



Effects of low- versus high-dose fluticasone propionate/formoterol fumarate combination therapy on AMP challenge in asthmatic patients: A double-blind, randomised clinical trial



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ABSTRACT

Background: The dose-response relationship between two dose levels of fluticasone/formoterol (flutiform[®], 100/10 µg and 500/20 µg) was evaluated in asthmatic patients. Non-invasive inflammatory markers were used including adenosine monophosphate (AMP) challenge (primary endpoint), and sputum eosinophils and fractional exhaled nitric oxide (FeNO) (secondary endpoints).

Methods: Patients aged ≥18 years with forced expiratory volume in 1 s (FEV₁) ≥60% predicted and who required a dose of <60 mg AMP to elicit a 20% drop in FEV₁ (AMP PD₂₀) were randomised in this incomplete block, crossover study to receive 2 of 3 treatments b.i.d.: fluticasone/formoterol 500/20 µg (high dose), 100/10 µg (low dose) or placebo, during 2 periods of 28 ± 3 days each, separated by 2–3 weeks. AMP challenges were performed pre-dose and 12 h after last dose at the end of each treatment period. A series of *post hoc* analyses were performed only in patients allocated to *both* fluticasone/formoterol doses, who completed the study and had evaluable AMP PD₂₀ data for both treatments (“fluticasone/formoterol subgroup”). Changes in AMP PD₂₀ FEV₁, percentage sputum eosinophils and FeNO levels (Day 1 vs Day 28) between treatments were compared by an analysis of covariance (ANCOVA).

Results: Sixty-two patients were randomised and 46 completed the study. Fifteen patients received both high- and low-dose fluticasone/formoterol (*post hoc* subgroup). The difference in AMP PD₂₀ for the overall population was not statistically significant between high- and low-dose fluticasone/formoterol (LS mean fold difference: 1.3; *p* = 0.489), although both dose levels were superior to placebo: high-dose vs placebo LS mean fold difference: 4.4, *p* < 0.001; low-dose vs placebo LS mean fold difference: 3.5, *p* < 0.001. In the *post hoc* subgroup, the difference in AMP PD₂₀ between the doses was statistically significant in favour of the high-dose (LS mean fold difference: 2.4, *p* = 0.012). Other inflammatory parameters (sputum eosinophil counts and FeNO) showed small differences and statistically non-significant changes between high- and low-dose fluticasone/formoterol.

Conclusions: A significant dose-response was found between low- and high-dose fluticasone/formoterol in the *post hoc* subgroup (patients who received both doses), but not in the overall population, with the higher dose demonstrating a greater reduction in airway responsiveness to AMP.

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1. Introduction

Effective long-term control of persistent asthma is frequently achieved with an inhaled corticosteroid (ICS) in combination with a long-acting β₂-agonist (LABA). Significant reductions in exacerbations with ICS and LABA combinations versus higher dosages of ICS

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or combinations of an ICS with other therapeutic agents have been demonstrated in clinical studies [1]. These findings have driven the development of several ICS/LABA combination inhalers.

The inhaled combination therapy *flutiform*[®] contains the ICS, fluticasone propionate (fluticasone), and the LABA, formoterol fumarate (formoterol). Both constituent components have favourable pharmacological and mechanistic properties compared to others in their respective classes. Fluticasone is highly lipophilic hence exhibits prolonged contact with the airway epithelium [2,3] and tissue retention compared to more hydrophilic ICSs [4,5]. It is also resistant to CYP 3A5-mediated intra-pulmonary degradation [6] (which has been implicated in corticosteroid resistance) unlike beclomethasone and budesonide [7,8]. Formoterol (unlike salmeterol) has been shown to reverse ICS insensitivity under oxidative stress [9], disposal of formoterol (but not salmeterol) from smooth muscle cells is inhibited by ICS (thereby increasing local formoterol concentrations) [10], and cytokine-induced inhibition of formoterol (but not salmeterol) is completely reversed by ICS co-administration [11].

Fluticasone/formoterol combination therapy has been developed in 3 dosage strengths (50/5 µg, 125/5 µg and 250/10 µg per actuation), based on the doses approved for other fluticasone- and formoterol-containing products. Nonetheless only limited dose-response data in asthma are available for fluticasone/formoterol [12] or for other ICS/LABA combinations [13–18]. This is related to the shallow dose-response that exists for conventional clinical endpoints, particularly spirometric outcomes, routinely employed in dose-finding and pivotal registration studies. It remains important therefore to investigate a dose-response relationship to support the rationale for dose escalation.

Compared to spirometric endpoints, airway hyper-responsiveness (AHR) to adenosine 5'-monophosphate (AMP) [19], is a sensitive marker for defining dose-response for ICSs or ICS/LABA combinations [20–23]. Other ICS-sensitive inflammatory biomarkers include fractional exhaled nitric oxide (FeNO) [24–27] and sputum eosinophils [26,28] which have also been shown to exhibit a dose-response [24–28].

This is only one of two studies that aimed to evaluate the dose-response relationship between two dose levels of fluticasone/formoterol (100/10 µg b.i.d. and 500/20 µg b.i.d.) and the first on non-invasive inflammatory markers including AMP challenge (primary endpoint) and sputum eosinophils and FeNO (secondary endpoints) in asthmatic patients.

2. Methods

2.1. Participants

Eligible patients were ≥18 years, non-/ex-smokers (<10 pack years), with a pre-bronchodilator forced expiratory volume in 1 s (FEV₁) ≥ 60% predicted and required a dose of <60 mg AMP to elicit a 20% drop in FEV₁ (AMP PD₂₀). Key exclusion criteria included: other clinically significant respiratory or cardiac diseases, medications considered likely to interfere with the study outcomes, hospitalisation or ER attendance or respiratory tract infections within 4 weeks of screening. The following medications were not allowed during study and needed to be discontinued before screening: omalizumab (6 months), systemic corticosteroids (12 weeks), antihistamines (2 weeks), cromones and leukotriene receptor antagonists (1 week). Patients were recruited at 7 sites in Germany. The study was performed in accordance with the Declaration of Helsinki, the ICH Harmonised Tripartite Guidelines for Good Clinical Practice (GCP), and with applicable regulatory requirements. The study protocol was reviewed by the central ethics committee (Schleswig Holstein). All patients gave written informed consent

prior to screening. EudraCT Number: 2009-009873-87; clinicaltrials.gov identifier: NCT00995800.

2.2. Study design

This was a multi-centre, double-blind, randomised, incomplete block, crossover study evaluating the effects of two dose levels of fluticasone/formoterol (100/10 µg b.i.d. and 500/20 µg b.i.d.) and placebo on AHR to AMP and other markers of airway inflammation (Fig. 1).

The study consisted of an initial 14–21 day wash-out period, followed by two double-blind 28-day treatment periods separated by a second 14–21 day wash-out period. At the start and end of each treatment period, FeNO measurement was followed by spirometry, AMP challenge and sputum induction. Given the length of both wash-out and treatment periods, an incomplete block, 2-treatment period design was considered more feasible than a complete-block approach. During the wash-out periods patients took only salbutamol (Ventolin[®] Evohaler[®]) as required. Patients were contacted by telephone on Day 27 of each treatment period to ensure that the last dose of study medication was taken that evening (12 h prior to the Day 28 visit). A follow-up telephone call took place 7–10 days after last dosing.

2.3. Study treatment and dose rationale

Randomisation was performed using a validated system that assigned eligible patients to receive 2 of the 3 study treatments below in 1 of 6 sequences:

- Fluticasone/formoterol 250/10 µg 2 puffs b.i.d. (500/20 µg b.i.d.)
- Fluticasone/formoterol 50/5 µg 2 puffs b.i.d. (100/10 µg b.i.d.)
- Placebo 2 puffs b.i.d.

All treatments were administered via the AeroChamber Plus[®] spacer device. The doses of fluticasone/formoterol were selected as a pairwise dose-response was considered unlikely to be seen without a four- to five-fold ICS dose difference [20,27–30].

2.4. Methods

FeNO was measured with a NIOX-MINO[™] analyser (Aerocrine, Solna, Sweden) per current guidelines [31]. Only one satisfactory measurement was performed [32]. Spirometry was performed in accordance with the American Thoracic Society/European Respiratory Society Guidelines (2005) [33]. Predicted values for adults were calculated according to Quanjer et al., 1993 [34]. If baseline FEV₁ was ≥60% predicted, AMP challenge (0.39–800 mg) was performed using the 5 deep breath dosimeter method, until a AMP PD₂₀ was reached or the highest dose inhaled, according to a previously described protocol [35]. Pre-challenge, patients refrained from short-acting β₂-agonists (SABAs) and LABAs for at least 6 and 12 h, respectively. After each inhalation, the patient held their breath at total lung capacity for 2–3 s. After each cycle of 5 breaths, a lung function test was performed in duplicate, 2 min after the last deep breath [36]. If FEV₁ values were more than 5% apart spirometry was repeated. The best post-saline FEV₁ was used as a baseline. At the start of the second treatment period, AMP PD₂₀ had to be within 1.5 doubling doses of that at the start of treatment period 1 to ensure comparability between periods. After AMP challenge, patients were given 200 µg salbutamol followed by sputum induction and processing per standardised protocols [37–39]. Sputum processing was performed by a trained nurse or physician at each site. Eosinophil counts were assessed from 400 non-squamous nucleated cells on stained (HemaColor[®], Merck,

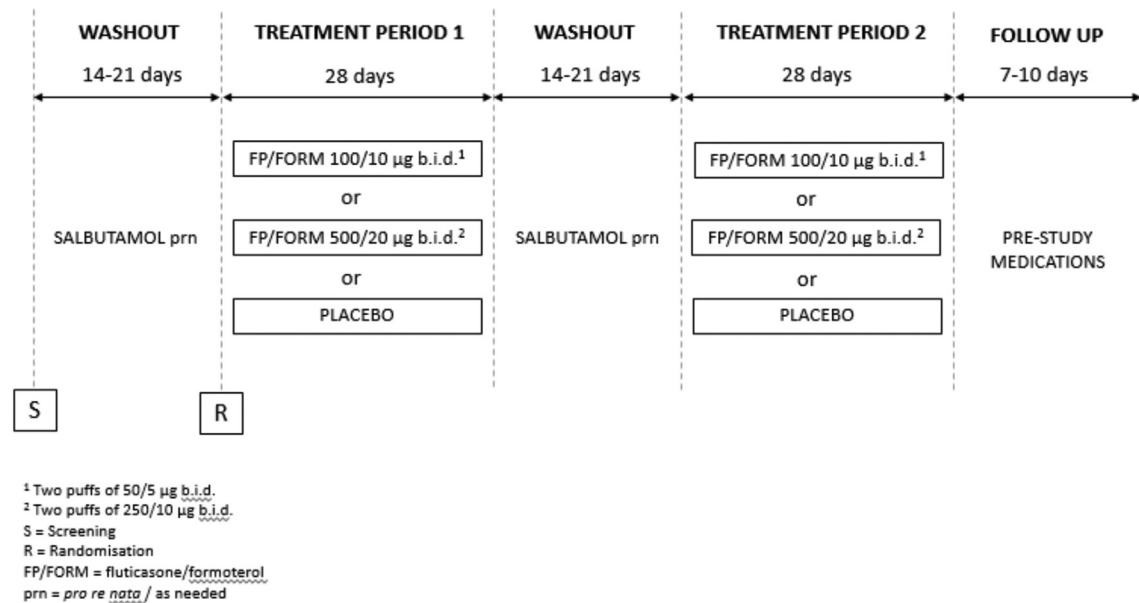


Fig. 1. Study design.

Darmstadt, Germany) cytospin slides. Cell differentials were expressed as a percentage of non-squamous cells.

Safety and tolerability were evaluated by monitoring adverse events (AEs), vital signs, clinical laboratory tests, lung function and electrocardiograms (ECGs).

Throughout the study patients kept a daily record of peak expiratory flow rate (PEFR), study medication use, rescue medication use, asthma symptom scores, and sleep disturbance due to asthma, which was reviewed at the end of each treatment period.

In case of uncontrolled asthma, the patient was withdrawn from the study.

The primary efficacy endpoint was the change in AMP PD₂₀ from baseline (Day 1) to the end of each treatment period (Day 28). Secondary efficacy variables included changes in FeNO levels, sputum eosinophil counts, lung function and symptom scores.

2.5. Statistical analysis

The primary (efficacy) analysis population was the full analysis set (FAS), defined as all randomised patients who received at least one dose of study treatment and had at least one post-dose AMP PD₂₀ measurement. Safety data were based upon all patients who received at least one dose of study treatment and had at least one post-dose safety assessment.

The sample size was based on an incomplete block design, with 3 treatments and 2 periods, and was calculated using simulations [20,22,40–43]. A sample size of 54 evaluable patients conferred 90% power to detect a difference in AMP PD₂₀ of 2 doubling doses between high and low dose levels of fluticasone/formoterol. This assumed a within-patient standard deviation (SD) of 2.3 doubling doses, a between-patient SD of 3.3 doubling doses, and a two-sided alpha of 0.05.

A series of *post hoc* analyses were performed only in patients allocated to both fluticasone/formoterol doses, who completed the study and had evaluable AMP PD₂₀ data for both treatments (“fluticasone/formoterol subgroup”).

Changes in AMP PD₂₀, percentage sputum eosinophils and FeNO levels from baseline (Day 1) to Day 28 were compared between treatments using an analysis of covariance (ANCOVA) with

treatment, baseline value and treatment period as fixed effects and study centre as a random effect. AMP PD₂₀ data were naturally log transformed before analysis and back-transformed for results. Other secondary endpoints were reported using descriptive statistics.

3. Results

3.1. Patients

A total of 62 patients (33 male/29 female) were randomised to 1 of the 6 treatment sequences, all of whom received at least one dose of treatment. Forty-six (74.2%) patients completed the study and 16 (25.8%) discontinued prematurely (Fig. 2). The most common reason for early discontinuation (in 10 patients) was because the Period 1 AMP PD₂₀ was either not calculable due to missing AMP challenge data or could not be reproduced at the start of Period 2. Fifteen patients received both fluticasone/formoterol dose levels, completed the study and produced evaluable AMP PD₂₀ data (i.e., constituted the *post hoc* fluticasone/formoterol subgroup).

Patient demographics and baseline characteristics for the study population are provided in Table 1 for the FAS and in Table 2 for the subgroup receiving both doses of fluticasone/formoterol. Baseline characteristics in the subgroup were similar to those in the overall FAS, although mean baseline AMP PD₂₀ levels were lower in the subgroup.

3.2. Primary efficacy analysis

In the overall FAS, the largest increase in AMP PD₂₀ was seen with the high dose fluticasone/formoterol, yielding nearly a 5-fold increase in AMP PD₂₀, compared to an almost 4-fold increase with the low dose. No change in AMP PD₂₀ was observed with placebo (Fig. 3).

The 1.3-fold difference in effect between high and low fluticasone/formoterol dose levels was statistically non-significant ($p = 0.489$) (Table 3). Both fluticasone/formoterol dose levels were superior to placebo, with approximately a 4-fold absolute difference or a 2-fold doubling dose difference between the active

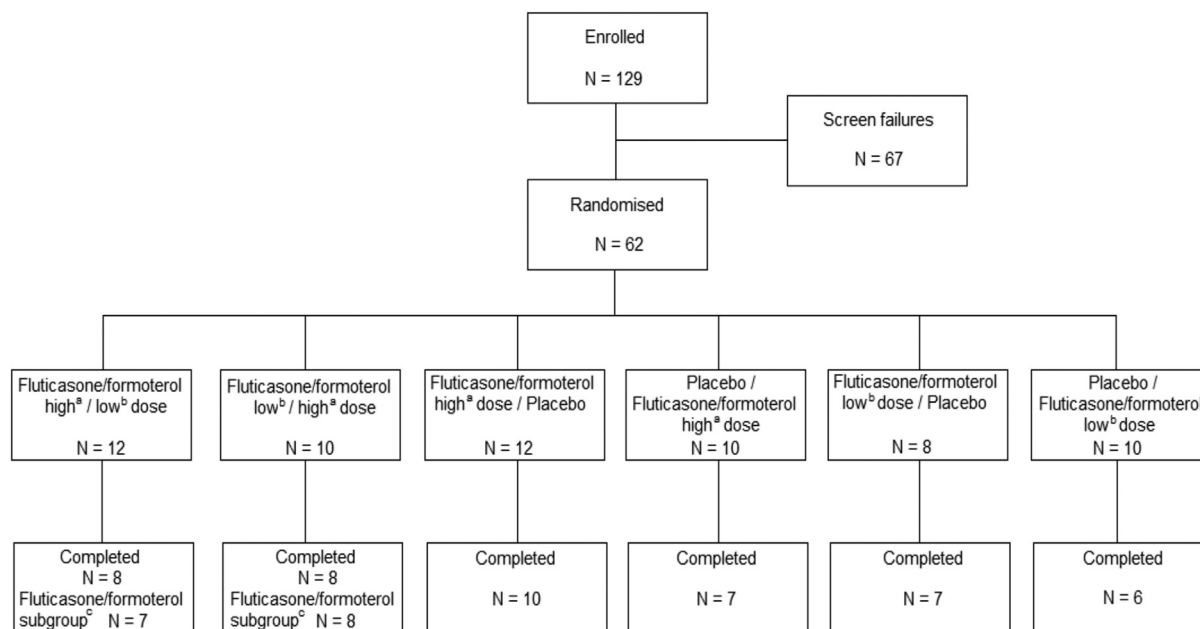


Fig. 2. Patient Flow Diagram. ^aFluticasone/formoterol 500/20 µg b.i.d. dose level. ^bFluticasone/formoterol 100/10 µg b.i.d. dose level. ^cFluticasone/formoterol subgroup of patients allocated to receive both fluticasone/formoterol dose levels (in either high/low or low/high sequences), who completed study, with evaluable AMP PD₂₀ data for both treatments.

Table 1
Patient baseline^a characteristics – FAS.

		Fluticasone/formoterol high dose	Fluticasone/formoterol low dose	Placebo
	N	36	32	32
Age (years)	Mean (SD)	39.6 (12.3)	39.4 (13.8)	40.7 (13.6)
Gender	Male/Female	17/19	16/16	20/12
FEV ₁ predicted [%]	Mean (SD)	88.9 (14.1)	87.2 (11.7)	88.0 (14.9)
FeNO (ppb)	Mean (SD)	36.6 (30.7)	46.5 (45.9)	44.5 (37.0)
Sputum eosinophils [%]	Mean (SD)	4.8 (4.4)	4.7 (4.0)	3.5 (3.6)
AMP PD ₂₀ [mg]	Geometric mean	7.9	6.6	15.3

^a Baseline represents the pre-treatment value, whether at the start of Treatment Period 1 or Treatment Period 2 dependent upon the treatment sequence, for each treatment group.

treatments and placebo.

In the fluticasone/formoterol subgroup there was a statistically significant AMP PD₂₀ dose-response, in favour of the high dose. The AMP PD₂₀ increased 11-fold on high dose fluticasone/formoterol, compared to an approximate 5-fold increase on the low-dose (Fig. 4); a 2.4-fold difference in absolute terms, or 1.2-fold doubling dose difference (Table 4).

3.3. Secondary efficacy analysis

3.3.1. Percentage of eosinophils in induced sputum

In the FAS there were no significant differences between any

treatments in terms of the percentage of sputum eosinophils (Table 5).

Differences between high and low dose fluticasone/formoterol in terms of changes in sputum eosinophil counts from baseline were more evident in the fluticasone/formoterol subgroup (LS mean difference: −2.2; *p* = 0.189) than in the overall FAS population (LS mean difference: −0.4; *p* = 0.666), albeit still non-significant (Tables 5 and 6).

3.3.2. Exhaled nitric oxide (FeNO)

In the overall FAS, FeNO decreased to a similar extent (from approximately 40 to 20 ppb) with both low and high doses of

Table 2
Patient baseline^a characteristics – Subgroup of patients who received both doses of fluticasone/formoterol.

		Fluticasone/formoterol high dose	Fluticasone/formoterol low dose
	N	15	15
Age (years)	Mean (SD)	37.5 (12.2)	37.5 (12.2)
Gender	Male/Female	7/8	7/8
FEV ₁ predicted [%]	Mean (SD)	84.0 (10.8)	85.9 (10.7)
FeNO (ppb)	Mean (SD)	44.0 (42.6)	41.8 (36.4)
Sputum eosinophils [%]	Mean (SD)	6.0 (5.7)	4.1 (4.5)
AMP PD ₂₀ [mg]	Geometric mean	3.8	4.1

^a Baseline represents the pre-treatment value, whether at the start of Treatment Period 1 or Treatment Period 2 dependent upon the treatment sequence, for each treatment group.

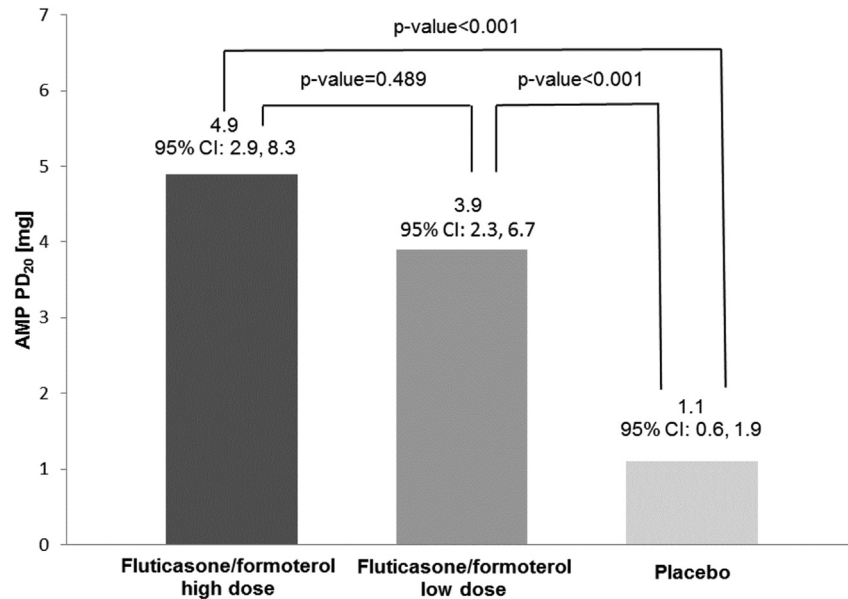


Fig. 3. AMP PD₂₀ [mg]: LS mean fold change from Baseline to End of Treatment – FAS.

Table 3

AMP PD₂₀ [mg]: Change from Baseline to End of Treatment – FAS.

Treatment	LS mean fold change (95% CI)	
Fluticasone/formoterol high dose (n = 34)	4.9 (2.9, 8.3)	
Fluticasone/formoterol low dose (n = 31)	3.9 (2.3, 6.7)	
Placebo (n = 31)	1.1 (0.6, 1.9)	
Comparison	Absolute fold difference (95% CI), p-value	Doubling dose difference (95% CI)
Fluticasone/formoterol high dose vs low dose	1.3 (0.7, 2.4), p = 0.489	0.3 (–0.6, 1.3)
Fluticasone/formoterol high dose vs placebo	4.4 (2.2, 8.5), p < 0.001	2.1 (1.2, 3.1)
Fluticasone/formoterol low dose vs placebo	3.5 (1.7, 7.0), p < 0.001	1.8 (0.8, 2.8)

n = number of patients with data available.

fluticasone/formoterol, from baseline to end of treatment, i.e., did not show any dose-response. Reductions in FeNO with fluticasone/formoterol were significantly greater than those observed with placebo which showed no significant change from baseline (Table 7).

FeNO results in the fluticasone/formoterol subgroup were very

similar to those in the overall FAS analysis. Again no dose difference was evident (Table 8).

3.4. Other secondary efficacy endpoints

In the overall FAS, effects with high dose fluticasone/formoterol

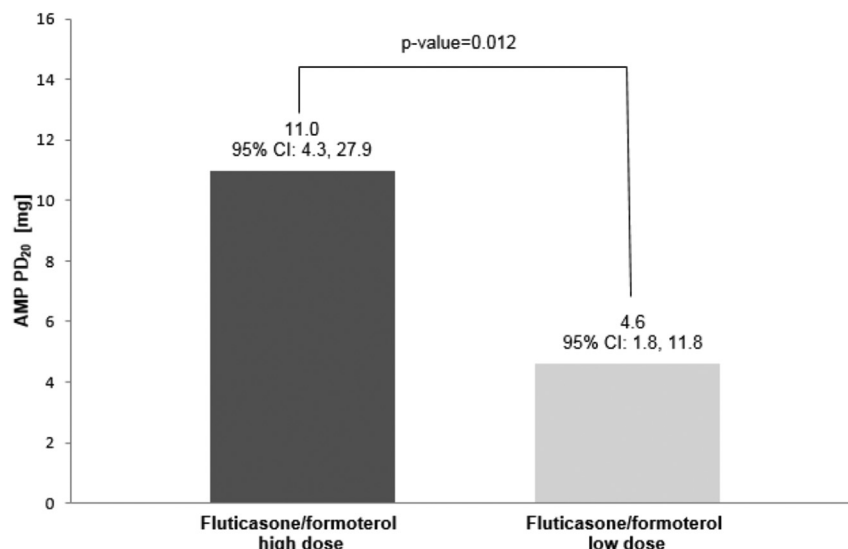


Fig. 4. AMP PD₂₀ [mg]: LS mean fold change from Baseline to End of Treatment – Subgroup of patients who received both dose levels of fluticasone/formoterol.

Table 4AMP PD₂₀ [mg]: Change from Baseline to End of Treatment - Subgroup of patients who received both dose levels of fluticasone/formoterol.

Treatment	n	LS mean fold change (95% CI)	Absolute fold difference (95% CI), p-value	Doubling dose difference (95% CI)
Fluticasone/formoterol high dose	15	11.0 (4.3, 27.9)	2.4 (1.3, 4.5), p = 0.012	1.2 (0.3, 2.2)
Fluticasone/formoterol low dose	15	4.6 (1.8, 11.8)		

n = number of patients with data available.

Table 5

Percentage of sputum eosinophils: Change from Baseline to End of Treatment - FAS.

Treatment	LS mean change (95% CI)
Fluticasone/formoterol high dose (n = 25)	−0.9 (−2.1, 0.3)
Fluticasone/formoterol low dose (n = 26)	−0.5 (−1.7, 0.7)
Placebo (n = 20)	−0.2 (−1.6, 1.1)
Comparison	LS mean difference (95% CI), p-value
Fluticasone/formoterol high dose vs low dose	−0.4 (−2.0, 1.3), p = 0.666
Fluticasone/formoterol high dose vs placebo	−0.6 (−2.4, 1.2), p = 0.477
Fluticasone/formoterol low dose vs placebo	−0.3 (−2.1, 1.5), p = 0.756

n = number of patients with data available.

Table 6

Percentage of Sputum Eosinophils: Change from Baseline to End of Treatment - Subgroup of patients who received both dose levels of fluticasone/formoterol.

Treatment	N	LS mean (95% CI)	LS mean difference (95% CI), p-value
Fluticasone/formoterol high dose	11	−1.8 (−4.2, 0.6)	−2.2 (−5.6, 1.2), p = 0.189
Fluticasone/formoterol low dose	11	0.4 (−2.0, 2.8)	

Table 7

Exhaled nitric oxide [ppb]: Change from Baseline to End of Treatment - FAS.

Treatment	LS mean (95% CI)
Fluticasone/formoterol high dose (n = 36)	−18.1 (−24.3, −11.8)
Fluticasone/formoterol low dose (n = 31)	−21.3 (−28.0, −14.7)
Placebo (n = 32)	−5.5 (−12.0, 1.1)
Comparison	LS mean difference (95% CI), p-value
Fluticasone/formoterol high dose vs low dose	3.3 (−5.8, 12.4), p = 0.477
Fluticasone/formoterol high dose vs placebo	−12.6 (−21.6, −3.6), p = 0.007
Fluticasone/formoterol low dose vs placebo	−15.9 (−25.2, −6.6), p = 0.001

numerically exceeded those with the low dose for the majority of the remaining secondary endpoints, although differences were not statistically significant (change from baseline in forced expiratory flow at 25–75% (FEF_{25–75}), diary PEFR, asthma symptom scores, sleep disturbance scores and rescue medication use). Results in the fluticasone/formoterol subgroup largely echoed those in the overall population, although between-treatment differences were accentuated for a number of endpoints. For further details of these results please refer to the Supplementary Material (Tables S1 and S2).

3.5. Safety

The incidence of adverse events (AEs) was 23.1%, 37.5% and 32.4% in the high dose, low dose and placebo treatment periods, respectively. The corresponding incidences of respiratory system events (the most frequent class of AEs) were 10.3%, 15.6% and 10.8%, respectively. Two patients (1 treated with placebo and 1 with the low dose of fluticasone/formoterol) were withdrawn due to asthma exacerbations. There were no clinically significant changes in

laboratory results, ECGs or in vital signs.

4. Discussion

To date, there is limited literature available reporting dose-response either on conventional clinical indices [12,13] or on inflammatory endpoints [36]. This was the first study using non-invasive biomarkers designed to explore a dose-response relationship between high (500/20 µg b.i.d.) and low (100/10 µg b.i.d.) doses of fluticasone/formoterol in terms of the effect on AMP PD₂₀. Although the highest dose of fluticasone/formoterol produced the largest increase in AMP PD₂₀, the absolute 1.3 fold difference between the high and low doses was statistically non-significant in the overall study population. Similarly, two other anti-inflammatory endpoints, i.e. the change in percent sputum eosinophils and FeNO from baseline to end of treatment failed to show a dose-response.

Subsequently, a *post hoc* analysis was performed in the subgroup of 15 patients who received *both* doses of fluticasone/

Table 8

Exhaled Nitric Oxide [ppb]: Change from Baseline to End of Treatment – Subgroup of patients who received both dose levels of fluticasone/formoterol.

Treatment	n	LS mean (95% CI)	LS mean difference (95% CI), p-value
Fluticasone/formoterol high dose	15	−19.9 (−25.9, −13.9)	−0.6 (−7.9, 6.8), p = 0.869
Fluticasone/formoterol low dose	15	−19.3 (−25.3, −13.4)	

formoterol (i.e., the high and the low dose) and had evaluable AMP PD₂₀ data for both treatment periods. The rationale underlying the subgroup analysis was that between-patient variability resulting from the incomplete block study design may have obscured between-treatment differences, ideally assessed within the same individuals. The *post hoc* analysis demonstrated a significant dose-response relationship for AMP PD₂₀ between the two doses with an absolute fold difference of 2.4. Although a non-significant trend was seen for the reduction in sputum eosinophils, no dose-response relationship could be established for FeNO levels. These results underscore the fact that between-patient variability in the overall population analysis may have obscured between-treatment differences.

Our findings confirm and extend previously published data where the majority of studies successfully showing ICS dose-response relationship on an (in)direct bronchial challenge have been performed in a complete block design [29,36,40] while only very few parallel group studies were able to find a dose-response relationship [20]. Other reasons that no dose-response relationship was found for the secondary inflammatory outcomes, i.e., sputum eosinophils and FeNO, may have included inadequate baseline values at inclusion and between-centre variability. Indeed, approximately 50% of the randomised patients had a normal, i.e., <2%, sputum eosinophil count at baseline, whilst the mean baseline eosinophil count in the study population was 4.8%. This compares to mean baseline values of approximately 9% and 14% in the studies of Kelly et al. [28] and Nolte et al. [44], respectively. Both studies mandated elevated baseline sputum eosinophil counts as an inclusion criterion and both showed a dose-response relationship for sputum eosinophils over an ICS dose range broadly comparable to that employed in the present study.

The same is true for FeNO: values < 25 ppb suggest that eosinophilic inflammation and/or responsiveness to corticosteroids is less likely [45]. In the present study, approximately one third of patients had a FeNO level <25 ppb at study entry despite not taking ICS. Previous studies successfully demonstrating pairwise differences in FeNO reduction between different ICS doses have generally required elevated baseline FeNO levels, such as those conducted by Nolte and colleagues [44] and O'Connor et al. [36].

Apart from these potential methodological drawbacks, other factors may also have affected our sputum eosinophil and FeNO outcomes. In a number of studies ICS effects on sputum eosinophil count and/or FeNO tend to plateau at approximately 200 µg of fluticasone-equivalents/day, whilst further incremental effects on AHR to indirect (or direct) stimuli may be seen at 2–4-fold higher ICS doses [20,22,26,28,46]. One study, comparing 100, 400 and 1600 µg daily doses of ciclesonide, may be particularly instructive. Nineteen of 23 patients for whom sputum eosinophil data are presented, exhibited baseline counts in excess of 2.5% [20]. Despite elevated baseline counts, effects on sputum eosinophils plateaued at 400 µg of ciclesonide, whilst the greatest attenuation of AHR to AMP was evidenced at the highest ciclesonide dose of 1600 µg. Another study, in which patients with very high baseline sputum eosinophil counts (mean values of approximately 27%) were recruited, demonstrated the exquisite sensitivity of sputum eosinophils (and to a lesser extent of FeNO) to low doses of ICS [28]. In this study, patients were administered 50, 100, 200 and 400 µg of fluticasone via a pMDI in a sequential, incremental fashion with each dose given for one week. Almost 87% of the reduction in sputum eosinophils attained at 400 µg fluticasone was already evident at the 50 µg dose. For FeNO, 65% of the maximum observed reduction was already seen at the lowest ICS dose. In contrast, effects on AHR to methacholine showed much clearer and greater separation across the dose ranges [28].

In summary, dose-response relationship for AMP PD₂₀ was

found in the *post hoc* fluticasone/formoterol subgroup receiving both doses, but not in the overall study population. These findings are most plausibly attributed to between-subject variability resulting from the incomplete block study design. Furthermore, enrolment of patients without elevated baseline values of sputum eosinophils and FeNO may at least partly account for the lack of dose-response relationship for these inflammatory markers in both the overall population and the *post hoc* fluticasone/formoterol subgroup. It may also be that inherent differences exist between these indices in terms of their capacity to reflect ICS dose-response over a broad dose range, which may reflect differences in the cell types implicated in each endpoint and/or differences in the regional expression of these cells.

5. Conclusions and recommendations

Using an incomplete block design, no dose-response relationship could be established for AMP PD₂₀, or for the secondary inflammatory markers, FeNO and sputum eosinophils in the overall study population, in contrast to the subgroup receiving both study medication doses. Therefore, our study outcomes are potentially informative for the design of future studies evaluating dose-response of anti-inflammatory compounds. We recommend that such studies preferentially employ complete block crossover designs, mandate elevated baseline levels of inflammatory markers of interest and consider AHR to an indirect challenge as the primary outcome variable.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.pupt.2016.02.003>.

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Appendix A. Supplementary Material

Table S1 Secondary endpoints - Change from Baseline to End of Treatment – FAS

Secondary Endpoints (continued)	Fluticasone/ formoterol high dose	Fluticasone/ formoterol low dose	Placebo
Change from baseline (Day1) to end treatment (Day 28) [Mean (SD)]			
FEV ₁ [L]	0.293 (0.343)	0.274 (0.270)	0.018 (0.248)
PEFR [L/min]	53.47 (66.65)	52.519 (55.89)	-3.6 (60.37)
FVC [L]	0.107 (0.297)	0.142 (0.223)	-0.006 (0.248)
FEF ₇₅ [L/sec]	0.215 (0.300)	0.150 (0.334)	0.029 (0.204)
FEF ₅₀ [L/sec]	0.773 (0.756)	0.586 (0.620)	0.018 (0.450)
FEF ₂₅ [L/sec]	1.165 (1.013)	0.923 (1.161)	-0.031 (0.765)
FEF ₂₅₋₇₅ [L/sec]	0.549 (0.555)	0.402 (0.624)	0.051 (0.370)
Morning daily PEFR [L/min]	58.11 (59.17)	42.19 (40.99)	-17.6 (57.90)
Evening daily PEFR [L/min]	52.72 (51.55)	42.36 (41.32)	-18.03 (54.58)
Asthma symptom scores	-0.43 (0.71)	-0.25 (0.42)	-0.10 (0.25)
Sleep disturbance scores	-0.27 (0.58)	-0.19 (0.33)	-0.08 (0.28)
Percentage of rescue medication-free days	36.16 (42.16)	29.95 (34.87)	3.33 (24.51)
Severe exacerbations of asthma, n (%)	0 (0.0)	1 (3.1)	0 (0.0)
Discontinuations due to lack of efficacy, n (%)	0 (0.0)	0 (0.0)	0 (0.0)
Rescue medication: number of uses per day, Mean (SD)	0.44 (0.9)	0.60 (1.0)	0.87 (1.2)
Rescue medication: percentage of study days, Mean (SD)	18.9 (30.1)	23.8 (30.6)	34.8 (37.9)

Table S2 Secondary endpoints - Change from Baseline to End of Treatment – Subgroup Treated with both High and Low Dose fluticasone/formoterol

Secondary Endpoints	Fluticasone/ formoterol high dose	Fluticasone/ formoterol low dose
Change from baseline (Day1) to end treatment (Day 28) [Mean (SD)]		
FEV ₁ [L]	0.385 (0.359)	0.277 (0.236)
PEFR [L/min]	57.64 (53.832)	62.80 (51.439)
FVC [L]	0.130 (0.332)	0.194 (0.250)
FEF ₇₅ [L/sec]	1.333 (1.015)	0.850 (1.256)
FEF ₅₀ [L/sec]	0.837 (0.720)	0.552 (0.533)
FEF ₂₅ [L/sec]	0.263 (0.302)	0.064 (0.397)
FEF ₂₅₋₇₅ [L/sec]	0.651 (0.571)	0.248 (0.643)
Morning daily PEFR [L/min]	58.17 (43.23)	43.50 (50.48)
Evening daily PEFR [L/min]	53.36 (47.85)	41.54 (42.64)
Asthma symptom scores	-0.58 (0.64)	-0.35 (0.53)
Sleep disturbance scores	-0.39 (0.59)	-0.11 (0.29)
Percentage of rescue medication-free days	38.78 (42.83)	35.24 (43.49)