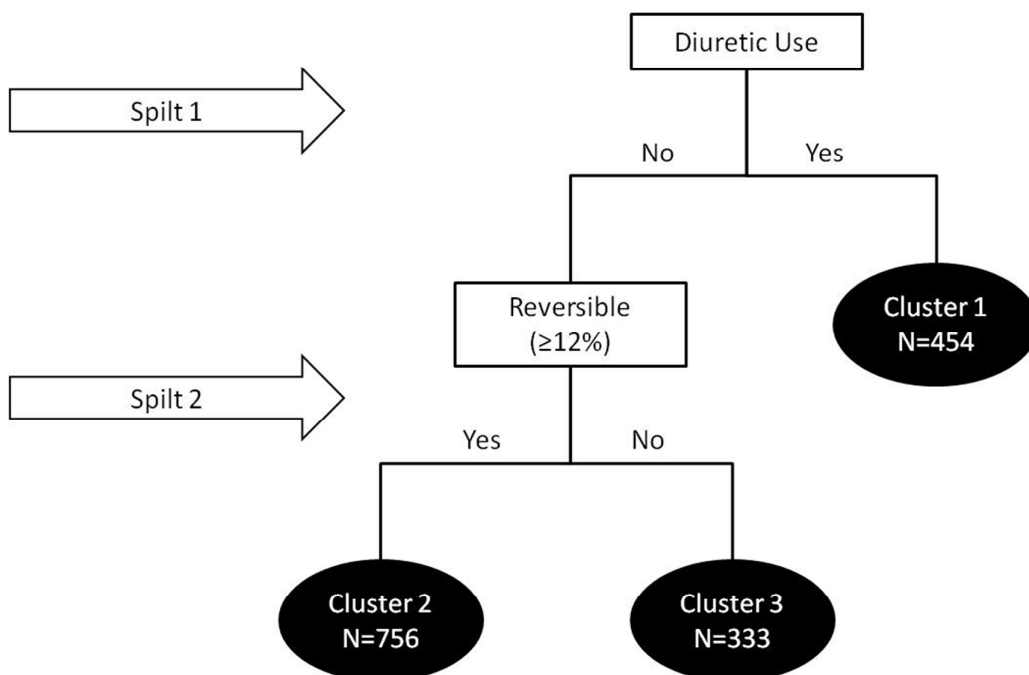
FINAL DRAFT

522 **Figures**

523 **Figure 1 Interaction tree generated by supervised cluster analysis**

524

Figure 1



525
526

527

View only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43

Spilt 1

Spilt 2

Diuretic Use

No

Yes

**Reversible
(≥12%)**

**Cluster 1
N=454**

Yes

No

**Cluster 2
N=756**

**Cluster 3
N=333**



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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2012-001838.R1
Article Type:	Research
Date Submitted by the Author:	08-Mar-2013
Complete List of Authors:	DiSantostefano, Rachael; GlaxoSmithKline, Li, Hao; GlaxoSmithKline, Rubin, David; GlaxoSmithKline, Stempel, David; GlaxoSmithKline,
Primary Subject Heading:	Respiratory medicine
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Chronic airways disease < THORACIC MEDICINE, Exacerbation, Long acting beta2 agonist, inhaled corticosteroid

SCHOLARONE™
Manuscripts

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 **Which patients with chronic obstructive pulmonary disease benefit from the addition**
4 **of an inhaled corticosteroid to their bronchodilator? A cluster analysis**
5
6
7

8
9 Rachael L DiSantostefano,¹ Hao Li,¹ David Rubin,¹ David Stempel¹

10
11 ¹*GlaxoSmithKline, Research Triangle Park, Durham, North Carolina, USA*
12
13

14
15 **Correspondence to**

16 R L DiSantostefano

17 GlaxoSmithKline,

18 5 Moore Drive

19 Research Triangle Park,

20 Durham, North Carolina,

21 27709, USA

22 email; rachael.l.disantostefano@gsk.com

23 Tel: 00 1 919 483 9237

24 Fax: 00 1 919 549 7459

25 **Current Word Count:** 3419

26
27 **Key Words:** COPD, Exacerbation, Cluster Analysis, Long-acting beta-2 agonist, inhaled
28 corticosteroid
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

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 $\geq 12\%$ 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 β_2 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.

FINAL DRAFT – REVIEW REVISION FINAL

ABSTRACT

Objective: To identify subsets of chronic obstructive pulmonary disease (COPD) patients who are more protected from exacerbations with the use of an inhaled corticosteroid/long-acting β_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 FEV₁ reversibility $\geq 12\%$ (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 FEV₁ 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 FEV₁ 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 (FEV₁) alone is an insufficient measure of disease severity¹. Importantly, the revised GOLD strategy document also recommends therapy with an inhaled corticosteroid/ long-acting β_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 function², 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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA (salmeterol/fluticasone
4 propionate [SFC]).^{14 15} The objective of this cluster analysis was to identify patients who
5 benefit most from the addition of ICS to bronchodilator therapy in terms of the reduction of
6
7 the mean annual rate of moderate/severe exacerbations for SFC compared to SAL.
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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 FEV₁ ≤50% of predicted, a pre-bronchodilator FEV₁/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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 The cluster analysis aimed to find subgroups in the study subjects that had
4 similar baseline characteristics and with maximum treatment differences for mean
5 yearly moderate/severe exacerbation risk ratio (RR).
6
7

8
9 Subjects included in the cluster analysis were required to have the following
10 baseline variables: FEV1 % predicted, FEV1 reversibility stratum (yes/no for $\geq 12\%$
11 improvement and ≥ 200 mL), time on treatment, and geographical region. Reversibility
12 following administration of 4 puffs of albuterol was determined prior to randomization to
13 treatment, following completion of the 4-week FSC 250/50 run-in period. Missing values
14 for the remaining baseline variables were imputed during cluster analysis as the
15 median for continuous/ordinal variables, or the most frequent value for categorical
16 variables. The baseline characteristics are listed in table 1. Baseline medications were
17 classified by the Anatomical Therapeutic Chemical (ATC) Classification System, controlled
18 by the World health organization. Medication are classified based on the organ or system
19 they affect and/or their therapeutic and chemical characteristics.¹⁷ When medications
20 could have more than one ATC code, the second-level ATC code corresponding to the
21 patient-supplied indication was evaluated to classify the medication (e.g, aspirin as a
22 platelet inhibitor versus analgesic would be assigned to B01 Antithrombotic vs. N02
23 Analgesic).
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38

39
40 Baseline characteristics were examined before inclusion in the model to ensure that
41 there was no significant co-linearity that may influence the cluster analysis. Co-linearity was
42 assessed by creating a correlation tree, and any two variables with an R^2 of ≥ 0.7 were
43 examined. The variables considered most clinically relevant were retained. St. George's
44 Respiratory Questionnaire (SGRQ) Impact and Activity scores were removed from cluster
45 analysis since they highly correlated with the total score.
46
47
48
49
50
51
52
53

54 **Modelling to define the tree: supervised analysis**

55
56 Modified recursive partitioning techniques were used to perform the supervised
57 subgroup analysis. The frequency of each variable was examined to identify sparse values
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 prior to inclusion into the tree. As the minimal subgroup size (terminal node) was set at 100,
4
5 all categorical variables were required to have at least 100 subjects in a response category
6
7 in order to be considered for the recursive partitioning algorithm. Variables with several
8
9 responses were collapsed into fewer categories as appropriate, such that all categories had
10
11 at least 100 subjects (eg, exacerbations requiring hospitalisation in the past year) or
12
13 eliminated from consideration during the cluster analysis (race, anti-hemorrhagics, anti-
14
15 hypertensives [eg, anti-adrenergics and smooth muscle agents], vasodilators, and
16
17 vasoprotectives [eg, topical haemorrhoid treatments and anti-varicose therapy]).

18
19 The best split of the tree was determined by maximising the subgroups according to
20
21 treatment interaction effect, and subgroup membership was then assigned to each patient
22
23 based on the selected tree. Internal validation was performed by using a split sample, so that
24
25 a random sample of 50% of the patients was selected to create the tree and the remaining
26
27 half was used for the computation of rate ratios and confidence intervals (CIs) to test
28
29 statistical significance.

30
31 Generalised linear models using a negative binomial function were used to compare
32
33 the likelihood of having an exacerbation by examining treatment by subgroup interaction.
34
35 The model was adjusted for study baseline FEV₁ % predicted, FEV₁ reversibility stratum
36
37 (yes/no for ≥12% and 200 mL post-bronchodilator change), time on treatment and
38
39 geographical region (8 regions), which was considered a random effect. The algorithm used
40
41 in the study maximises treatment differences (mean moderate/severe exacerbation rates for
42
43 SFC vs SAL) among subgroups. The RR for each cluster was estimated using linear
44
45 contrast, and rate ratios plus 95% CIs were used to estimate the differences in annual mean
46
47 moderate/severe exacerbation rates for each cluster. The programme was completed in the
48
49 R statistical package.¹⁸

50
51 Clusters were clinically characterised based on the tree. Descriptive statistics were
52
53 used to present the baseline differences in clinical features among clusters; proportions
54
55 were used for categorical variables, and medians with interquartile ranges were used for
56
57 continuous variables. The χ^2 test was used to examine the statistical differences among the
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

For peer review only

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 FEV₁ 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 $\geq 12\%$ reversibility threshold as 11.5% was close to the $\geq 12\%$ component of the ERS/ATS threshold for reversibility, although it should be noted that this definition also requires a volume response of ≥ 200 mL¹⁹. 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 FEV₁ % predicted, FEV₁ reversibility stratum (yes/no for $\geq 12\%$ and 200 mL post-bronchodilator change), and region. Only baseline FEV₁% 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 $\geq 12\%$ FEV₁ reversibility based on the model. The first cluster

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 (cluster 1) identified subjects treated with diuretics (predominantly furosemide).
4
5 Approximately half of the diuretic use reported (n=282) was for hypertension, with the
6
7 remaining use for unspecified oedema, coronary artery disease, and/or congestive heart
8
9 failure. No other sub-populations were identified in this cluster. In patients not using diuretics
10
11 at baseline two further clusters were defined based on the presence or absence of FEV₁
12
13 reversibility. Cluster 2 patients exhibited reversibility, defined as a post-bronchodilator
14
15 change of $\geq 12\%$. Cluster 3 patients did not exhibit reversibility, ie, a post-bronchodilator
16
17 change in FEV₁ of $< 12\%$. Compared to SAL, significant reductions in the rate of
18
19 moderate/severe exacerbations were observed with SFC therapy in cluster 1 (44%
20
21 reduction) and cluster 2 (33% reduction). Similar reductions were not observed in cluster 3
22
23 (figure 2)
24

25 Baseline demographics that were significantly different across clusters are presented
26
27 in table 3. Subjects in cluster 1 tended to be older, had a higher BMI, were more likely to be
28
29 former smokers than current smokers, and had the greatest smoking pack-year history.
30
31 Cluster 1 subjects also had a higher prevalence of treatment for comorbidities (eg,
32
33 cardiovascular disease [CVD], hypertension, diabetes) than clusters 2 and 3. Subjects in
34
35 cluster 3 had a higher % predicted FEV₁ compared with those in clusters 1 and 2, whereas
36
37 those in cluster 2 had the lowest % predicted FEV₁. No difference was observed in the
38
39 baseline incidence of moderate/severe exacerbations between the clusters.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

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 COPD²¹ and 2) if CVD is a driver for COPD exacerbation occurrence²² 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 established⁶ and it has recently been suggested, through use of a rigorous assessment of co-morbidity, that patients with CVD and metabolic

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 syndrome form discrete clusters of COPD patients¹² which are represented in our analysis in
4 Cluster 1. Another assessment of co-morbidity in COPD has found that certain CVDs
5 increase the risk of all-cause mortality in COPD;²⁵ Although the TORCH study failed to show
6 a significant effect ($p=0.052$) of FP/SAL versus placebo for all-cause mortality²⁶ a
7 subsequent analysis of CV-related mortality and AEs found a positive effect of FP/SAL
8 versus SAL in terms of CV-related outcomes²⁷ which further implies a potential benefit of
9 ICS in COPD patients with co-morbid CVD.
10
11
12
13
14
15
16

17 It is also possible that cluster 1 (diuretic) was predisposed to an increased
18 exacerbation risk as a consequence of heart failure, especially as compared with cluster 2
19 and 3 more patients in cluster 1 were in receipt of anti-thrombotics, beta-blockers and
20 cardiac therapy, all of which suggest a greater degree of heart failure in cluster 1 compared
21 with the other clusters. Heart failure can be aggravated by increased aortic stiffness, a
22 marker of cardiovascular risk found in greater prevalence among COPD patients than in the
23 general population.²⁸ Dransfield *et al*²⁹ recently found that SFC lowered aortic pulse wave
24 velocity (aPWV), a marker of aortic stiffness, in COPD patients with elevated aPWV.
25
26
27
28
29
30
31
32

33 Another possible explanation of the lower rate in moderate/severe exacerbations with
34 SFC over SAL in cluster 1 reflects the direct activity of the concomitant diuretic therapy.
35 Recent studies have examined the effectiveness of diuretics in the treatment of chronic
36 respiratory diseases, in particular furosemide (which was the predominantly used diuretic in
37 cluster 1). Mechanistically, furosemide inhibits inflammatory cytokines³⁰ and enhances the
38 anti-inflammatory impact of ICS.³¹ Clinically it has been shown to alleviate exertional
39 dyspnoea in COPD,³² and to protect against bronchoconstriction in asthma.^{33 34 35}
40
41
42
43
44
45
46
47

48 The findings in cluster 1 relating to co-morbidity in COPD are of particular interest
49 given recent publications on the prevalence and impact of co-morbidity in COPD^{12 25 36} and
50 the concept of multiple morbidities and their impact on clinical practice.³⁷ Certainly our
51 findings and others^{11 12} suggest that patients with multiple diseases may benefit from a
52 different approach to management than those with a single disease, a concept which has
53 recently been raised as a major issue in primary care.³⁸
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 In cluster 2 (reversibility $\geq 12\%$) a significant effect of SFC was also observed over
4 SAL in terms of a lower rate of moderate/severe exacerbations. There was also reversibility
5 in Cluster 1 (median, 18.6%). Subjects exhibiting reversibility have been shown to have
6 greater improvement in lung function compared to those without reversibility which could
7 explain the significant effect of SFC relative to SAL in terms of a lower rate of
8 moderate/severe exacerbations.³⁹ Recent data suggest that an improvement in lung function
9 of 100 mL relates to a reduction in exacerbation rate of 12%,⁴⁰ while a 12% increase in
10 exacerbation rate has been reported for each 100 mL loss of lung function.⁸ The effect of
11 SFC in COPD⁴¹ has been shown to provide a significantly greater effect on lung function in
12 reversible versus irreversible subjects. This suggests a potential mechanism for the lower
13 rate of moderate/severe exacerbations in cluster 1 and cluster 2 for patients receiving SFC.
14 The rationale for this is that a greater improvement in lung function is typically associated
15 with a greater effect on exacerbations (e.g. Jones *et al*⁴⁰).

16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 testing³⁹ 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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 active asthma. It has been suggested that Asthma and COPD form part of the same
4 disease continuum⁴⁴ and though this is a controversial concept, the idea of an asthma-
5 COPD overlap syndrome may give insight into the response to combination ICS/LABA.^{45 46}
6
7 Forty percent and 59% of patients in Clusters 1 and 2, respectively, were $\geq 12\%$ reversible
8 and had an increase of $\geq 200\text{mL}$ in FEV₁. However, the reversibility stratum ($\geq 12\%$ and
9 $\geq 200\text{mL}$) was adjusted for in the overall negative binomial model examining mean annual
10 exacerbation rates and was found to be not statistically significant, suggesting that any
11 impact of reversibility as determined by both $\geq 12\%$ and $\geq 200\text{mL}$ was minimal.
12
13

14
15 A number of recent studies have investigated COPD heterogeneity, and have
16 identified independent factors such as dyspnoea, airway inflammation, and asthma-like
17 features,^{10 47} or subgroups associated with differential outcome.^{48 49} However, only one¹¹ has
18 validated the COPD subtypes identified against clinically meaningful outcomes. Garcia-
19 Aymerich *et al*¹¹ identified three clusters of subjects, comprising those with severely impaired
20 lung function, those with more mildly impaired lung function and, importantly, those with
21 more mildly impaired lung function *and* evidence of cardiovascular disorders, obesity,
22 diabetes and systemic inflammation. The clusters identified in the present study align to
23 some extent with those already identified, such as increased reversibility and the presence
24 of CVD. This suggests a convergence of COPD subtypes that warrants further examination.
25
26

27
28 Cluster analysis is limited due to its retrospective nature and the fact that it is limited
29 to assessing only the categorical variables collected at baseline. In addition, the splitting into
30 groups is automated by the computer-driven algorithm to maximise treatment differences,
31 and is not necessarily robust, thus external validation is warranted. Because this analysis
32 includes only those patients with a history of exacerbation, it is also difficult to generalise to
33 subjects with COPD who do not have a history of exacerbation.
34
35

36
37 In conclusion, cluster analysis of subjects taking part in two exacerbation studies of
38 SFC versus SAL identified three distinct groups of COPD subjects based on diuretic use and
39 reversibility. These subjects varied in their response with subjects in two of the three groups
40 experiencing a greater reduction in the annual rate of moderate/severe exacerbations with
41
42
43
44
45
46
47
48
49
50
51

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 SFC versus SAL. Those in the remaining group received no additional benefit in terms of
4
5 reduction in the annual moderate/severe exacerbation rate over that provided by SAL alone.
6
7 This study highlights the future potential for a personalised medicine approach to the
8
9 treatment of patients with COPD. It additionally suggests how this methodology can be used
10
11 to generate potential hypothesis for future studies.
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

Acknowledgements

Editorial support in the form of development of the manuscript first draft under the guidance of the corresponding author and co-authors, editorial suggestions to draft versions of this paper in collaboration with the corresponding author and co-authors, assembling tables and figures, collating author comments, copyediting, fact checking, referencing, and graphic services was provided by Geoff Weller, PhD at Gardiner-Caldwell Communications and was funded by GlaxoSmithKline.

Amanda Emmett, an employee of GlaxoSmithKline, provided consultation regarding the clinical trials data and statistical review of the results and subsequent manuscript. Ms. Emmett was the primary statistician on the two COPD exacerbation randomised control trials.

Statement of interest

All co-authors are employees and shareholders of GlaxoSmithKline.

Funding

Funded by GlaxoSmithKline.

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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 results. DS was involved in planning of analyses, interpretation of results. All authors have
4
5 read and approved the final manuscript.
6
7

8 9 **Licence Statement**

10
11 The Corresponding Author has the right to grant on behalf of all authors and does grant on
12
13 behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a
14
15 worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if
16
17 accepted) to be published in BMJ Open and any other BMJPGGL products to exploit all
18
19 subsidiary rights, as set out in our licence (
20
21 http://group.bmj.com/products/journals/instructions-for-authors/wholly_owned_licence.pdf)
22
23 and the Corresponding Author accepts and understands that any supply made under these
24
25 terms is made by BMJPGGL to the Corresponding Author.
26
27

28 29 **Data sharing**

30
31 No additional data available.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global Strategy for the Diagnosis, Management and Prevention of COPD*, 2011. Available from: <http://www.goldcopd.org/>.
2. Celli BR, Thomas NE, Anderson JA, *et al*. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med* 2008 Aug 15;178(4):332-8.
3. Spencer S, Calverley PM, Burge PS, Jones PW. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J* 2004 May;23(5):698-702.
4. Jones PW, Tabberer M, Chen WH. Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores. *BMC Pulm Med* 2011 Aug 11;11:42.
5. Agusti AG. COPD, a multicomponent disease: implications for management. *Respir Med* 2005 Jun;99(6):670-82.
6. Agusti A, Calverley P, Celli B, *et al*. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res* 2010;11:122.
7. Han MK, Agusti A, Calverley PM, *et al*. Chronic obstructive pulmonary disease phenotypes: the future of COPD. *Am J Respir Crit Care Med*. 2010 Sep 1;182(5):598-604.
8. Hurst JR, Vestbo J, Anzueto A. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *New Engl J Med* 2010;363:1128–38.
9. Cho MH, Washko GR, Hoffmann TJ, *et al*. Cluster analysis in severe emphysema subjects using phenotype and genotype data: an exploratory investigation. *Respir Res* 2010;11:30.
10. Roy K, Smith J, Kolsum U, *et al*. COPD phenotype description using principal components analysis. *Respir Res* 2009;10:41
11. Garcia-Aymerich J, Gomez F, Benet M, *et al*. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (COPD subtypes). *Thorax* 2011;66:430–37.
12. Vanfleteren LE, Spruit MA, Groenen M, *et al*. Clusters of Comorbidities Based on Validated Objective Measurements and Systemic Inflammation in Patients with Chronic Obstructive Pulmonary disease. *Am J Respir Crit Care Med*. 2013 Feb 7. [Epub ahead of print]
13. Su X, Tsai C-L, Wang H, Nickerson DM, Li B. Tree-based subgroup analysis via recursive partitioning. *J Machine Learning Res* 2009;10:141–58.
14. Anzueto A, Feldman G, Chinsky K, *et al*. Effect of fluticasone propionate/salmeterol (250/50) on COPD exacerbations and impact on patient outcomes. *J COPD* 2009;6:320–29.

FINAL DRAFT – REVIEW REVISION FINAL

15. Ferguson GT, Anzueto A, Fei R, *et al.* Effect of fluticasone propionate/salmeterol (250/50 mcg) or salmeterol (50 mcg) on COPD exacerbations. *Respir Med* 2008;102:1099–108.
16. American Thoracic Society Standards for diagnosis and care of patients with chronic obstructive pulmonary disease and asthma. *Am Rev Respir Dis.* 1987;136:225–44
17. WHO Collaborating Centre for Drug Statistics Methodology. Structure and principles of the Anatomical Therapeutic Chemical (ATC) classification system. Available at http://www.whocc.no/atc/structure_and_principles/ accessed 20th Feb 2013
18. Everitt B. An R and S-PLUS® companion to multivariate analysis. London, Springer-Verlag, 2005.
19. Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26: 948–968
20. Poulain M, Doucet M, Drapeau V, *et al.* Metabolic and inflammatory profile in obese patients with chronic obstructive pulmonary disease. *Chron Respir Dis* 2008;5:35–41
21. Man SF, Leipsic JA, Man JP, Sin DD. Is atherosclerotic heart disease in COPD a distinct phenotype? *Chest.* 2011 Sep;140(3):569-71
22. Niewoehner DE, Lokhnygina Y, Rice K, *et al.* Risk indexes for exacerbations and hospitalizations due to COPD. *Chest* 2007 Jan;131(1):20-8.
23. Chang CL, Robinson SC, Mills GD, *et al.* Biochemical markers of cardiac dysfunction predict mortality in acute exacerbations of COPD. *Thorax.* 2011 Sep;66(9):764-8.
24. Groenewegen KH, Postma DS, Hop WC, *et al.* Increased systemic inflammation is a risk factor for COPD exacerbations. *Chest* 2008 Feb;133(2):350-7.
25. Divo M, Cote C, de Torres JP, *et al.* Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2012;186(2):155-61.
26. Calverley PM, Anderson JA, Celli B, *et al.* Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med.* 2007;356(8):775-89
27. TORCH CV Calverley PM, Anderson JA, Celli B, *et al.* Cardiovascular events in patients with COPD: TORCH study results. *Thorax.* 2010;65(8):719-25.
28. Sabit R, Bolton CE, Edwards PH, *et al.* Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2007 Jun 15;175(12):1259-65.
29. Dransfield MT, Cockcroft JR, Townsend RR, *et al.* Effect of fluticasone propionate/salmeterol on arterial stiffness in patients with COPD. *Respir Med* 2011 Sep;105(9):1322-30.

FINAL DRAFT – REVIEW REVISION FINAL

- 1
- 2
- 3 30. Yuengsrigul A, Chin TW, Nussbaum E. Immunosuppressive and cytotoxic effects of
- 4 furosemide on human peripheral blood mononuclear cells. *Ann Allergy Asthma*
- 5 *Immunol* 1999;83:559–66.
- 6
- 7 31. Prandota J. Furosemide: progress in understanding its diuretic, anti-inflammatory,
- 8 and bronchodilating mechanism of action, and use in the treatment of respiratory
- 9 tract diseases. *Am J Ther* 2002;9:317–28.
- 10
- 11 32. Jensen D, Amjadi K, Harris-McAllister V, et al. Mechanism of dyspnoea relief and
- 12 improved exercise after furosemide inhalation in COPD. *Thorax* 2008;63:606–13.
- 13
- 14 33. Roger A, Botey J, Esevri JL, et al. Prevention of exercise-induced asthma in children
- 15 using low doses of inhaled furosemide. *J Invest Allergol Clin Immunol* 1993;3:300–3.
- 16
- 17 34. Novembre E, Frongia G, Lombardi E, et al. The preventative effect and duration of
- 18 action of two doses of inhaled furosemide on exercise-induced asthma in children. *J*
- 19 *Allergy Clin Immunol* 1995;96:906–9.
- 20
- 21 35. Melo RE, Sole D, Naspitz CK. Comparative efficacy of inhaled furosemide and
- 22 disodium cromoglycate in the treatment of exercise-induced asthma in children. *J*
- 23 *Allergy Clin Immunol* 1997;99:204–9.
- 24
- 25 36. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur*
- 26 *Respir J.* 2009;33(5):1165-85.
- 27
- 28 37. Barnett K, Mercer SW, Norbury M, et al. Epidemiology of multimorbidity and
- 29 implications for health care, research, and medical education: a cross-sectional
- 30 study. *Lancet.* 2012;380(9836):37-43.
- 31
- 32 38. Guthrie B, Payne K, Alderson P, et al. Adapting clinical guidelines to take account of
- 33 multimorbidity. *BMJ.* 2012;345:e6341
- 34
- 35 39. Hanania N, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in COPD.
- 36 *Chest* 2011;140:1055–63.
- 37
- 38 40. Jones PW, Donohue JF, Nedelman J, et al. Correlating changes in lung function with
- 39 patient outcomes in chronic obstructive pulmonary disease: a pooled analysis. *Respir*
- 40 *Res.* 2011 Dec 29;12:161.
- 41
- 42 41. Bleeker ER, Emmett A, Crater G, et al. Lung function and symptom improvement
- 43 with fluticasone propionate/salmeterol and ipratropium bromide/albuterol in COPD:
- 44 response by beta-agonist reversibility. *Pulm Pharmacol Ther* 2008 Aug;21(4):682-8.
- 45
- 46 42. Albert P, Agusti A, Edwards L, et al. Bronchodilator responsiveness as a phenotypic
- 47 characteristic of established chronic obstructive pulmonary disease. *Thorax*
- 48 2012;68(8):701-708
- 49
- 50 43. Soriano JB, Sin DD, Zhang X, et al. A pooled analysis of FEV1 decline in COPD
- 51 patients randomized to inhaled corticosteroids or placebo. *Chest* 2007
- 52 Mar;131(3):682-9.
- 53
- 54
- 55
- 56
- 57
- 58
- 59
- 60

FINAL DRAFT – REVIEW REVISION FINAL

- 1
2
3 44. Postma DS, Boezen HM. Rationale for the Dutch hypothesis. Allergy and airway
4 hyperresponsiveness as genetic factors and their interaction with environment in the
5 development of asthma and COPD. *Chest*. 2004 Aug;126(2 Suppl):96S-104S
6
7
8 45. de Oca MM, Halbert RJ, Lopez MV, *et al*. The chronic bronchitis phenotype in
9 subjects with and without COPD: the PLATINO study. *Eur Respir J*. 2012;40(1):28-
10 36.
11
12
13 46. Soler-Cataluña JJ, Cosío B, Izquierdo JL, *et al*. Consensus document on the overlap
14 phenotype COPD-asthma in COPD. *Arch Bronconeumol*. 2012;48(9):331-7.
15
16 47. Bafadhel M, McKenna S, Terry S, *et al*. Acute exacerbations of chronic obstructive
17 pulmonary disease: identification of biologic clusters and their biomarkers. *Am J*
18 *Respir Crit Care Med* 2011;184:662–71.
19
20 48. Burgel P-R, Paillasseur J-L, Caillaud D, *et al*. Clinical COPD phenotypes: a novel
21 approach using principal component and cluster analyses. *Eur Respir J*
22 2010;36:531–39.
23
24 49. Weatherall M, Travers J, Shirtcliffe PM, *et al*. Distinct clinical phenotypes of airways
25 disease defined by cluster analysis. *Eur Respir J* 2009;34:812–18.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

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 Antihaemorrhagics 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; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT – REVIEW REVISION FINAL

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)

¹Reversibility based on change in FEV₁ 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; FEV₁, forced expiratory volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT – REVIEW REVISION FINAL

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

FEV₁, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant; SD, Standard Deviation; ¹Reversibility based on change in FEV₁ from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 Figure Legends
4

5 Figure 1. Interaction tree generated by supervised cluster analysis
6

7 MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC =
8 Salmeterol/Fluticasone propionate combination
9

10 Figure 2. Pooled analysis of SFC effect on mean annual moderate/severe exacerbation rate
11 by cluster
12

13 ns = not significant ($p>0.05$); SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate
14 combination
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 **Which patients with chronic obstructive pulmonary disease benefit from the addition**
4 **of an inhaled corticosteroid to their bronchodilator? A cluster analysis**
5
6
7

8
9 Rachael L DiSantostefano,¹ Hao Li,¹ David Rubin,¹ David Stempel¹

10
11 ¹*GlaxoSmithKline, Research Triangle Park, Durham, North Carolina, USA*
12
13

14
15 **Correspondence to**

16 R L DiSantostefano

17 GlaxoSmithKline,

18 5 Moore Drive

19 Research Triangle Park,

20 Durham, North Carolina,

21 27709, USA

22
23 email; rachael.l.disantostefano@gsk.com
24
25

26
27 Tel: 00 1 919 483 9237

28
29 Fax: 00 1 919 549 7459
30
31

32
33 **Current Word Count:** [27393419](#)
34

35
36 **Key Words:** COPD, Exacerbation, Cluster Analysis, Long-acting beta-2 agonist, inhaled
37
38 corticosteroid
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

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 $\geq 12\%$ 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 \$\beta_2\$ agonist](#) bronchodilator ~~among COPD patients.~~

Strengths and limitations of this study: ~~Well characterised, pooled~~ [Pooled systematically collected](#) data from >1500 [well characterized](#) patients from ~~at 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.](#)

FINAL DRAFT – REVIEW REVISION FINAL

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 corticosteroid/long-acting β_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~~ at had primary ~~endpoint~~ endpoints of moderate/severe ~~exacerbation~~ 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 FEV₁ reversibility $\geq 12\%$ (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 FEV₁ 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 FEV₁

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 reversibility [>12% at baseline](#) were significantly more likely to experience a reduction in
4
5 COPD-associated exacerbations with SFC versus SAL [alone](#).
6

7 **Trial registration:** clinicaltrials.gov NCT00115492, NCT00144911
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only

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 (FEV₁) alone is an insufficient ~~marker~~measure of disease severity¹. Importantly, the revised GOLD strategy document also recommends therapy with an inhaled corticosteroid/ long-acting β_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 function², 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 complexityclinical 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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 those in other groups.¹² This is in contrast to subgroup analysis, where populations are pre-
4 defined and then statistical testing is applied to identify differences.¹³
5
6

7 In the present study, cluster analysis was conducted using data pooled from two
8 clinical [trials](#)¹³⁻¹⁴ that studied differences in exacerbation rates in COPD patients
9 randomly assigned to either a LABA (salmeterol [SAL]) or to ICS/LABA
10 (salmeterol/fluticasone propionate [SFC]).^{14 15} The objective of [this](#) cluster analysis was
11 to identify patients who benefit most from the addition of ICS to bronchodilator therapy [by](#)
12 [maximising treatment differences within a cluster for in terms of the reduction of the](#)
13 [mean annual rate of moderate/severe exacerbations for SFC compared to SAL.](#)
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 FEV₁ ≤50% of predicted, a pre-bronchodilator FEV₁/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 algorithm¹² 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](#)~~

1
2
3 exacerbates during the study. Subjects with a history of or current clinically significant
4 cardiac arrhythmias, uncontrolled/unstable congestive heart failure, uncontrolled
5 hypertension, or unstable angina were excluded from the study. Subjects (n=36) with
6
7
8 protocol violations or missing data required for the primary model were excluded (n=1543
9
10
11 analysed vs n=1579 ~~enrolled~~).

Cluster analysis methodology

12
13
14
15
16
17 The annual moderate/severe exacerbation rate was entered into a cluster
18 analysis using an interaction tree algorithm to maximise the identification of subgroups
19 showing differences in their response to SFC and SAL treatment.¹³

20
21 The cluster analysis aimed to find subgroups in the study subjects that had
22 similar baseline characteristics and with maximum treatment differences for mean
23 yearly moderate/severe exacerbation risk ratio (RR).

24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
Subjects included in the cluster analysis were required to have the following
baseline variables ~~comprising~~: FEV1 % predicted, FEV1 reversibility stratum (yes/no
for $\geq 12\%$ 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

[\(e.g. aspirin as a platelet inhibitor versus analgesic would be assigned to B01 Antithrombotic vs. N02 Analgesic\).](#)

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.

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 The model was adjusted for study baseline FEV₁ % predicted, FEV₁ reversibility stratum
4 (yes/no for ≥12% and 200 mL post-bronchodilator change), time on treatment and
5
6 geographical region (8 regions), which was considered a random effect. The algorithm used
7
8 in the study maximises treatment differences (mean moderate/severe exacerbation rates for
9
10 SFC vs SAL) among subgroups. The RR for each cluster was estimated using linear
11
12 contrast, and rate ratios plus 95% CIs were used to estimate the differences in annual mean
13
14 moderate/severe exacerbation rates for each cluster. The programme was completed in the
15
16
17 R statistical package.⁴⁵¹⁸

18
19 Clusters were clinically characterised based on the tree, ~~using descriptive~~
20
21 ~~statistics.~~ Descriptive statistics were used to present the baseline differences in clinical
22
23 features among clusters; proportions were used for categorical variables, and medians with
24
25 interquartile ranges were used for continuous variables. The χ^2 test was used to examine the
26
27 statistical differences among the subgroups for categorical variables and the non-parametric
28
29 Wilcoxon Rank sum test was performed to test the statistical differences among the
30
31 subgroups for continuous variables.
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 [studies¹³⁻¹⁴](#) [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 FEV₁ 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 [mL¹⁶](#) [mL¹⁹](#). 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 FEV₁ % predicted, FEV₁ reversibility stratum \(yes/no for ≥12% and 200 mL post-bronchodilator change\), and region. Only baseline FEV₁% of predicted was statistically significant \(p<0.01\) in the final model.](#)

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 Three final COPD clusters were defined (figure 1) based on use of diuretics and the
4 presence or absence of $\geq 12\%$ FEV₁ reversibility [based on the model](#). The first cluster
5 (cluster 1) identified subjects treated with diuretics (predominantly furosemide).
6
7 Approximately half of the diuretic use reported (n=282) was for hypertension, with the
8 remaining use for unspecified oedema, coronary artery disease, and/or congestive heart
9 failure. No other sub-populations were identified in this cluster. In patients not using diuretics
10 at baseline two further clusters were defined based on the presence or absence of FEV₁
11 reversibility. Cluster 2 patients exhibited reversibility, defined as a post-bronchodilator
12 change of $\geq 12\%$. Cluster 3 patients did not exhibit reversibility, ie, a post-bronchodilator
13 change in FEV₁ of $< 12\%$. Compared to SAL, significant reductions in the rate of
14 moderate/severe exacerbations were observed with SFC therapy in cluster 1 (44%
15 reduction) and cluster 2 (33% reduction). Similar reductions were not observed in cluster 3
16
17 ([table 3](#))–[figure 2](#))
18
19
20
21
22
23
24
25
26
27
28
29

30 Baseline demographics that were significantly different across clusters are presented
31 in [table 43](#). Subjects in cluster 1 tended to be older, had a higher BMI, were more likely to be
32 former smokers than current smokers, and had the greatest smoking pack-year history.
33
34 Cluster 1 subjects also had a higher prevalence of treatment for comorbidities (eg,
35 cardiovascular disease [CVD], hypertension, diabetes) than clusters 2 and 3. Subjects in
36 cluster 3 had a higher % predicted FEV₁ compared with those in clusters 1 and 2, whereas
37 those in cluster 2 had the lowest % predicted FEV₁. No difference was observed in the
38 baseline incidence of moderate/severe exacerbations between the clusters.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 COPD¹⁷ COPD²¹ and 2) if CVD is a driver for COPD exacerbation ~~occurrence~~¹⁸ occurrence²² 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

1
2
3 risk of a COPD exacerbation^{8 2024} and this logic supports the value of the addition of an ICS
4
5 (FP) in cluster 1.
6

7 The heterogeneity of COPD is well established⁶ and it has recently been suggested,
8
9 through use of a rigorous assessment of co-morbidity, that patients with CVD and metabolic
10 syndrome form discrete clusters of COPD patients¹² which are represented in our analysis in
11 Cluster 1. Another assessment of co-morbidity in COPD has found that certain CVDs
12 increase the risk of all-cause mortality in COPD;²⁵ Although the TORCH study failed to show
13 a significant effect (p=0.052) of FP/SAL versus placebo for all-cause mortality²⁶ a
14 subsequent analysis of CV-related mortality and AEs found a positive effect of FP/SAL
15 versus SAL in terms of CV-related outcomes²⁷ which further implies a potential benefit of
16 ICS in COPD patients with co-morbid CVD.
17
18
19
20
21
22
23
24

25 It is also possible that cluster 1 (diuretic) was predisposed to an increased
26
27 exacerbation risk as a consequence of heart failure-, especially as compared with cluster 2
28 and 3 more patients in cluster 1 were in receipt of anti-thrombotics, beta-blockers and
29 cardiac therapy, all of which suggest a greater degree of heart failure in cluster 1 compared
30 with the other clusters. Heart failure can be aggravated by increased aortic stiffness, a
31
32 marker of cardiovascular risk found in greater prevalence among COPD patients than in the
33
34 general population.²⁴²⁸ Dransfield *et al*²² al²⁹ recently found that SFC lowered aortic pulse
35
36 wave velocity (aPWV), a marker of aortic stiffness, in COPD patients with elevated aPWV.
37
38
39
40
41

42 Another possible explanation of the lower rate in moderate/severe exacerbations with
43
44 SFC over SAL in cluster 1 reflects the direct activity of the concomitant diuretic therapy.
45
46 Recent studies have examined the effectiveness of diuretics in the treatment of chronic
47
48 respiratory diseases, in particular furosemide (which was the predominantly used diuretic in
49
50 cluster 1). Mechanistically, furosemide inhibits inflammatory cytokines²³ cytokines³⁰ and
51
52 enhances the anti-inflammatory impact of ICS.²⁴³¹ Clinically it has been shown to alleviate
53
54 exertional dyspnoea in COPD,²⁵³² and to protect against bronchoconstriction in asthma.²⁶⁻²⁷
55
56

57 2833 34 35
58
59
60

1
2
3 The findings in cluster 1 relating to co-morbidity in COPD are of particular interest
4 given recent publications on the prevalence and impact of co-morbidity in COPD^{12 25 36} and
5 the concept of multiple morbidities and their impact on clinical practice.³⁷ Certainly our
6 findings and others^{11 12} suggest that patients with multiple diseases may benefit from a
7 different approach to management than those with a single disease, a concept which has
8 recently been raised as a major issue in primary care.³⁸

9
10
11
12
13
14
15 In cluster 2 (reversibility $\geq 12\%$) a significant effect of SFC was also observed over
16 SAL in terms of a lower rate of moderate/severe exacerbations. There was also reversibility
17 in Cluster 1 (median, 18.6%). Subjects exhibiting reversibility have been shown to have
18 greater improvement in lung function compared to those without reversibility²⁹ which could
19 explain the significant effect of SFC relative to SAL in terms of a lower rate of
20 moderate/severe exacerbations.³⁹ Recent data suggest that an improvement in lung function
21 of 100 mL relates to a reduction in exacerbation rate of 12%,³⁰⁴⁰ while a 12% increase in
22 exacerbation rate has been reported for each 100 mL loss of lung function.⁸ The effect of
23 SFC in COPD³⁴ COPD⁴¹ has been shown to provide a significantly greater effect on lung
24 function in reversible versus irreversible subjects. This suggests a potential mechanism for
25 the lower rate of moderate/severe exacerbations in cluster 2 patients receiving SFC-1 and
26 cluster 2 for patients receiving SFC. The rationale for this is that a greater improvement in
27 lung function is typically associated with a greater effect on exacerbations (e.g. Jones *et*
28 *al*⁴⁰).

29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45 The role of reversibility as a distinguishing feature in COPD has recently been
46 questioned. While it is apparent that COPD subjects can be more or less reversible, there is
47 considerable within-patient variability both on single testing³⁹ and at testing on multiple
48 occasions.⁴² Subject reversibility will vary over time, such that over 1 year only 4% of
49 patients were reversible on every occasion tested.⁴² As such it has been shown that while
50 the percentage of reversible patients is between 20-30% in any given population at any time
51 (as was the case in these studies), the actual patients who are reversible may change.
52
53
54
55
56
57
58
59
60

1
2
3 Despite the limitations with reversibility, there is evidence that subjects who are more
4 reversible are likely to have a more robust bronchodilator response to treatment than those
5 who are less reversible.^{39,41} None of the subjects in cluster 3 exhibited reversibility by
6 definition, and this together with the highest prevalence of current smokers (which is known
7 to attenuate ICS effects in COPD)^{32,43} may explain why no difference was observed between
8 SFC and SAL in this cluster.

9
10
11 Although the studies excluded subjects with current asthma based on the
12 investigator's judgment, subjects could have had a history of asthma but not a diagnosis of
13 active asthma. It has been suggested that Asthma and COPD form part of the same
14 disease continuum⁴⁴ and though this is a controversial concept, the idea of an asthma-
15 COPD overlap syndrome may give insight into the response to combination ICS/LABA.^{45,46}
16 Forty percent and 59% of patients in Clusters 1 and 2, respectively, were $\geq 12\%$ reversible
17 and had an increase of $\geq 200\text{mL}$ in FEV₁. However, the reversibility stratum ($\geq 12\%$ and
18 $\geq 200\text{mL}$) was adjusted for in the overall negative binomial model examining mean annual
19 exacerbation rates and was found to be not statistically significant, suggesting that any
20 impact of reversibility as determined by both $\geq 12\%$ and $\geq 200\text{mL}$ was minimal.

21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
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 one³⁶ one¹¹ has validated the COPD subtypes identified against clinically meaningful outcomes. Garcia-Aymerich *et al*³⁶ al¹¹ 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.

1
2
3 Cluster analysis is limited due to its retrospective nature and the fact that it is limited
4 to assessing only the categorical variables collected at baseline. In addition, the splitting into
5 groups is automated by the computer-driven algorithm to maximise treatment differences,
6
7 and is not necessarily robust, thus external validation is warranted. [Because this analysis](#)
8
9 [includes only those patients with a history of exacerbation, it is also difficult to generalise to](#)
10
11 [subjects with COPD who do not have a history of exacerbation.](#)
12
13
14

15 In conclusion, cluster analysis of subjects taking part in two exacerbation studies of
16 SFC versus SAL identified three distinct groups of COPD subjects based on diuretic use and
17 reversibility. These subjects varied in their response with subjects in two of the three groups
18 experiencing a greater reduction in the annual rate of moderate/severe exacerbations with
19 SFC versus SAL. Those in the remaining group received no additional benefit in terms of
20 reduction in the annual moderate/severe exacerbation rate over that provided by SAL alone.
21 This study highlights the future potential for a personalised medicine approach to the
22 treatment of patients with COPD. It additionally suggests how this methodology can be used
23 to generate potential hypothesis for future studies.
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Acknowledgements

Editorial support in the form of development of the manuscript first draft under the guidance of the corresponding author and co-authors, editorial suggestions to draft versions of this paper in collaboration with the corresponding author and co-authors, assembling tables and figures, collating author comments, copyediting, fact checking, referencing, and graphic services was provided by Geoff Weller, PhD at Gardiner-Caldwell Communications and was funded by GlaxoSmithKline.

Amanda Emmett, an employee of GlaxoSmithKline, provided consultation regarding the clinical trials data and statistical review of the results and subsequent manuscript. Ms. Emmett was the primary statistician on the two COPD exacerbation randomised control trials.

Statement of interest

All co-authors are employees and shareholders of GlaxoSmithKline.

Funding

Funded by GlaxoSmithKline.

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

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 methodological expertise. DR was involved in planning the analyses, interpretation of
4
5 results. DS was involved in planning of analyses, interpretation of results.
6
7

8 9 **Licence Statement**

10
11 The Corresponding Author has the right to grant on behalf of all authors and does grant on
12
13 behalf of all authors, an exclusive licence (or non-exclusive for government employees) on a
14
15 worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if
16
17 accepted) to be published in BMJ Open and any other BMJPGGL products to exploit all
18
19 subsidiary rights, as set out in our licence (
20
21 http://group.bmj.com/products/journals/instructions-for-authors/wholly_owned_licence.pdf)
22
23 and the Corresponding Author accepts and understands that any supply made under these
24
25 terms is made by BMJPGGL to the Corresponding Author.
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD). *Global Strategy for the Diagnosis, Management and Prevention of COPD*, 2011. Available from: <http://www.goldcopd.org/>.
2. Celli BR, Thomas NE, Anderson JA, *et al*. Effect of pharmacotherapy on rate of decline of lung function in chronic obstructive pulmonary disease: results from the TORCH study. *Am J Respir Crit Care Med* 2008 Aug 15;178(4):332-8.
3. Spencer S, Calverley PM, Burge PS, Jones PW. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J* 2004 May;23(5):698-702.
4. Jones PW, Tabberer M, Chen WH. Creating scenarios of the impact of COPD and their relationship to COPD Assessment Test (CAT™) scores. *BMC Pulm Med* 2011 Aug 11;11:42.
5. Agusti AG. COPD, a multicomponent disease: implications for management. *Respir Med* 2005 Jun;99(6):670-82.
6. Agusti A, Calverley P, Celli B, *et al*. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res* 2010;11:122.
7. Han MK, Agusti A, Calverley PM, *et al*. Chronic obstructive pulmonary disease phenotypes: the future of COPD. *Am J Respir Crit Care Med*. 2010 Sep 1;182(5):598-604.
8. Hurst JR, Vestbo J, Anzueto A. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *New Engl J Med* 2010;363:1128–38.
9. Cho MH, Washko GR, Hoffmann TJ, *et al*. Cluster analysis in severe emphysema subjects using phenotype and genotype data: an exploratory investigation. *Respir Res* 2010;11:30.
10. Roy K, Smith J, Kolsum U, *et al*. COPD phenotype description using principal components analysis. *Respir Res* 2009;10:41
11. Garcia-Aymerich J, Gomez F, Benet M, *et al*. Identification and prospective validation of clinically relevant chronic obstructive pulmonary disease (COPD subtypes). *Thorax* 2011;66:430–37.
12. [Vanfleteren LE, Spruit MA, Groenen M, *et al*. Clusters of Comorbidities Based on Validated Objective Measurements and Systemic Inflammation in Patients with Chronic Obstructive Pulmonary disease. *Am J Respir Crit Care Med*. 2013 Feb 7. \[Epub ahead of print\]](#)
13. Su X, Tsai C-L, Wang H, Nickerson DM, Li B. Tree-based subgroup analysis via recursive partitioning. *J Machine Learning Res* 2009;10:141–58.
14. Anzueto A, Feldman G, Chinsky K, *et al*. Effect of fluticasone propionate/salmeterol (250/50) on COPD exacerbations and impact on patient outcomes. *J COPD* 2009;6:320–29.

FINAL DRAFT – REVIEW REVISION FINAL

- 1
2
3 | 14-15. Ferguson GT, Anzueto A, Fei R, *et al.* Effect of fluticasone
4 | propionate/salmeterol (250/50 mcg) or salmeterol (50 mcg) on COPD exacerbations.
5 | *Respir Med* 2008;102:1099–108.
6 |
7 | [16. American Thoracic Society Standards for diagnosis and care of patients with chronic
8 | obstructive pulmonary disease and asthma. *Am Rev Respir Dis.* 1987;136:225–44](#)
9 |
10 | [17. WHO Collaborating Centre for Drug Statistics Methodology. Structure and principles
11 | of the Anatomical Therapeutic Chemical \(ATC\) classification system. Available at
12 | \[http://www.whocc.no/atc/structure_and_principles/\]\(http://www.whocc.no/atc/structure_and_principles/\) accessed 20th Feb 2013](#)
13 |
14 |
15 | 15-18. Everitt B. An R and S-PLUS® companion to multivariate analysis. London,
16 | Springer-Verlag, 2005.
17 |
18 | 16-19. Pellegrino R, Viegi G, Brusasco V, *et al.* Interpretative strategies for lung
19 | function tests. *Eur Respir J* 2005; 26: 948–968
20 |
21 | [20. Poulain M, Doucet M, Drapeau V, *et al.* Metabolic and inflammatory profile in obese
22 | patients with chronic obstructive pulmonary disease. *Chron Respir Dis* 2008;5:35–41](#)
23 |
24 | 17-21. Man SF, Leipsic JA, Man JP, Sin DD. Is atherosclerotic heart disease in
25 | COPD a distinct phenotype? *Chest.* 2011 Sep;140(3):569-71
26 |
27 | 18-22. Niewoehner DE, Likhnygina Y, Rice K, *et al.* Risk indexes for exacerbations
28 | and hospitalizations due to COPD. *Chest* 2007 Jan;131(1):20-8.
29 |
30 | 19-23. Chang CL, Robinson SC, Mills GD, *et al.* Biochemical markers of cardiac
31 | dysfunction predict mortality in acute exacerbations of COPD. *Thorax.* 2011
32 | Sep;66(9):764-8.
33 |
34 | 20-24. Groenewegen KH, Postma DS, Hop WC, *et al.* Increased systemic
35 | inflammation is a risk factor for COPD exacerbations. *Chest* 2008 Feb;133(2):350-7.
36 |
37 | [25. Divo M, Cote C, de Torres JP, *et al.* Comorbidities and risk of mortality in patients
38 | with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.*
39 | 2012;186\(2\):155-61.](#)
40 |
41 | [26. Calverley PM, Anderson JA, Celli B, *et al.* Salmeterol and fluticasone propionate and
42 | survival in chronic obstructive pulmonary disease. *N Engl J Med.* 2007;356\(8\):775-89](#)
43 |
44 | [27. TORCH CV Calverley PM, Anderson JA, Celli B, *et al.* Cardiovascular events in
45 | patients with COPD: TORCH study results. *Thorax.* 2010;65\(8\):719-25.](#)
46 |
47 |
48 | 21-28. Sabit R, Bolton CE, Edwards PH, *et al.* Arterial stiffness and osteoporosis in
49 | chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2007 Jun
50 | 15;175(12):1259-65.
51 |
52 | 22-29. Dransfield MT, Cockcroft JR, Townsend RR, *et al.* Effect of fluticasone
53 | propionate/salmeterol on arterial stiffness in patients with COPD. *Respir Med* 2011
54 | Sep;105(9):1322-30.
55 |
56 |
57 |
58 |
59 |
60 |

FINAL DRAFT – REVIEW REVISION FINAL

- 1
2
3 | ~~23-30.~~ Yuengsrigul A, Chin TW, Nussbaum E. Immunosuppressive and cytotoxic
4 | effects of furosemide on human peripheral blood mononuclear cells. *Ann Allergy*
5 | *Asthma Immunol* 1999;83:559–66.
6
7 | ~~24-31.~~ Prandota J. Furosemide: progress in understanding its diuretic, anti-
8 | inflammatory, and bronchodilating mechanism of action, and use in the treatment of
9 | respiratory tract diseases. *Am J Ther* 2002;9:317–28.
10
11 | ~~25-32.~~ Jensen D, Amjadi K, Harris-McAllister V, Webb KA, O'Donnell DE.
12 | Mechanism of dyspnoea relief and improved exercise after furosemide inhalation in
13 | COPD. *Thorax* 2008;63:606–13.
14
15 | ~~26-33.~~ Roger A, Botey J, Esevri JL, *et al.* Prevention of exercise-induced asthma in
16 | children using low doses of inhaled furosemide. *J Invest Allergol Clin Immunol*
17 | 1993;3:300–3.
18
19 | ~~27-34.~~ Novembre E, Frongia G, Lombardi E, *et al.* The preventative effect and
20 | duration of action of two doses of inhaled furosemide on exercise-induced asthma in
21 | children. *J Allergy Clin Immunol* 1995;96:906–9.
22
23 | ~~28-35.~~ Melo RE, Sole D, Naspitz CK. Comparative efficacy of inhaled furosemide
24 | and disodium cromoglycate in the treatment of exercise-induced asthma in children.
25 | *J Allergy Clin Immunol* 1997;99:204–9.
26
27 | [36. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur*](#)
28 | [Respir J. 2009;33\(5\):1165-85.](#)
29
30 | [37. Barnett K, Mercer SW, Norbury M, *et al.* Epidemiology of multimorbidity and](#)
31 | [implications for health care, research, and medical education: a cross-sectional](#)
32 | [study. *Lancet.* 2012;380\(9836\):37-43.](#)
33
34 | [38. Guthrie B, Payne K, Alderson P, *et al.* Adapting clinical guidelines to take account of](#)
35 | [multimorbidity. *BMJ.* 2012;345:e6341](#)
36
37
38
39 | ~~29-39.~~ Hanania N, Celli BR, Donohue JF, Martin UJ. Bronchodilator reversibility in
40 | COPD. *Chest* 2011;140:1055–63.
41
42 | ~~30-40.~~ Jones PW, Donohue JF, Nedelman J, *et al.* Correlating changes in lung
43 | function with patient outcomes in chronic obstructive pulmonary disease: a pooled
44 | analysis. *Respir Res.* 2011 Dec 29;12:161.
45
46 | ~~31-41.~~ Bleeker ER, Emmett A, Crater G, *et al.* Lung function and symptom
47 | improvement with fluticasone propionate/salmeterol and ipratropium
48 | bromide/albuterol in COPD: response by beta-agonist reversibility. *Pulm Pharmacol*
49 | *Ther* 2008 Aug;21(4):682-8.
50
51 | ~~32-42.~~ Albert P, Agusti A, Edwards L, *et al.* Bronchodilator responsiveness as a
52 | phenotypic characteristic of established chronic obstructive pulmonary disease.
53 | *Thorax* 2012;68(8):701-708
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

1
2
3 | ~~33~~.43. Soriano JB, Sin DD, Zhang X, *et al*. A pooled analysis of FEV1 decline in
4 COPD patients randomized to inhaled corticosteroids or placebo. *Chest* 2007
5 Mar;131(3):682-9.
6
7

8 | [44. Postma DS, Boezen HM. Rationale for the Dutch hypothesis. Allergy and airway
9 hyperresponsiveness as genetic factors and their interaction with environment in the
10 development of asthma and COPD. *Chest*. 2004 Aug;126\(2 Suppl\):96S-104S](#)
11

12 | [45. de Oca MM, Halbert RJ, Lopez MV, *et al*. The chronic bronchitis phenotype in
13 subjects with and without COPD: the PLATINO study. *Eur Respir J*. 2012;40\(1\):28-
14 36.](#)
15

16 | [46. Soler-Cataluña JJ, Cosío B, Izquierdo JL, *et al*. Consensus document on the overlap
17 phenotype COPD-asthma in COPD. *Arch Bronconeumol*. 2012;48\(9\):331-7.](#)
18

19 | ~~34~~.47. Bafadhel M, McKenna S, Terry S, *et al*. Acute exacerbations of chronic
20 obstructive pulmonary disease: identification of biologic clusters and their
21 biomarkers. *Am J Respir Crit Care Med* 2011;184:662–71.
22

23 | ~~35~~.48. Burgel P-R, Paillasseur J-L, Caillaud D, *et al*. Clinical COPD phenotypes: a
24 novel approach using principal component and cluster analyses. *Eur Respir J*
25 2010;36:531–39.
26

27 | ~~36~~.49. Weatherall M, Travers J, Shirtcliffe PM, *et al*. Distinct clinical phenotypes of
28 airways disease defined by cluster analysis. *Eur Respir J* 2009;34:812–18.
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FINAL DRAFT – REVIEW REVISION FINAL

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
	Antihaemorrhagics
	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; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; OCS, Oral Corticosteroid. QOL, quality of life; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT – REVIEW REVISION FINAL

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)

¹Reversibility based on change in FEV₁ 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; FEV₁, forced expiratory volume in 1 second; SGRQ, St. George's Respiratory Questionnaire.

FINAL DRAFT – REVIEW REVISION FINAL

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

FEV₁, forced expiratory volume in 1 second; ACE, angiotensin converting enzyme; ns, not significant; SD, Standard Deviation; ¹Reversibility based on change in FEV₁ from baseline following 4 puffs (360 µg) albuterol, defined as a ≥12% and ≥200 mL increase.

1
2
3 Figure Legends

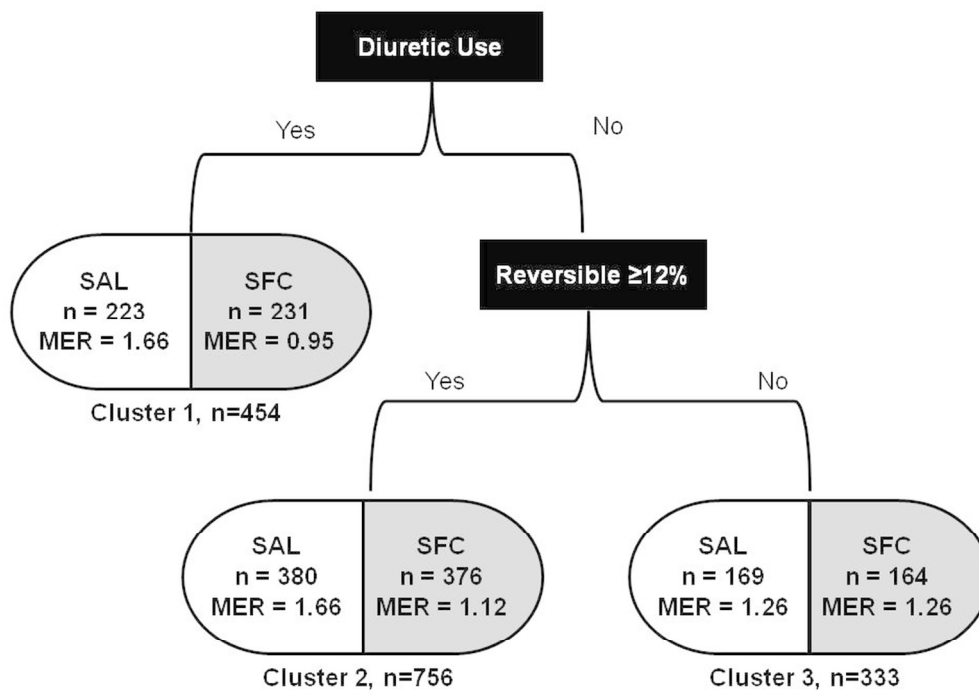
4
5 Figure 1. Interaction tree generated by supervised cluster analysis

6
7 MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC =
8 Salmeterol/Fluticasone propionate combination
9

10
11 Figure 2. Pooled analysis of SFC effect on mean annual moderate/severe exacerbation rate
12 by cluster

13
14 ns = not significant (p>0.05); SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate
15 combination
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only



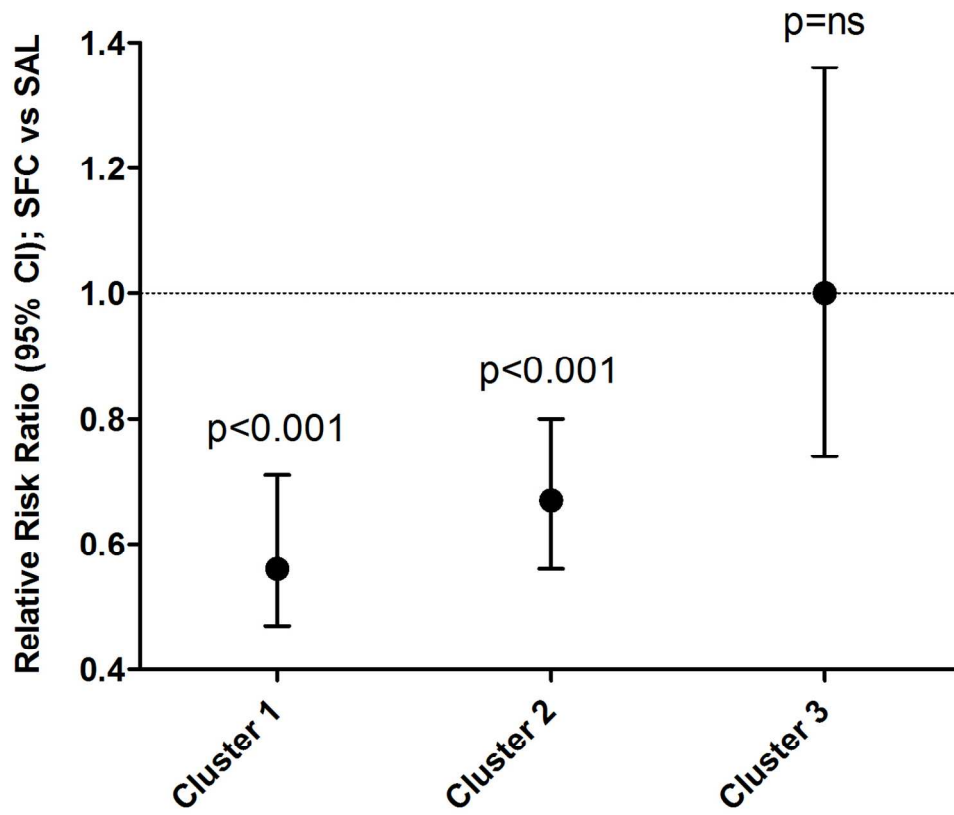
MER = Mean annual moderate/severe Exacerbation Rate

MER = Mean annual rate of moderate/severe exacerbations; SAL = Salmeterol; SFC = Salmeterol/Fluticasone propionate combination 119x90mm (300 x 300 DPI)

ew only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



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

For peer review only