ORIGINAL ARTICLE

Blood eosinophils and inhaled corticosteroid/long-acting β-2 agonist efficacy in COPD

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ABSTRACT

Objective We performed a review of studies of fluticasone propionate (FP)/salmeterol (SAL) (combination inhaled corticosteroid (ICS)/long-acting β2-agonist (LABA)) in patients with COPD, which measured baseline (pretreatment) blood eosinophil levels, to test whether blood eosinophil levels ≥2% were associated with a greater reduction in exacerbation rates with ICS therapy.

Methods Three studies of ≥1-year duration met the inclusion criteria. Moderate and severe exacerbation rates were analysed according to baseline blood eosinophil levels (<2% vs ≥2%). At baseline, 57–75% of patients had ≥2% blood eosinophils. Changes in FEV_1 and St George's Respiratory Questionnaire (SGRQ) scores were compared by eosinophil level.

Results For patients with ≥2% eosinophils, FP/SAL was associated with significant reductions in exacerbation rates versus tiotropium (INSPIRE: n=719, rate ratio (RR)=0.75, 95% CI 0.60 to 0.92, p=0.006) and versus placebo (TRISTAN: n=1049, RR=0.63, 95% CI 0.50 to 0.79, p<0.001). No significant difference was seen in the <2% eosinophil subgroup in either study (INSPIRE: n=550, RR=1.18, 95% CI 0.92 to 1.51, p=0.186; TRISTAN: n=354, RR=0.99, 95% CI 0.67 to 1.47, p=0.957, respectively). In SCO30002 (n=373), no significant effects were observed (FP or FP/SAL vs placebo). No relationship was observed in any study between eosinophil subgroup and treatment effect on FEV_1 and SGRQ.

Discussion Baseline blood eosinophil levels may represent an informative marker for exacerbation reduction with ICS/LABA in patients with COPD and a history of moderate/severe exacerbations.

Key messages

What is the key question?
► Is a pretreatment blood eosinophil level of ≥2% (vs <2%) associated with a greater reduction in COPD exacerbation rate with inhaled corticosteroids/long-acting β2-agonist combination?

What is the bottom line?
► A retrospective analysis of data from three randomised, controlled trials of at least 1-year duration supported the hypothesis that there is greater response to fluticasone propionate/salmeterol, compared with placebo or long-acting anti-muscarinic agents, in individuals with a pretreatment blood eosinophil level of ≥2% compared with those with a pretreatment blood eosinophil level of <2%.

Why read on?
► A blood test for eosinophil levels could be employed as a simple biomarker for clinical decision-making in patients with moderate-to-severe COPD and a history of exacerbations.

INTRODUCTION

Inhaled corticosteroids (ICS) are an important treatment for COPD.[1] Exacerbations, defined as acute worsening of symptoms necessitating treatment with antibiotics and/or systemic corticosteroids or hospitalisation, are a key determinant of COPD morbidity, mortality and healthcare costs.[2] Compared with placebo, ICS such as fluticasone propionate (FP) and budesonide reduce exacerbations by up to 20% as monotherapy, and up to 30% in combination with a long-acting β2-agonist (LABA).[3–5] National and international guidelines on the management of COPD[1 6] recommend that patients with COPD at risk of exacerbations receive ICS/LABA maintenance therapy.

The TOwards a Revolution in COPD Health (TORCH) study showed that ICS treatment was associated with an increased risk of non-fatal pneumonia in patients with FP-treated COPD,[7] now recognised as an ICS class effect.[8] The reconsideration of potential risks associated with ICS treatment, weighed against its known benefits, has motivated the search for biomarkers to inform COPD treatment decisions.[9]

A predictive marker for ICS (or ICS/LABA) effectiveness in preventing COPD exacerbations could aid clinical decision-making by identifying patients likely to gain the most benefit from ICS-based treatment. Blood eosinophil count may provide such a marker. Studies have demonstrated associations between airway eosinophilia and exacerbations of chronic bronchitis[10] and COPD.[11] Exacerbations are heterogeneous, presenting as one of four distinct phenotypes, and airway eosinophilia in the stable state was found to be predictive of subsequent exacerbation phenotype.[12] Relationships between sputum eosinophilia and steroid responsiveness in COPD have also been

reported.[13] [14] The use of systemic corticosteroids in patients experiencing acute exacerbations of COPD has shown greater benefit in patients with a blood eosinophil level of ≥2% versus those with <2%.[15] [16] There is also evidence for an association between airway eosinophilia with response to systemic corticosteroids for FEV_1[14] [17] and quality of life.[13] A recent retrospective analysis of data from two parallel 1-year studies of once-daily ICS/LABA, fluticasone furoate (FF)/vilanterol (VI) in patients with moderate-to-very severe COPD showed a greater reduction of moderate and severe exacerbations in patients with a blood eosinophil level ≥2% vs <2% when treated with FF/VI compared with VI alone.[18] [19]

To investigate the potential of blood eosinophil level as a marker for the preventive efficacy of ICS or ICS/LABA on exacerbations, we reanalysed data from studies comparing ICS or ICS/LABA combination therapy (FP/salmeterol (SAL)) with a long-acting antimuscarinic (LAMA), LABA or placebo according to baseline eosinophil categories.

METHODS
Study selection
Parallel-group, double-blind, randomised clinical trials of FP or FP/SAL that included at least one non-ICS comparator and were at least 24 weeks in duration were identified in the GSK Clinical Study Register and reviewed for inclusion in this retrospective analysis. Studies in which blood eosinophil levels were not recorded at baseline or screening (ie, before randomisation) were excluded. In total, six studies met the criteria, three of which were ≥1 year in duration: INSPIRE (SCO40036; NCT00361959),[20] TRISTAN (SFCB3024)[3] and SCO30002.[21] One additional study was identified,[22] but was excluded, as eosinophil data were only available in a subset of subjects.

Analysis population
The primary analysis population of each study was used as the analysis population for this retrospective analysis.

The 2-year INSPIRE (SCO40036) trial was designed to study exacerbations and compared twice-daily FP/SAL 500/50 µg with once-daily tiotropium 18 µg in 1323 patients with severe or very severe COPD (post-bronchodilator FEV_1 <50% predicted).[20] Patients had a history of COPD exacerbations in the year prior to randomisation. The primary outcome variable was the rate of exacerbations requiring treatment with systemic corticosteroids or antibiotics, or necessitating hospitalisation.

TRISTAN (SFCB3024) was a 1-year study comparing twice-daily FP/SAL 500/50 µg with its monocomponents and placebo in 1465 patients with moderate-to-severe COPD (pre-bronchodilator FEV_1 25–70% predicted).[3] Patients had a history of ≥1 treated exacerbation/year in the 3 years prior to trial entry. The primary outcome variable was change from baseline in pretreatment FEV_1 (after patients had abstained from bronchodilators for ≥6 h and from study medication for ≥12 h) at study end. Number of moderate and severe exacerbations was a secondary endpoint.

SCO30002, another 1-year study, compared twice-daily FP/SAL 500/50 µg with FP 500 µg and placebo in 387 patients with moderate-to-severe COPD (FEV_1 ≤70% predicted) with and without a history of exacerbation. The primary endpoint was time to first moderate/severe exacerbation. Number of moderate and severe exacerbations was a secondary endpoint.

Three additional studies met the selection criteria, and were each 24 weeks in duration. SFCA3006 compared the same daily FP/SAL dose as the longer-duration studies (FP/SAL 500/50 µg) with its monocomponents and placebo.[23] SFCA3007 and SCO100470 compared FP/SAL 250/50 µg with its monocomponents and placebo, and with SAL alone, respectively.[24] [25] These studies included the following numbers of patients with eosinophil count data: 670 (SFCA3006), 716 (SFCA3007) and 1020 (SCO100470). In SFCA3006 and SFCA3007, subjects were withdrawn after an exacerbation necessitating hospitalisation, oral corticosteroid treatment, or having >3 antibiotic-treated exacerbations; in SCO100470 patients were withdrawn if they had >1 moderate, or ≥1 severe, exacerbations. Since moderate and severe exacerbation rates, our primary parameter of interest, could not be calculated from these 24-week studies, the available data from these studies are presented in online supplementary material.

With the exception of SCO30002, blood eosinophil levels were measured by a central laboratory using standard cell counting procedures. SCO30002 used local laboratories.

Statistical analysis
The retrospective analysis was conducted in accordance with a predefined analysis plan (see online supplementary material). The comparators were such that pooling of the data was not considered appropriate. The last pre-randomisation drug blood eosinophil level was used to dichotomise study participants according to blood eosinophil level of <2% vs ≥2%. In an additional preplanned analysis, an absolute eosinophil count of 200/mm^3 was used to dichotomise the data. A post hoc analysis of a 3% cut-off level was also carried out. The main outcome of interest was moderate/severe exacerbation rate (≥1-year studies only). Time to first moderate/severe exacerbation, change from baseline in FEV_1, rate of decline in FEV_1 (TRISTAN and INSPIRE only), and St George's Respiratory Questionnaire (SGRQ) score were analysed, data permitting. Weighted mean FEV_1 over the study period was analysed to provide a single on-treatment assessment (post hoc to the analysis plan). The primary treatment comparisons of interest were FP/SAL versus non-ICS comparators (SAL, tiotropium or placebo) and FP versus placebo. SAL versus placebo and FP/SAL versus FP were examined, but were not predefined comparisons of interest.

Moderate/severe exacerbation rates were analysed, for studies of ≥1 year in duration, using a negative binomial model, with number of recorded on-treatment moderate/severe exacerbations per patient as the response variable. Explanatory variables were treatment group, sex, % predicted FEV_1 at baseline, frequency of prior exacerbations (0 (SCO30002 only), 1, ≥2) within the past year (data not available for TRISTAN), eosinophil subgroup, and eosinophil subgroup by treatment interaction. Log treatment exposure per patient was included as an offset variable. Point estimates and 95% CI for treatment differences were obtained for treatment comparisons of interest. A post hoc analysis of rate of exacerbations requiring oral corticosteroids or antibiotics by eosinophil level was carried out.

Analysis of time to first moderate/severe exacerbation was performed using a Cox's proportional hazards model including covariates for treatment group, sex, % predicted FEV_1 at baseline, frequency of prior exacerbations (INSPIRE, SCO30002, SCO100470), eosinophil subgroup, and eosinophil subgroup by treatment interaction. HRs for treatment comparisons of interest were calculated together with associated 95% CI and p values. Kaplan–Meier survival probability estimates were calculated for each treatment and eosinophil subgroup.

FEV_1 was analysed using data as collected in each study: trough (INSPIRE, SCO100470), pre-bronchodilator (SFCB3024; SCO30002), post-bronchodilator (TRISTAN) and pre-dose (SFCA3006, SFCA3007). Weighted mean FEV_1 over

the duration of the study was derived from available data by calculating the area under the curve using the trapezoidal rule. Treatment differences in weighted mean FEV_1 were analysed using an analysis of covariance with covariates of age, sex, baseline FEV_1, treatment group, eosinophil subgroup, and treatment group by eosinophil subgroup interaction. Point estimates and 95% CIs of difference in FEV_1 were obtained for treatment comparisons of interest.

SGRQ data were analysed using mixed model repeated measures including covariates of age, sex, baseline SGRQ, treatment group, eosinophil group, nominal day, and pairwise interactions of day by baseline, eosinophil group and treatment group, and the three-way interaction of day by treatment by eosinophil. Point estimates and 95% CIs were calculated for treatment comparisons of interest at each nominal day.

Statistical analyses were performed using the Harmonisation of Analysis & Reporting Program (HARP) system (GSK, Harlow, UK) using SAS V.9.1.3 or later.

RESULTS
Patient demographics and baseline characteristics
COPD patient demographics were generally similar across the three ≥1-year studies (tables 1–3), although patients in INSPIRE had a lower post-bronchodilator % predicted FEV_1. A total of 1269 (INSPIRE), 1403 (TRISTAN) and 373 (SCO30002) patients had eosinophil data available. Most participants were male, aged ≥60 years, and with a smoking history averaging ≥ approximately 40 pack-years. Approximately half of patients were current smokers. In all three studies, a greater proportion of patients with eosinophils <2% vs ≥2% were current smokers (tables 1–3). No other consistent trends between eosinophil categories in demographic or baseline characteristics were observed, including previous exacerbation history.

The proportions of patients categorised into each eosinophil subgroup were consistent across the treatment arms in all three studies (tables 1–3). A larger overall proportion of patients had eosinophil levels ≥2% in the TRISTAN study than in INSPIRE or SCO30002 (figure 1). Additional demographic and baseline data, for the ≥1-year and 24-week studies, are provided in online supplementary tables S1 and S2 and figure S1. For TRISTAN, where eosinophil measurements were available at multiple time points throughout the study, most patients were categorised in the same category (<2% vs ≥2%) at baseline and at week 24 or 52 (see online supplementary figure S2).

Moderate/severe exacerbation rate
Across all three ≥1-year studies, numerically greater percentage reductions in moderate/severe exacerbation rates in patients receiving FP/SAL versus placebo and LAMA were observed in those with baseline eosinophil levels ≥2% vs <2% (figure 2). This was also seen for FP and SAL versus placebo in those studies having these arms. The adjusted mean exacerbation rates by treatment according to baseline eosinophil subgroup (<2%, ≥2%) are reported in online supplementary table S3. An analysis of INSPIRE and TRISTAN according to a 3% blood eosinophil level cut-off is reported in online supplementary table S4. An analysis of exacerbation rate in INSPIRE, TRISTAN and SCO30002 is reported in figure 3 (time to first moderate/severe exacerbation according to a 200/mm^3 absolute eosinophil count cut-off is reported in online supplementary figure S5).

In INSPIRE (excluding a prior history of exacerbations as a covariate), a reduction of 25% in annual moderate/severe exacerbation rate with FP/SAL versus tiotropium was observed in the ≥2% eosinophils subgroup (p=0.006). In the <2% eosinophils subgroup, the rate of these exacerbations was 18% higher in patients receiving FP/SAL than in those who received tiotropium, although these treatment differences did not reach statistical significance (p=0.186). When prior history of exacerbations was included as a covariate, the overall change in exacerbations on treatment was less pronounced, but the trend was similar for the ≥2% eosinophils subgroup versus the <2% baseline eosinophils group (18% reduction vs 7% increase, respectively) (figure 2). There was an imbalance in treatment arms for history of exacerbations (in the <2% eosinophils group, 26% of FP/SAL-treated patients had >2 exacerbations in the last 12 months, compared with 18% of tiotropium-treated patients (table 1)).

In TRISTAN, exacerbation rates with FP/SAL were compared with FP and SAL alone and with placebo; FP and SAL were also compared with placebo. For all comparisons, relatively greater reductions were observed in patients with eosinophils ≥2% vs

Table 1 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: INSPIRE (SCO40036)

Characteristic	FP/SAL		Tiotropium	
	<2%	≥2%	<2%	≥2%
n	263	371	287	348
Age, mean (SD)	64.1 (8.79)	64.3 (8.06)	64.8 (8.05)	64.4 (8.46)
Male (%)	79	82	81	86
Current smokers (%)	42	35	41	36
Pack-years, median (range)	38.0 (10–140)	38.0 (–50–201)*	38.0 (4–248)	35.0 (3–151)
Post-BD FEV$_1$% predicted, mean (SD)	38.8 (8.14)	39.3 (8.34)	39.3 (9.20)	39.5 (8.67)
FEV$_1$% reversibility, mean (SD)	2.4 (3.14)	2.2 (4.29)	2.6 (4.34)†	2.7 (4.44)
Moderate/severe exacerbations in prior 12 months, n (%)				
0	69 (26)	111 (30)	89 (31)	84 (24)
1	75 (29)	111 (30)	92 (32)	97 (28)
2	51 (19)	71 (19)	55 (19)	92 (26)
>2	68 (26)	78 (21)	51 (18)	75 (22)

*Negative value was recorded in dataset.
†n=285.
BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

Table 2 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: TRISTAN (SFCB3024)

Characteristic	FP/SAL		FP		SAL		Placebo	
	<2%	≥2%	<2%	≥2%	<2%	≥2%	<2%	≥2%
n	93	248	94	266	86	269	81	266
Age, mean (SD)	63.1 (8.49)	62.8 (8.80)	63.3 (8.64)	63.7 (8.57)	64.4 (8.38)	62.8 (8.47)	60.3 (8.23)	64.3 (8.50)
Male (%)	77	76	61	72	56	74	68	76
Current smokers (%)	56	50	64	49	57	50	62	44
Pack-years, median (range)	40.0 (10–124)	40.0 (10–159)	35.5 (10–105)	40.0 (10–110)	44.5 (10–140)	40.0 (10–135)	41.2 (12–131)	40.0 (10–150)
Post-BD FEV$_1$% predicted, mean (SD)	52.4 (14.51)	51.0 (14.58)	50.5 (13.89)	51.8 (14.46)	49.1 (13.91)	50.4 (13.95)	49.1 (14.50)*	51.3 (13.98)
FEV$_1$% reversibility, mean (SD)	3.4 (3.38)	3.9 (3.60)	2.5 (3.25)	3.9 (5.95)	3.6 (3.85)	3.3 (3.72)	3.6 (3.52)*	3.5 (4.59)

*n=80.
BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

<2% (figure 2). For the comparisons of FP/SAL, FP and SAL versus placebo, statistically significant reductions in the rate of exacerbations were observed in the ≥2% eosinophils subgroup (n=1048): FP/SAL 37% (p<0.001); FP 28% (p=0.005); SAL 30% (p=0.002). Smaller reductions versus placebo were observed in the <2% subgroup (n=353): FP/SAL 1%; FP 18%; SAL 14%; statistical significance was not achieved for any treatment comparison in this smaller subgroup. Comparisons of FP/SAL versus FP or SAL did not achieve significance in either subgroup.

In the SCO30002 study, although the 95% CIs for all comparisons were wide because of the small sample size, FP/SAL and FP were associated with a lower exacerbation rate in the ≥2% eosinophils subgroup, but the reverse was seen in patients with eosinophil level <2%.

An analysis of the rate of exacerbations requiring antibiotics or oral corticosteroids by a 2% eosinophil level cut-off is reported in online supplementary tables S5 and S6.

Time to first moderate/severe exacerbation
Time to first moderate/severe exacerbation was analysed for all studies (1-year studies: see online supplementary figure S3; 24-week studies: see online supplementary figure S4). In all

three ≥1-year studies, for all comparisons of FP/SAL versus placebo, versus SAL alone, versus FP alone or versus tiotropium, none of the treatment comparisons in the ≥2% eosinophils subgroup were statistically significant for time to first moderate/severe exacerbation. In the SCO30002 study, a statistically significantly (p=0.037) shorter time to first exacerbation in the FP/SAL group versus placebo was observed in the <2% group.

An analysis of time to first moderate/severe exacerbation for INSPIRE, TRISTAN and SCO30002 according to a 200/mm^3 absolute eosinophil count cut-off is reported in online supplementary figure S5.

Secondary analyses (FEV$_1$ and SGRQ)
The magnitude of the treatment differences in FEV$_1$, and weighted mean FEV$_1$ during the study, between patients with eosinophil level <2% vs ≥2% were similar in all of the ≥1-year studies. In TRISTAN, comparisons of FP/SAL versus FP, SAL or placebo for weighted mean FEV$_1$ favoured FP/SAL in both subgroups. Likewise, in comparisons of weighted mean FEV$_1$ for FP or SAL versus placebo, the active treatment was favoured in both subgroups. There was no evidence of a treatment difference in either subgroup for INSPIRE and SCO30002 (see online supplementary figure S6). Findings from analysis of the

Table 3 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: SCO30002

Characteristic	FP/SAL		FP		Placebo	
	<2%	≥2%	<2%	≥2%	<2%	≥2%
n	45	84	50	74	50	70
Age, mean (SD)	64.4 (9.08)	63.7 (10.50)	63.0 (9.44)	65.1 (8.13)	66.9 (9.02)	64.7 (8.62)
Male (%)	87	82	84	81	74	84
Current smokers (%)	47	40	52	39	38	34
Pack-years, median (range)	37.5 (10–90)*	35.0 (10–300)	30.5 (10–150)	38.8 (10–300)	39.0 (10–88)	30.0 (10–108)
Post-BD FEV$_1$% predicted, mean (SD)	55.3 (11.20)	56.9 (14.07)	56.5 (11.38)	57.5 (13.46)†	55.6 (9.48)	56.2 (12.06)
FEV$_1$% reversibility, mean (SD)	3.4 (3.06)	2.6 (4.22)	2.8 (3.49)	3.6 (3.90)†	3.5 (3.50)	3.5 (3.59)
Moderate/severe exacerbations in prior 12 months, n (%)						
0	17 (38)	23 (28)‡	19 (38)	27 (36)	18 (36)	29 (41)
1	9 (20)	22 (27)‡	10 (20)	14 (19)	9 (18)	14 (20)
2	8 (18)	20 (24)‡	11 (22)	16 (22)	10 (20)	17 (24)
>2	11 (24)	18 (22)‡	10 (20)	17 (23)	13 (26)	10 (14)

*n=43.
†n=73.
‡n=83.
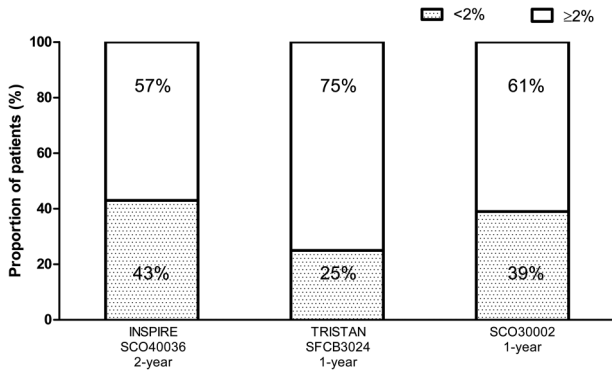BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

Figure 1 Proportion of all patients with baseline blood eosinophil level <2% and ≥2% in ≥1-year studies of fluticasone propionate (FP)/salmeterol (SAL) in patients with COPD.

24-week studies suggested similar changes in both subgroups (see online supplementary figure S7). Similarly, for the two ≥1-year studies for which change from baseline SGRQ data was analysed, there were no treatment differences for FP/SAL versus any comparator in change from baseline SGRQ score in either eosinophil subgroup (see online supplementary figure S8).

Reversibility
No relationship between bronchodilator reversibility and eosinophil level was observed (see online supplementary figure S9).

Safety
The incidence of pneumonia in the six studies did not appear to have any clear relationship with eosinophil level (<2% vs ≥2%) (see online supplementary figure S10).

DISCUSSION
We examined blood eosinophil levels as a potential biomarker for reduction in exacerbation frequency with ICS/LABA versus LAMA or placebo using data from six randomised, controlled trials of FP/SAL in patients with COPD, including three that followed patients for at least 1 year. The 2% threshold for high versus low blood eosinophil level (at baseline) was chosen for consistency with previous studies.[15][19] The results of all studies were consistent with the hypothesis that there is a greater reduction in exacerbation rate with ICS/LABA, compared with placebo or LAMA, in individuals with a pretreatment blood eosinophil level ≥2%.

The proportion of patients with a pretreatment eosinophil level ≥2% was 50–75% across all six studies. All studies
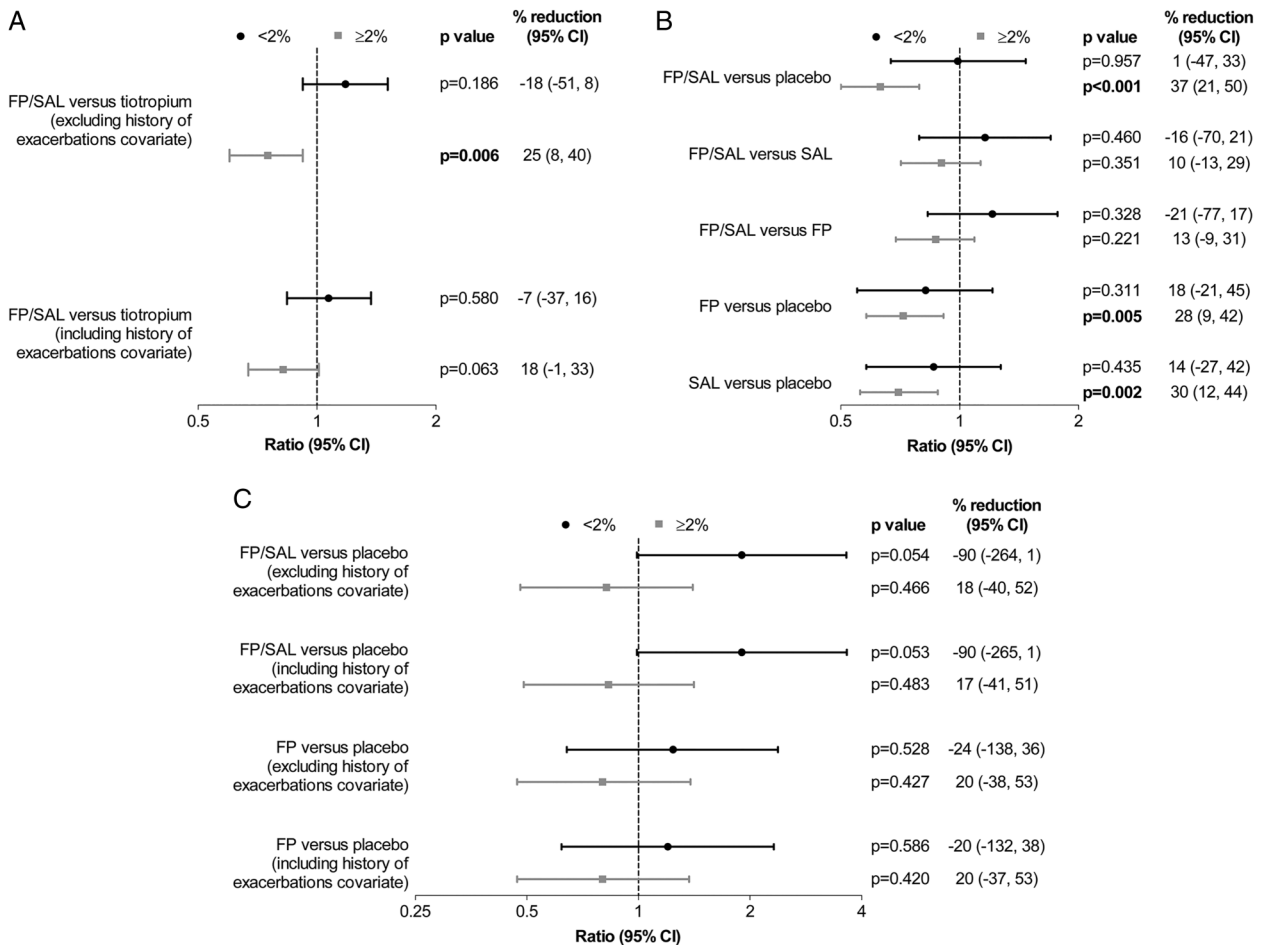
Figure 2 Percentage reduction in moderate/severe exacerbation rates with fluticasone propionate (FP)/salmeterol (SAL) and monocomponents for treatment comparisons of interest in ≥1-year studies by percentage baseline blood eosinophil level in (A) INSPIRE, (B) TRISTAN and (C) SCO30002. Analysis performed using a negative binomial regression model with covariates of either treatment, gender, history of exacerbations, baseline % predicted FEV$_1$, percentage eosinophil group and treatment by percentage eosinophils interaction or treatment, gender, baseline % predicted FEV$_1$, percentage eosinophil group and treatment by percentage eosinophils interaction. <1 favours FP or FP/SAL; >1 favours tiotropium/placebo. Note: statistically significant comparisons (p<0.05) shown in bold font.
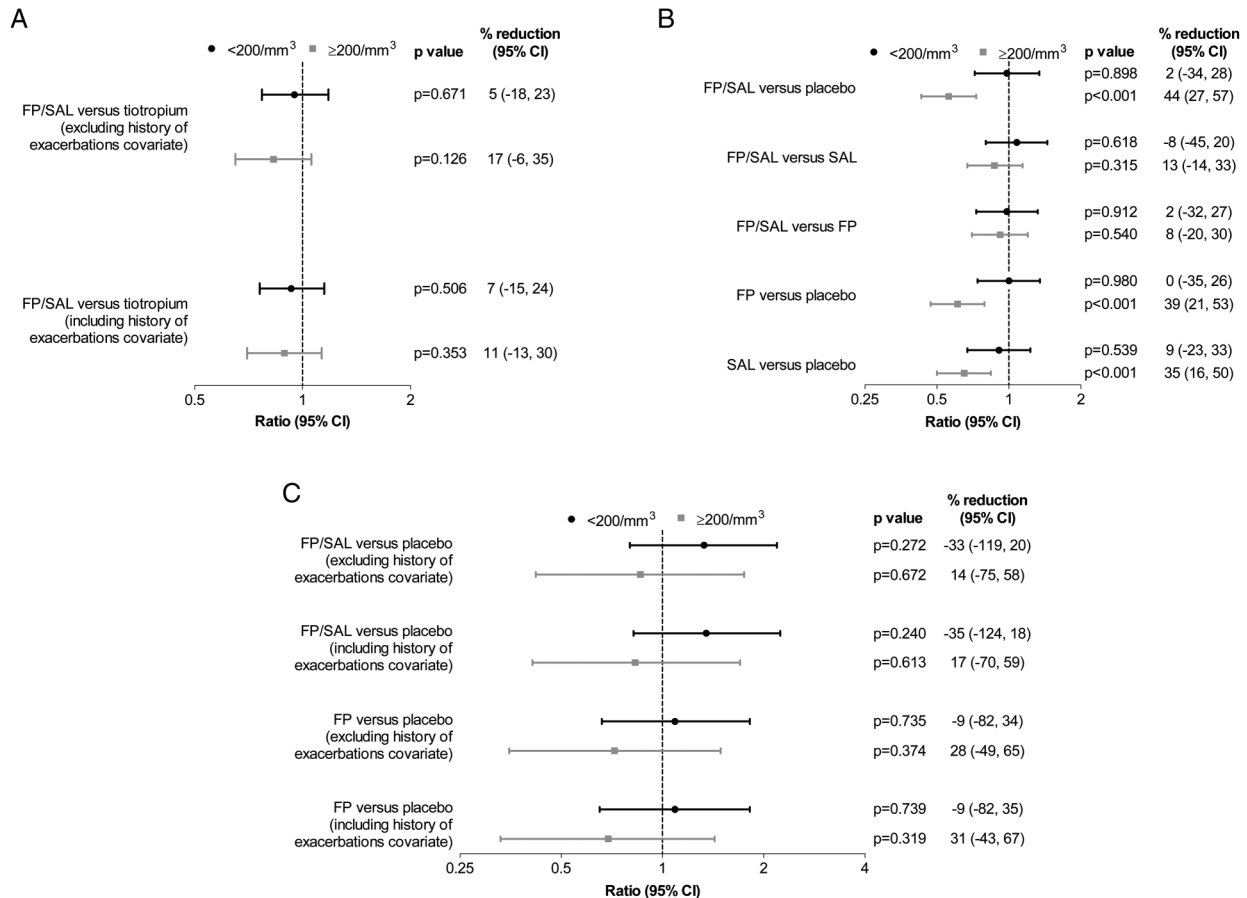
Figure 3 Percentage reduction in moderate/severe exacerbation rates with fluticasone propionate (FP)/salmeterol (SAL) and monocomponents for treatment comparisons of interest in ≥1-year studies by absolute baseline blood eosinophil count in (A) INSPIRE, (B) TRISTAN and (C) SCO30002. Analysis performed using a negative binomial regression model with covariates of either treatment, gender, history of exacerbations, baseline % predicted FEV₁, percentage eosinophil group and treatment by percentage eosinophils interaction or treatment, gender, baseline % predicted FEV₁, percentage eosinophil group and treatment by percentage eosinophils interaction.

recruited patients with well-defined COPD with a smoking history averaging approximately 40 pack-years, so the inadvertent inclusion of patients with asthma is unlikely to account for any variation in baseline eosinophil levels. In TRISTAN, which had the highest proportion of patients with elevated eosinophils, the majority of patients (88%) were not atopic in either subgroup. Most clinical characteristics, such as age, sex, smoking history and baseline lung function, showed no discernible differences between those with a pretreatment eosinophil level of <2% vs ≥2%; however, there was a slightly higher proportion of current smokers in the <2% vs ≥2% subgroup across all six studies.

Analysis of data from the 2-year INSPIRE study was indicative of greater efficacy with twice-daily FP/SAL 500/50 µg (vs tiotropium) in reducing the rate of moderate/severe exacerbations in patients with blood eosinophils ≥2% vs <2% (both with and without including prior exacerbations as a covariate (1, ≥2)). In the 1-year TRISTAN study, FP/SAL 500/50 µg was more effective (vs placebo) in reducing the rate of moderate/severe exacerbations in patients with blood eosinophils ≥2% vs <2%. In TRISTAN, FP and SAL alone were significantly more effective than placebo in the ≥2% group, but not in the <2% group. Stratification by blood eosinophil level ≥3% vs <3% showed similar trends for TRISTAN. However, for INSPIRE, differences were smaller in magnitude. This result may be due to the imbalance in exacerbation history between the tiotropium and FP/SAL groups, which makes

interpretation difficult despite correction for this in the analysis. These findings are consistent with another recent retrospective study in which data from two parallel randomised trials of FF/VI, once-daily ICS/LABA, in 3177 patients with moderate-to-severe COPD and a 1-year history of exacerbation[18] were reanalysed according to eosinophil count, using the same 2% threshold as in the present study.[19] Across all FF/VI doses, FF/VI reduced exacerbation rates by 29% compared with VI alone (p<0.001) in patients with ≥2% eosinophils, and by 10% (p=0.283) in patients with <2% eosinophils.[18] The findings with ICS or ICS/LABA across our studies appear to be consistent, the only exception being in the TRISTAN study, in which the observation of reduced exacerbation rates in patients with ≥2% blood eosinophils receiving SAL alone versus placebo was unexpected. Unfortunately, there is no other trial measuring blood eosinophils with a LABA-only group of sufficient size and duration to corroborate this finding.

When exacerbations requiring oral corticosteroids were considered, reductions were seen for FP/SAL versus placebo in patients with low and high eosinophil levels, but the magnitude of reduction was greater in the ≥2% group in TRISTAN. In INSPIRE, reductions in exacerbations requiring oral corticosteroids for FP/SAL versus tiotropium were only seen in the ≥2% group. For exacerbations requiring antibiotics in INSPIRE, tiotropium was favoured over FP/SAL in the <2% group (in the ≥2% group there was no evidence of a difference between treatments).

Analysis of the SCO30002 study did not show any significant relationship between pretreatment blood eosinophil levels and treatment. With a sample size less than one-third that of the other two ≥1-year studies, the study had insufficient power to show treatment differences in the eosinophil subgroups, compared with the other two studies, as the wide 95% CI margins illustrate. Furthermore, this study used local laboratories for measuring eosinophils.

The interpretation of the time to first moderate/severe exacerbation analysis was limited by lack of power resulting in wide 95% CI margins. While no treatment comparison was statistically significant, numerical trends across all three ≥1-year studies were consistent with the exacerbation rate analysis, and are suggestive of relatively greater efficacy with FP/SAL and its components versus placebo or tiotropium in the ≥2% eosinophil subgroup. No evidence for an effect of eosinophil levels on response to ICS/LABA in terms of lung function or health-related quality of life was found.

A strength of our analysis is that the studies contributing the majority of patients (INSPIRE and TRISTAN) measured pretreatment blood eosinophil levels using a central laboratory system. Furthermore, the patients were selected in accordance with strict inclusion criteria and had well-characterised COPD. A uniform, consensus definition of COPD exacerbations[26] was used in all studies. Although this was a retrospective analysis, the analysis plan was defined prospectively before testing of the new hypotheses. A limitation of the analysis was that in most studies there was only a single measurement of eosinophil levels before study treatment, and intrapatient variability of eosinophil levels in COPD is not known. Post hoc analysis of eosinophil levels in TRISTAN, where repeated measurements were available, showed some variability between measurements for individual patients. We chose to stratify our population by a blood eosinophil count of 2%, as previous publications identified the high sensitivity of this cut-off point for the presence of a raised sputum eosinophil count.[12 13] In addition, our previous analysis demonstrated clear differences in the response to additional ICS using this cut-off point.[18] Post hoc analysis of this and earlier studies[19] shows a graded response to additional ICS by baseline blood eosinophil count, whether expressed as a differential or absolute count, and supports a cut-off point of 2%. However, we acknowledge that, as with other biomarkers, the optimum cut-off point depends on detailed knowledge of measurement characteristics, the question being asked, and the likely response to that question. We also recognise that data from prospective studies are required to further validate this cut-off point. Our findings are relevant to a population of patients with a history of exacerbations who would be considered for treatment with an ICS-containing regimen. Whether the findings can be extrapolated to a wider population with COPD and whether stratification by blood eosinophil count results in more effective use of ICS than other stratification strategies are important areas for further study.

In conclusion, our analysis suggests that an informative relationship exists between pretreatment blood eosinophil levels and reduction in the frequency of COPD exacerbations with ICS/LABA treatment. Blood eosinophil levels represent a potentially important biomarker that could aid treatment decision-making in patients with moderate-to-severe COPD. Prospective studies are required to explore these findings further.

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