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Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations

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ABSTRACT: One important goal of asthma treatment is to reduce exacerbations. The current authors investigated if the use of sputum cell counts to guide treatment would achieve this goal.

A total of 117 adults with asthma were entered into a multicentre, randomised, parallel group-effectiveness study for two treatment strategies over a 2-yr period. In one strategy (the clinical strategy: CS) treatment was based on symptoms and spirometry. In the other (the sputum strategy: SS) sputum cell counts were used to guide corticosteroid therapy to keep eosinophils $\leqslant$2%; symptoms and spirometry were used to identify clinical control, exacerbations and other treatments. Patients were blind to sputum cell counts in both strategies and physicians were blind in the CS, thus removing bias. First, the minimum treatment to maintain control was identified in 107 patients (Phase 1) and then this treatment was continued (Phase 2) for the remaining of the 2 yrs. The primary outcomes were the relative risk reduction for the occurrence of the first exacerbation in Phase 2 and the length of time without exacerbation. The current authors also examined the type and severity of exacerbations and the cumulative dose of inhaled steroid needed.

The duration and number of exacerbations in Phase 1 were similar in both groups. In Phase 2 there were a 126 exacerbations of which 79 occurred in the CS (62.7%) and 47 (37.3%) in the SS groups. The majority of the 126 exacerbations (101; 80.1%) were mild. The majority of the 102 exacerbations, where sputum examination was performed before any treatment (n=70), were noneosinophilic. In the SS patients, the time to the first exacerbation was longer (by 213 days) especially in those considered to need treatment with a long acting β_2-agonist (by 490 days), the relative risk ratio was lower (by 49%), and the number of exacerbations needing prednisone was reduced (5 *versus* 15). This benefit was seen mainly in patients needing treatment with inhaled steroid in a daily dose equivalent to fluticasone $>$250 μg, and was due to fewer eosinophilic exacerbations. The cumulative dose of corticosteroid during the trial was similar in both groups.

Monitoring sputum cell counts was found to benefit patients with moderate-to-severe asthma by reducing the number of eosinophilic exacerbations and by reducing the severity of both eosinophilic and noneosinophilic exacerbations without increasing the total corticosteroid dose. It had no influence on the frequency of noneosinophilic exacerbations, which were the most common exacerbations.

KEYWORDS: Asthma exacerbations, asthma treatment, induced sputum cell counts

Asthma is characterised by variable airflow limitation, which is validated by spirometry or measurements of airway responsiveness and is treated by bronchodilators [1]. It is also associated with airway inflammation, which is traditionally considered to be eosinophilic and is treated by avoidance of any causes and by anti-inflammatory medications of which corticosteroids are the most effective [2]. This treatment of the inflammation also reduces variable airflow limitation and airway hyperresponsiveness. At present, the airway inflammation is only objectively measured in clinical practice in a few academic centres.

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The most comprehensive measurement of airway inflammation is spontaneous or induced sputum cell counts [3]. This measurement has become established worldwide in research [4]. The test is noninvasive or relatively non invasive respectively and has excellent reliability, validity and responsiveness [3]. Its application in research has emphasised the heterogeneity of airway inflammation in each of the common airway conditions of asthma [5, 6], chronic obstructive pulmonary disease (COPD) [7, 8] and chronic cough [9].

The inflammation (a bronchitis) can be eosinophilic, neutrophilic, both or neither, and its recognition is important in diagnosing and treating the illness. For example, eosinophilic bronchitis, which occurs in patients with or without asthma and in some patients who have COPD, is responsive to corticosteroid treatment, and current evidence suggests that when there is no eosinophilia the condition is not clinically responsive to corticosteroid treatment [7–11]. There is only, at best, a poor correlation between sputum (or bronchial biopsy or lavage) eosinophils and symptoms or physiological abnormalities [12, 13]. As a result, accurate clinical recognition of airway inflammation is poor [14] emphasising the need to measure it in clinical practice.

Support for the use of sputum cell counts to improve treatment was provided by a recent report by GREEN *et al.* [15]. The group performed a single-centre randomised controlled trial with a 1-yr duration in 74 patients with corticosteroid-dependent asthma. They compared the efficacy of treatment to reduce exacerbations when this was monitored by symptoms and spirometry in one arm *versus* these indices and sputum eosinophils (to be kept <3%) in the other arm of the study. The sputum eosinophils were used to guide corticosteroid treatment. During the study, if control was maintained for 2 months, a further attempt to reduce corticosteroid treatment was made. There were a large number of severe exacerbations but these were three-fold less in the sputum arm. The types of exacerbations were not examined.

The present study was conceived and started several months before the study by GREEN *et al.* [15]. Its aim was to compare the effect of determining treatment with or without the use of sputum cell counts on the number and type of exacerbations. Patients were blind to sputum cell counts in both arms and physicians were blind in the clinical arm, thus removing bias. The primary outcomes were the relative risk reduction for the occurrence of the first exacerbation and the length of time without exacerbation. In addition, the type of airway inflammation at exacerbations was measured along with the clinical severity. The current authors were also able to examine the usefulness of monitoring sputum cell counts in relation to the overall severity of asthma, defined by the minimum dose of inhaled steroid to maintain control.

METHODS

Patients

Patients aged 18–70 yrs with asthma, whose minimum treatment to maintain control had not been determined in the previous 6 months, were recruited from the chest clinics of three Canadian and one Brazilian academic centres (table 1). All had symptoms of asthma for a minimum of 1 yr. At entry into the study asthma was confirmed objectively by the demonstration of variable airflow limitation [1]. The severity of the asthma, which was defined by the minimum corticosteroid treatment needed to maintain control [16], was not established until later in the study. All patients were either nonsmokers or exsmokers (<10 pack-yrs) for >6 months. None of the patients had other lung diseases or a history of noncompliance with treatment. The Research Ethics Board of each participating centre approved the study and each patient signed written informed consent.

Study design

This was a multicentre, randomised, parallel group, effectiveness study of two treatment strategies over a 2-yr period (fig 1).

At baseline, subject characteristics, positive allergy skin tests, asthma symptoms and their severity, medications, asthma quality of life questionnaire (AQoL), pre- and post-salbutamol spirometry in addition to methacholine airway responsiveness (provocative concentration causing a 20% drop in the forced expiratory volume in one second (FEV_1): PC_{20}) and induced sputum cell counts were documented. Eligible subjects at each centre were stratified by the duration of the asthmatic symptoms ($\leqslant$20 or >20 yrs), inhaled corticosteroid dose (equivalent to fluticasone $\leqslant$500 or >500 $\mu g \cdot day^{-1}$) and FEV_1 ($\leqslant$70 or >70% predicted). They were then randomised off site in blocks of four to one of two treatment strategies. In one strategy, the clinical strategy (CS), treatment was guided by symptoms and spirometry. In the other, the sputum strategy (SS), the dose of inhaled steroid was guided solely by induced sputum eosinophils to be kept within the normal range at $\leqslant$2.0% [17], while symptoms and spirometry were used to identify clinical control, exacerbations and other treatment. In both strategies the patients were blind to the strategy allocation and to sputum cell counts. In the CS the investigators were blind to the sputum cell counts. The study consisted of two phases (fig 1). In Phase 1 the objective was to determine the minimum treatment to maintain asthma control for a period of 1 month. In Phase 2 the objective was to measure the outcomes of maintaining this minimum treatment for the remainder of the study duration (2 yrs from the baseline visit).

The primary outcomes were the relative risk reduction for the occurrence of the first exacerbation in Phase 2 and the length of time without exacerbation. Secondary outcomes included: the type and severity of exacerbations; the usefulness of monitoring sputum cell counts in relation to the overall severity of asthma, defined by the minimum dose of inhaled steroid to maintain control; and the cumulative dose of inhaled steroid needed in Phase 2 adjusted for its duration.

Study definitions

Control

Control was defined as daytime symptoms <4 $days \cdot week^{-1}$, night-time symptoms < $1 \cdot week^{-1}$, need for short-acting β_2-agonist (SABA) <4$\cdot week^{-1}$ and $FEV_1 \geqslant 80\%$ of personal best [1], and in the sputum arm this plus eosinophils $\leqslant$2%. Clinical control was required to be maintained in every week of the preceding month but the questionnaire at each study visit only requested information over the preceding week. Patients were supplied with a telephone number and could have a nonscheduled visit at any time if there was an increase in their asthma symptoms or lack of improvement or control after

TABLE 1 Characteristics of patients eligible for analysis[#]

	Baseline		Maintenance visit	
	CS	SS	CS	SS
Subjects n	52	50	52	50
Clinical characteristics				
Age, yrs	43.5 (13.9)	46.0 (13.8)		
Sex, male %	28.8	30		
Duration of asthma yrs	19.3 (12.2)	20.0 (16.7)		
Atopy	90.2	90.2		
Symptoms score[¶]	5.4 (1.1)	5.6 (1.0)	5.9 (0.9)	6.2 (0.8)
Pre BD FEV_1[+]	78.7 (18.9)	78.4 (18.3)	81.1 (17.0)	82.4 (15.5)
Pre BD FEV_1/SVC	69.8 (10.4)	69.3 (12.1)	71.7 (11.5)	72.3 (11.0)
ΔFEV_1 after BD[§]	18.3 (12.5)	21.4 (12.5)	7.1 (6.5)	7.6 (6.9)
Methacholine PC_{20} mg·mL^{-1}[ƒ]	0.82 (5.7)	0.89 (4.0)	1.5 (3.4)[§§]	1.4 (4.6)[§§]
Asthma treatment				
IS	88.5	86.0	92.3	94.0
IS dose μg·day^{-1}[##]	500 (0–2000)	500 (0–4000)	625 (0–2000)	750 (0–3000)
LABA	34.6	26.0	39.4	36.0
Antileukotriene	7.7	10.0	7.7	12.0
Prednisone	1.9	4.0	0	2.0
Other asthma medication[¶¶]	3.8	6.0	3.8	2.0
Nasal steroid	20.8	26.0	34.6	30.0
Induced sputum[##]				
Total cell count $\times 10^6$·g^{-1}	2.9 (0.2–266.7)	2.7 (0.4–48.9)	3.8 (0.5–62.5)	3.3 (0.4–23.8)
Neutrophils	26.0 (2.3–94.2)	35.5 (2.0–86.3)	37.0 (4.0–94.55)	41.8 (2.0–94.5)
Eosinophils	2.0 (0–79.0)	2.0 (0–71.0)	1.2 (0–53.0)	1.0 (0–2.0)
Eosinophilia ⩾3%	41.2	30.0	39.6	2.1[ƒƒ]

Data are presented as percentages and presented as mean±SD for continuous and percentages for dichotomous variables, unless otherwise stated. CS: clinical strategy; SS: sputum strategy; Atopy: means ⩾1 positive allergy skin-prick test with a wheal of >2 mm than the negative control; Pre: before use; BD: bronchodilator (salbutamol); FEV_1: forced expiratory volume in one second; SVC: slow vital capacity; PC_{20}: provocative concentration causing a 20% drop in FEV_1; IS: inhaled steroid; LABA: long acting β_2-agonist; [#]: only subjects who achieved maintenance (see fig. 1). [¶]: symptoms score varied from 1 (a very great deal of discomfort or distress) to 7 (no discomfort or distress) and is the mean of 7 individual scores; [+]: FEV_1 predicted values from CRAPO *et al.* [23] and are prebronchodilator; [§]: n=16 in CS and n=14 in SS; [ƒ]: data presented as geometric mean (geometric SD), n=36 in CS and n=36 in SS; [##]: data presented as median (minimum–maximum); [¶¶]: other asthma medication included theophylline or cromone; [§§]: data from the visit at 6 months in the study, n=36 in the CS and n=33 in the SS (of whom 92 and 83%, respectively, were on maintenance treatment at this visit); [ƒƒ]: $p<0.001$ within SS at different time points and between treatment strategies at maintenance.

treatment of an exacerbation or an episode of respiratory infection.

Exacerbations

Exacerbations were regarded as synonymous with the loss of symptomatic control. They were defined in both arms by worsening (from control values) of symptoms requiring increased use of SABA by ⩾4 extra puffs·day^{-1} for a minimum of 48 h, or by nocturnal symptoms, or early morning wakening due to respiratory symptoms two or more times in 1 week, with or without a reduction in FEV_1 of at least 20%. A severe exacerbation of asthma was defined as one requiring ingested treatment with prednisone, as judged by the investigator.

Minimum treatment

Minimum treatment was considered the minimum dose of inhaled steroid to maintain asthma control in Phase 1 for 1 month. The current authors used it synonymously with maintenance treatment.

Study treatment

The study treatment was directed by two physicians in each of the four centres in an effectiveness (rather than efficacy) manner, so as to make it more relevant to routine clinical practice. Treatment followed the Canadian Asthma Consensus Group Guidelines [1].

In both CS and SS the strategy management included the following. 1) Avoidance strategies when they were relevant for allergy to inhaled allergens and intolerance to nonsteroid anti-inflammatory drugs. 2) Patient education regarding treatment and adherence to medications; the later was reinforced at each visit of Phases 1 and 2, although compliance was not formally examined. 3) Review of factors that could affect control, such as rhinosinusitis, major nasal polyps and gastro-oesophageal reflux and their treatment. The individual

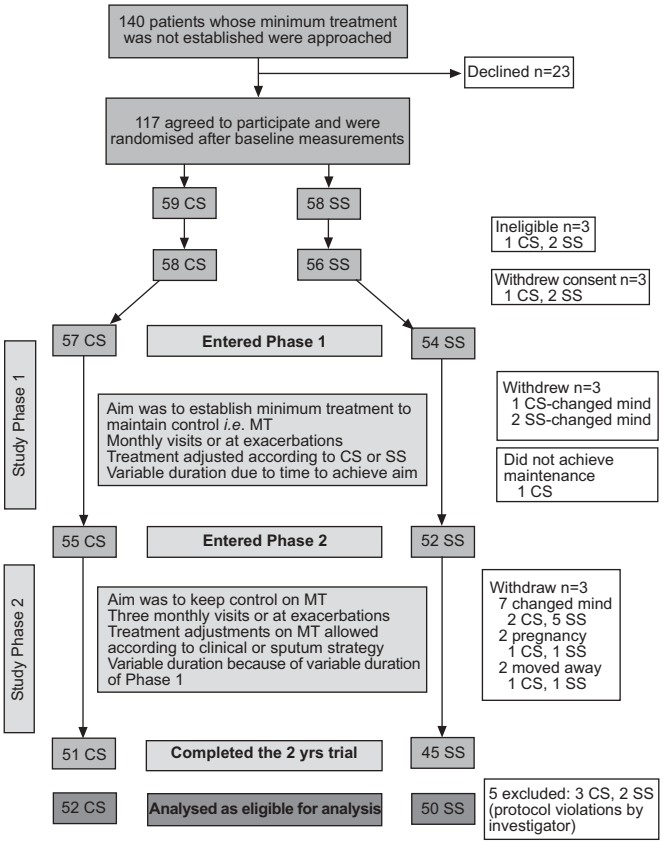

FIGURE 1. Study design and profile. CS: clinical strategy; SS: sputum strategy; MT: maintenance treatment.

medications or inhaler device used were left to the discretion of the treating physician and patient's preference. Changes in treatment were made within 24 h after sputum induction to ensure that the patients remained blind to their treatment strategy. Guidance to the use of medications was as follows.

In Phase 1 patients were seen at intervals of 1 month (within 2 h of the time of the baseline visit) and at the time of any exacerbation. Symptoms and their severity over the past week, medications, pre- and post-bronchodilator spirometry and induced sputum cell counts were recorded. The asthma was controlled if necessary and the minimum treatment to maintain control was determined (table 2). The only difference between the treatment in the two strategies resulted from the sputum cell counts, which were used in the SS primarily to guide (up or down) the dose of inhaled steroid needed. The cell counts also influenced other treatment. Specifically, if they were normal but symptoms were still uncontrolled, they indicated that another cause for the symptoms needed to be considered and treated. If this was considered to be variable airflow limitation improved by salbutamol, a long acting β_2-agonist (LABA) or leukotriene antagonist was added. Also, if the total and differential cell counts showed an intense neutrophilia (total cell count being $\geqslant 25 \times 10^6 \cdot g^{-1}$ and neutrophils $\geqslant 65\%$), suggestive of bacterial infection (18), an antibiotic was added. In contrast optimising the dose of inhaled steroids in the CS was exclusively based on symptoms and spirometry. LABA or leukotriene antagonist could be added if it was considered that the steroid dose was adequate but symptoms required a SABA $\geqslant 2 \cdot day^{-1}$. Once control was achieved for 4 weeks, the dose of inhaled steroid was reduced two-fold at 1-month intervals (and discontinued once it was equivalent to fluticasone 125 $\mu g \cdot day^{-1}$) until there was or was not an exacerbation. If there was an exacerbation, the dose of inhaled steroid was increased two- or four-fold to re-establish control and subsequently maintained at two-fold above the exacerbation dose. If control was maintained for 1 month this was the maintenance dose or minimum treatment. The study visit where maintenance dose or minimum treatment was established was called the maintenance visit and the end of Phase 1. The time taken to identify the minimum treatment varied between patients. At the end of Phase 1 the asthma severity in each patient was graded by the dose of corticosteroid required [16].

In Phase 2, the maintenance dose of corticosteroid was maintained and patients were seen every 3 months and at exacerbations for the remainder of the 2 yrs. At each visit the same measurements were made as in Phase 1. In addition, AQoL and methacholine PC_{20} were determined at 6, 12, 18 and 24 months from the baseline visit. Adjustments of the corticosteroid dose were transient at the time of exacerbations in both strategies or, in SS, when sputum eosinophils were >2.0%. However, the maintenance inhaled steroid dose could be re-adjusted permanently in the CS if there was a persistent clinical deterioration that did not meet the definition of an exacerbation or in the SS if there was a persistent eosinophilia, or in either strategy if the dose of inhaled steroids seemed too high. Exacerbations were treated in the same way as in Phase 1.

In the CS if the exacerbation was not regarded as severe, the dose of inhaled steroid was increased two or four-fold to re-establish control; an antibiotic was added if the sputum was purulent. In the SS, if sputum eosinophils were not increased, the corticosteroid dose was not increased. Instead, additional bronchodilator treatment was given or a course of antibiotics was started if the cell counts suggested a bacterial infection [14, 18]. In both strategies a course of prednisone could be given if the physician was concerned with severity. Once symptomatic control was re-established for 2 weeks, any increase in dose of steroid was returned to the minimum maintenance level.

Procedures

Patient characteristics were documented by a structured questionnaire. Allergy skin tests were performed by the modified skin prick technique [19] with 14 common allergen extracts. Symptoms (shortness of breath, tightness of the chest, wheeze, cough and sputum, nocturnal and early morning awakenings) were scored using a validated seven point Likert scale, with a score of one being the worst and seven the best [20]. AQoL was assessed using the self administered Asthma Quality of Life Questionnaire [21]. Spirometry was performed according to the American Thoracic Society standards [22], before and 10 min after salbutamol 200 μg was inhaled through an Aerochamber (Trudell Medical International, London, ON, Canada). Reference values were taken from CRAPO *et al.* [23]. Methacholine inhalation tests were carried out by the tidal breathing method [24]. Sputum induction and processing for total and differential cell counts were performed by the methods described by PIZZICHINI *et al.* [25].

TABLE 2 Physicians guidelines for adjusting therapy

CS	SS
Phase 1 visits and adjustments to therapy each month or at exacerbations	
SABA	
Administer when needed	Administer when needed
ICS	
No ICS and controlled	
No ICS added	If sp-eos >2% add fluticasone 125 μg *b.i.d.* of equivalent, then treatment adjusted as for patients on ICS baseline.
	If sp-eos ⩽2% no ICS is added
On ICS and controlled	
Reduce ICS two-fold each visit until an exacerbation or fluticasone discontinued (after 125 μg·day^{-1})	If sp-eos >2% increase ICS dose two–four-fold
	If sp-eos ⩽2% reduce ICS two-fold each visit until sp-eos >2% or fluticasone discontinued (after 125 μg·day^{-1})
When ICS reduction is followed by an exacerbation	
Increase ICS dose two–four-fold to regain control and return dose prior to deterioration, this is the MT	If sp-eos >2% increase ICS dose two–four-fold[#] for 2 weeks then reduce to 2-fold above the previous dose, this is the MT
If on no ICS and uncontrolled	
Add fluticasone 125 μg *b.i.d.* or equivalent. Treatment adjusted as for patients on ICS at baseline	If sp-eos >2% add fluticasone 125 μg *b.i.d.* or equivalent. Then treatment adjusted as for patients with ICS at baseline. If sp-eos ⩽2% increase bronchodilator treatment
If on ICS and uncontrolled	
Increase ICS two–four-fold or add LABA or other treatments. When controlled treat as above for controlled asthma	If sp-eos >2% increase ICS dose two–four-fold. When controlled treat as above for controlled asthma. If sp-eos ⩽2% add LABA or other treatment
Exacerbation	
Control with two–four-fold increase in dose[#] until controlled for 2 weeks then reduce to two-fold above exacerbation dose. An antibiotic can be added if purulent sputum	If sp-eos >2% control with two–four-fold increase in dose[¶] until controlled for 2 weeks then reduce to two-fold above exacerbation dose
	If sp-eos ⩽2% add or increase LABA dose if the exacerbation is not severe or add antibiotic if cell count suggests bacterial infection
Phase 2 visits and adjustments to therapy every 3 months and exacerbations	
Maintain minimum treatment[+]	Maintain minimum treatment[§]
Adjust treatment for exacerbations as in Phase 1	Adjust treatment for exacerbations as in Phase 1 or for sp-eos >2% as in Phase 1

CS: clinical stategy; SS Sputum strategy; SABA: short acting β_2-agonist; ICS: inhaled corticosteroid; sp-eos: sputum eosinophils; MT maintenance therapy; LABA: long acting β_2-agonist. [#]: if exacerbation is considered severe by investigator a course of prednisone can be given; [¶]: If exacerbation is considered severe by investigator a course of prednisone can be given; [+]: maintenance could be readjusted if persistent clinical deterioration or if ICS dose was considered to be too high; [§]: maintenance could be readjusted if there was a persistent eosinophilia or if ICS dose was considered to be too high.

Analysis

Descriptive statistics were used to summarise the demographic characteristics of the patients. Continuous data were summarised by the arithmetic mean and standard deviation or the median and quartiles. Variables with skewed distribution (total cell count and eosinophils %) were log transformed before analysis. PC_{20} data were log transformed and reported as geometric mean (GM) and geometric standard deviation (GSD). Two tailed, unpaired independent t-tests or Chi-squared tests were used for cross-sectional comparisons between groups.

The primary analysis was based on the occurrence of exacerbations during Phase 2, so as to exclude those resulting from reducing the dose of inhaled steroid to establish the minimum to maintain asthma control in Phase 1. The sample size was calculated to give 90% power to detect a 15% reduction in the rate of exacerbations based on a two-sided test at the 5% level of a Poisson distribution for the incidence of exacerbations. Exacerbation rates were estimated from the Formoterol and Corticosteroids Establishing Therapy International Study Group study [26]. The exacerbations were regarded as severe if they required treatment with prednisone; the others were regarded as mild. The types of exacerbations were labelled as definitely eosinophilic if sputum eosinophils were ⩾3%, and noneosinophilic if sputum eosinophils were <3%. The current authors selected 3%, rather than >2%, because it is likely that there is a gray area around the cut-off point of 2%. Differences between 2 and 3% are subtle and 3% seems to be more clinically relevant with respect to short-term benefit from the addition of inhaled steroid treatment [7, 8, 10]. Relative risks (RR) and associated 95% confidence intervals were obtained by Cox regression analyses [27] for the time to the first exacerbation in Phase 2 and by multiple event analyses

based on Andersen-Gill models [28] with robust variance estimates to assess the effects of the SS on the rate of all exacerbations in Phase 2. The relative risk reduction (RRR) was also calculated. Tests of group differences based on these analyses gave p-values which were considered significant if $p<0.05$. Plots of the cumulative mean number of exacerbations over time were also constructed based on the Nelson-Aalen estimate [29]. The current authors cumulated the dose of inhaled steroid·day^{-1}·patient^{-1} in Phase 2, adjusted for its duration, averaged the results and investigated difference by two group comparisons. In addition to these analyses, a secondary analysis was directed at assessing group differences in exacerbations in Phase 2 in patients with very mild and mild asthma *versus* those with moderate and severe asthma, and in those with or without treatment with LABA. The impact of CS and SS treatments on exacerbations was also examined by the number of exacerbations·patient^{-1}·yr^{-1} on maintenance.

RESULTS

Randomisation and withdrawals

Between August, 1999 and September, 2000, 117 consecutive patients were randomised to the clinical or sputum strategies (fig. 1). Three patients were immediately found to be ineligible. Of the remainder, three dropped-out after the baseline assessment, three withdrew consent during Phase 1 and one never achieved the maintenance dose because medications were not adjusted by the physician. Among the 107 who achieved maintenance treatment, five were excluded due to investigator protocol violations during Phase 2. The decision to exclude patients from analysis was made by an adjudicator researcher who was not an investigator in the study and was blind to the treatment allocation.

Characteristics of patients at baseline

The baseline characteristics in the two treatment strategies were not different from one another in the 102 patients who were eligible for analysis at the end of Phase 1 (table 1; individual sputum data are shown in the online supplementary data; fig.E1) and in those in the 114 eligible randomised patients (data not shown).

Phase 1 results

The mean±SD time to establish maintenance was similar in the two treatment strategies (5.0±3.6 months in SS, 4.0±3.4 months in CS; p=0.2). The percentage of patients whose dose was reduced or increased in relation to baseline treatment was also similar in both groups (data shown in the online supplementary data; fig E2). As a result of the attempts to reduce corticosteroid dose to identify the minimum treatment, there were 85 exacerbations in 42 patients (46 and 39 in the SS and CS groups, respectively). Sputum cell counts were obtained before any additional steroid treatment in 81 (96.4%) of these; the proportion of eosinophilic exacerbations was similar in both strategies (51.2 and 48.6% in CS and SS, respectively; p=0.2).

At the end of Phase 1, the characteristics of patients between strategies did not differ with the exception of the percentage of patients with sputum eosinophilia ($p<0.001$; table 1; individual sputum data are shown in the online supplementary data; fig. 3). Maintenance treatment was also similar between strategies (table 1; fig. 2). The majority of patients could then

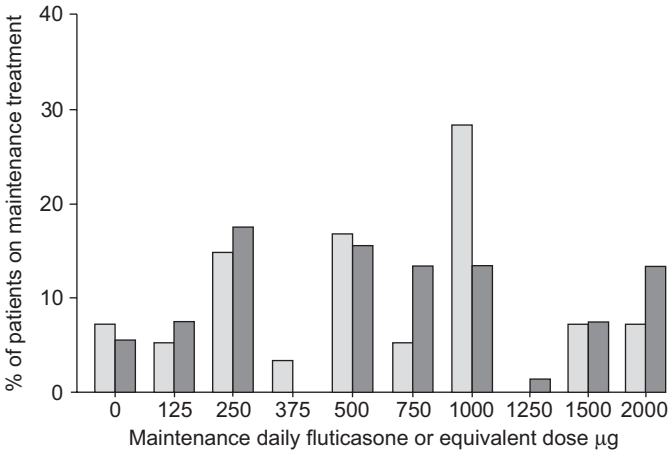

FIGURE 2. Maintenance inhaled steroid dose in clinical strategy (□) and sputum strategy (■). The distribution of each dose of inhaled corticosteroid was similar in both study groups.

be classified as having moderate-to-severe asthma because they needed fluticasone in a dose >250 μg·day^{-1} (or other steroid equivalent) to maintain asthma control (table 1; detailed data and dose are shown in the online supplementary data; table E1). These patients had a lower FEV_1 and required LABA more often than those with very mild-to-mild asthma (table 3).

Phase 2 results

The duration of Phase 2, expressed as mean (95% CI), was slightly but not significantly lower in the SS (1.4 (1.3–1.6) *versus* 1.6 (1.5–1.7) yrs; p=0.5). The duration was not different between patients of different asthma severity within or between study strategies (data not shown). The maintenance dose of inhaled steroid was permanently readjusted during Phase 2 in a minority of the patients in each strategy (online supplementary data table E2) and this did not affect the classification of asthma severity.

Number of exacerbations; primary outcomes

There were 126 exacerbations in 63 patients. The SS compared with the CS, resulted in fewer exacerbations (47 *versus* 79), fewer exacerbations·patient^{-1}·yr^{-1} on maintenance, 0.75 (0.4–1.1) *versus* 1.02 (0.7–1.3), p=0.04, more patients without exacerbations (48 *versus* 29%, p=0.04) particularly in those with moderate-to-severe asthma (45 *versus* 19%, p=0.02), an overall RR for the first exacerbation of 0.61 (0.37–1.02), p=0.06 (table 4) and a longer median time to the first exacerbation (607 *versus* 394 days; fig 3). These advantages were achieved with a similar mean cumulative inhaled steroid dose in Phase 2, adjusted for its duration, of 840 *versus* 780 μg. The relative risk of all exacerbations based on the multiple event analysis was 0.71 (0.45–1.12), p=0.14.

Influence of strategies on type of exacerbations

Induced sputum cell counts were obtained before any additional steroid treatment in 102 exacerbations (39 out of 47 and 63 out of 79 for SS and CS, respectively) and these were divided into eosinophilic and noneosinophilic (table 4; fig 4). The eosinophilic exacerbations were fewer in the SS (15.4

TABLE 3 Characteristics at maintenance visit according to asthma severity

	Very mild to mild		Moderate to severe	
	CS	SS	CS	SS
Clinical parameters				
Subjects n	15	16	37	34
Symptoms score	6.0±0.8	5.8±0.9	5.8±0.9	6.3±0.8
Pre BD FEV_1	95.0±12.2	90.0±11.6	76.2 ±15.7*	79.0±16.2*
Asthma treatment				
On inhaled steroid	73.3	81.2	100*	100*
On LABA	13.3	12.5	50.0*	47.1*
On antileukotriene	6.7	0	8.1	17.6
On prednisone	0	0	0	2.9
Other asthma medication	0	0	5.4	2.9
On nasal steroid	20.0	25.0	40.5	32.4
Induced sputum				
Total cell count $\times 10^6 \cdot g^{-1}$ #	3.0 (0.5–7.0)	3.9 (0.9–23.8)	4.3 (0.6–62.5)	3.1 (0.4–22.7)
Neutrophils#	37.0 (4.0–72.0)	52.0 (5.0–94.5)	37.0 (5.0–94.5)	40.0 (2.0–96.8)
Eosinophils#	3.0 (0–44.0)	1.0 (0–4.0)	0.7 (0–53.0)	0.6 (0–2.0)
Eosinophilia ⩾3%	53.3	6.7	33.3**	0**

Data are presented as mean±SD (for continuous variables) or % (for dichotomous variables), unless otherwise specified. CS: clinical stategy; SS: sputum strategy; Pre: before use; BD: bronchodilator (salbutamol); FEV_1: forced expiratory volume in one second; LABA: long acting β_2-agonist; #: data presented as median (minimum–maximum); *: p-value <0.05 within strategy between severity groups; **: p-value <0.01 within strategy between severity groups.

TABLE 4 Relative risk between sputum strategy and clinical strategy from Cox regression models for the time to the first exacerbation and Andersen-Gill models for the multiple exacerbations during maintenance phase

	Time to first exacerbation#			Multiple event analysis¶		
	RR	95% CI	p-value	RR	95% CI	p-value
All exacerbations	0.61	(0.37–1.02)	0.06	0.71	(0.45–1.12)	0.14
By type of exacerbation						
Eosinophilic	0.19	(0.05–0.83)	0.03	0.28	(0.10–0.74)	0.01
Noneosinophilic	0.82	(0.44–1.53)	0.53	1.07	(0.61–1.85)	0.82
By use of LABA						
Not on LABA	0.84	(0.44–1.63)	0.61	1.05	(0.62–1.79)	0.85
On LABA	0.40	(0.18–0.88)	0.02	0.53	(0.24–1.14)	0.11
By asthma severity						
Very mild to mild	0.99	(0.34–2.81)	0.98	1.34	(0.52–3.46)	0.54
Moderate to severe	0.51	(0.29–0.90)	0.02	0.63	(0.38–1.03)	0.07

RR: relative risks; 95% CI; 95% confidence intervals; LABA: long-acting β_2-agonists. #: RR is relative risk from a Cox regression model. ¶: RR is relative risk from an Anderson-Gill model. Asthma severity was based on minimum daily maintenance fluticasone equivalent dose, very mild: 0; mild: <250 μg; moderate: ⩾250–500 μg; severe: >500 μg.

versus 41.3%; p=0.006). There was an overall reduction of the first eosinophilic exacerbation as indicated by the RR of 0.19 (0.054–0.830), p=0.03 which corresponds to a RRR of 81%. The multiple event analyses yielded a significant, but less extreme, RR of 0.28 (0.10–0.74; p=0.01) for eosinophilic exacerbations. There was no significant benefit for noneosinophilic exacerbations based on either the time to the first event or the multiple event analyses. The results were similar when duration of maintenance treatment was taken into account by calculating the number of eosinophilic and noneosinophilic exacerbations·$patient^{-1} \cdot yr^{-1}$ (data are shown in the online supplementary data; table E3).

The current authors also compared the sputum cells in the noneosinophilic exacerbations with those in the eosinophilic exacerbations (fig. 4). The former were characterised by a higher total cell count (median (interquartile range)) of 13.6 (25.8) *versus* 5.0 (9.0) $\times 10^6 \cdot g^{-1}$; p=0.002) and a higher proportion

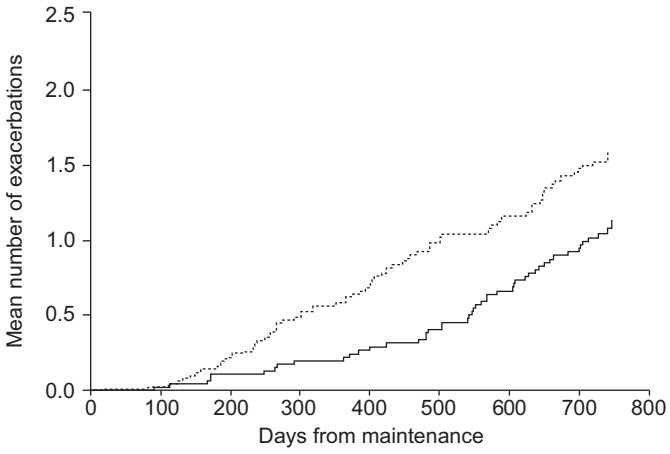

FIGURE 3. Mean number of exacerbations from maintenance visit to the end of the scheduled follow-up or withdrawal. For clinical strategy (·····; n=52 patients, 79 exacerbations) and sputum strategy (SS: ——; n=48, 47 exacerbations). The SS resulted in a reduction in the rate of exacerbations which was not statistically significant. (relative risk=0.71, 95% confidence interval=0.45–1.12, p=0.14).

of neutrophils (68.0 (43) *versus* 35.0 (51.1)%, p<0.001). By definition they had a lower percentage of eosinophils (0.3 (1.0) *versus* 10.0 (28.0)%; p <0.001). Clinically they had a similar increase in symptoms (1.4±1.3 *versus* 1.4±1.1; p=0.1) but a significantly smaller fall in pre-bronchodilator FEV_1 (0.08±0.3 *versus* 0.24±0.2 L; p=0.03) from that established at maintenance.

Exacerbations by overall asthma severity

The patients who benefited from monitoring by sputum were those with moderate-to-severe asthma; they had a RRR for asthma exacerbation of 49% (95% CI: 10–71), p=0.02, (table 5, fig. 5). In these patients the SS compared with the CS, resulted in an overall RR for the first exacerbation of 0.51 (0.29–0.90), p=0.02 (table 4), and a longer median time compared with the first exacerbation (559 *versus* 301 days). Those patients with very mild-to-mild asthma did not benefit. The number of exacerbations in the SS *versus* CS arms were 0.5 (10 and 90% percentiles: 0.1, 0.9) *versus* 0.6 (0.1, 1.0); p=0.9. The results were similar when duration of maintenance treatment was taken into account by calculating the number exacerbations·$patient^{-1}$·yr^{-1} by asthma severity (data are shown in the online supplement; table E3).

Exacerbations by use of long acting β_2-agonists

The current authors also examined the effect of treatment with LABA in the treatment strategies (table 4; fig. 6). The dose of inhaled steroid was similar in patients using LABA in both strategies as shown by median (10 and 90% percentiles) of 1,000 (250, 2000) μg in the SS and 1,000 (400, 2000) μg in CS. Hence, the rate of first exacerbation was significantly reduced in the SS among patients on LABA (RRR: 60%, RR: 0.40, 95% CI: 0.18–0.88; p=0.02) compared with those not on LABA. This difference was not observed in patients not on LABA (RR: 0.8; 95% CI: 0.4, 1.6) *versus* 0.7 (0.4, 1.0), p=0.1) and the median times to the first exacerbation were longer (728 *versus* 238). Based on the multiple event analyses, similar conclusions were made although the effects did not reach statistical significance for those on LABA (RR: 0.53, 95% CI: 0.24–1.14; p=0.11. The results were similar when duration of maintenance treatment was taken into account by calculating the number exacerbations·$patient^{-1}$·yr^{-1} by use of LABA (data are shown in the online supplementary data; table E3).

Severity of exacerbations

The majority of exacerbations were mild (table 5). Only 23 (18.3%) were severe requiring treatment with prednisone and most of these (78%) occurred in the CS, none required hospital admission. Sputum was obtained from 15 out of 23 severe exacerbations, before they were treated with prednisone; 10 were eosinophilic (nine CS and one SS).

DISCUSSION

In the current study the monitoring of sputum cell counts reduced the overall risk of exacerbations by 49%, it reduced the number of severe exacerbations by two-thirds and it prolonged the period without an exacerbation with no need for more treatment. These benefits were seen in patients with moderate-to-severe asthma and were due to a reduction of eosinophilic exacerbations. There was no effect on noneosinophilic exacerbations, which were the most common. The results support the use of sputum cell counts in the long-term treatment of asthma and identify how this reduces exacerbations.

TABLE 5 Characteristics of exacerbations in Phase 2

	Severe exacerbations			Mild exacerbations		
	CS	SS	p-value	CS	SS	p-value
Number n (%)	18 (22.9)	5 (10.6)	0.004	61 (77.1)	42 (89.4)	0.08
Symptoms score	3.5±0.4	3.9±0.9	NS	4.6±1.0	4.4±0.8	NS
Pre BD FEV_1 % pred	60.5±11.1)	63.8±19.4)	NS	74.7±16.9	82.0±16.9	0.03
Occurrence by asthma severity						
Very mild to mild	1 (9.1)	0	NS	10 (90.9)	13 (100)	NS
Moderate to severe	17 (25.0)	5 (14.7)	NS	51 (75.0)	29 (85.3)	NS

Data are presented as mean±SD or n (%), unless otherwise stated. CS: clinical strategy; SS: sputum strategy; Pre-BD FEV_1 % pred: forced expiratory volume in one second expressed as per cent predicted before administrating bronchodilator (salbutamol).

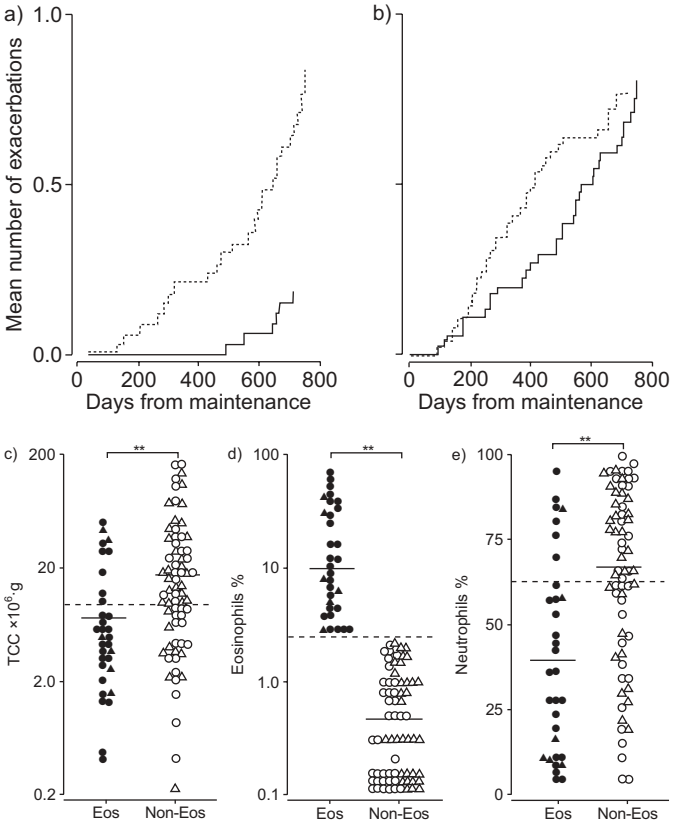

FIGURE 4. Analyses by exacerbation type: a) Mean number of eosinophilic exacerbations in clinical strategy (CS; (·····) patients (n=34, 26 exacerbations) and sputum strategy (SS; ——) patients (n=34, 6 exacerbations). b) Mean number of noneosinophilic exacerbations in CS (n=49, 37 exacerbations) and SS (n=48, 33 exacerbations) patients. Both a) and b) show data from maintenance to the end of the study or to withdrawal. SS reduced the rate of eosinophilic exacerbations by 72% (relative risk (RR): 0.28, 95% confidence interval: 0.10–0.74; p=0.01), but had no effect on the number of noneosinophilic exacerbations (RR=1.07, 95% CI=0.61–1.85; p=0.82). c)–e) Show individual plots for sputum total cell counts (TCCs) (c), eosinophils (d) and neutrophils (e) in the eosinophilic (Eos) and noneosinophilic (Non-Eos) exacerbations. ○: indicate Non-Eos exacerbations in CS; △: Non-eos exacerbations in SS; ●: Eos exacerbations in CS; ▲: Eos exacerbations in SS; - - - -: represent upper limit of normal values; ——: represent the median values. **: p-values <0.01 for comparisons between Eos and Non-Eos exacerbations. In comparison with Eos exacerbations, the Non-Eos exacerbations were characterised by a higher and higher percentage of neutrophils.

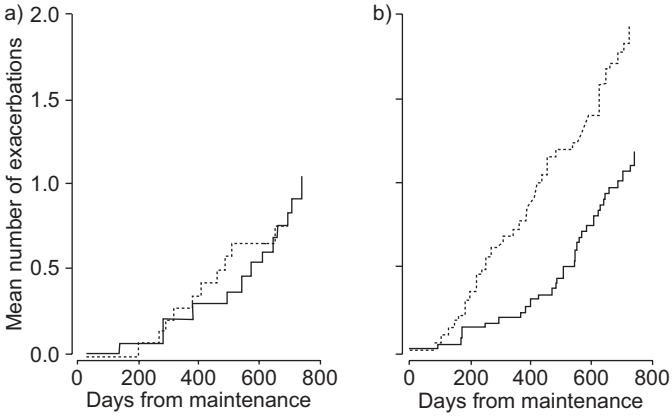

FIGURE 5. Analysis by severity of asthma defined by the minimum dose of inhaled steroid to maintain control: mean number of exacerbations from maintenance to the end of the study or to withdrawal in a) very mild to mild asthma for clinical strategy (CS) patients (·····; n=15, 11 exacerbations) and sputum strategy (SS) patients (——; n=15, 13 exacerbations) and in b) moderate-to-severe asthma for CS patients (n=37, 68 exacerbations) and SS patients (n=33, 34 exacerbations). While patients with very mild or mild asthma did not benefit from the SS (relative risk (RR)=1.34, 95% confidence interval=0.52–3.46, p=0.54) those with moderate-to-severe asthma had a 37% risk reduction for exacerbations after maintenance (RR=0.63, 95% CI=0.38, 1.03, p=0.07).

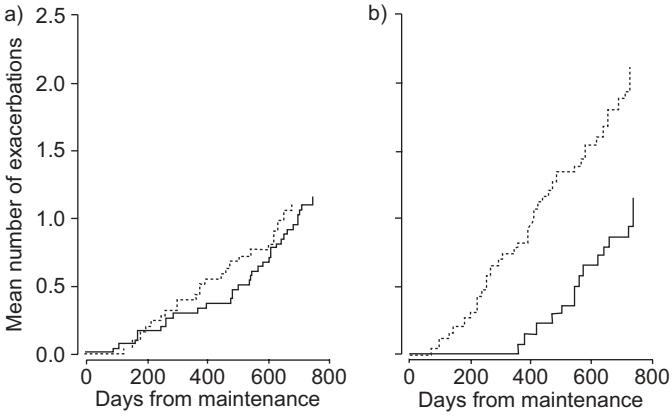

FIGURE 6. Analyses by use (necessity for) of long acting β_2-agonist (LABA). Mean number of exacerbations from maintenance to the end of the study or withdrawal in patients on LABA (a) with clinical strategy (CS, ·····) patients n=26, 52 exacerbations) and sputum strategy (SS, ——) patients (n=16, 15 exacerbations) and b) not on LABA with CS patients; n=26, 27 exacerbations) and SS patients (n=32, 32 exacerbations). Those on LABA in the SS had a 47% reduction in events (relative risk (RR)=0.53, 95% confidence interval=0.24–1.14; p=0.11) and those not on LABA had no reduction (RR=1.05, 95% CI=0.62–1.79; p=0.85).

The study differed in a number of ways from the study by GREEN *et al.* [15]. The current study was a multicentre study and recruited patients with asthma of more variable severity. Treatment was adjusted in an effectiveness rather than efficacy manner. The minimum treatment to maintain control was first identified and then continued for up to 2 yrs from the start. Despite these differences the current study's results confirm the considerable overall reduction of exacerbations without an increase in steroid treatment reported by GREEN *et al.* [15]. The results support the sensitivity of sputum eosinophilia which precedes clinical exacerbations [30–32]. The results also add the reason for the reduction in exacerbations, the severity of asthma that benefited and the more frequent occurrence of noneosinophilic exacerbations. There were fewer exacerbations than in the study by GREEN *et al.* [15] because the minimum dose of corticosteroid to maintain control was not reduced in the vast majority of patients. The exacerbations were also milder in severity because they were identified and treated early, based on symptoms and need for short-acting β-agonist rather than a required reduction in spirometry.

The current authors chose prevention of exacerbations as the most important clinical outcome in the management of asthma because it has the greatest impact on patient's quality of life, morbidity and healthcare utilisation. The primary outcomes

were the relative risk reduction for the occurrence of the first exacerbation and length of time without exacerbation, instead of the number of exacerbations·patient^{-1}·yr^{-1}, because in an analysis of repeated events the occurrence of the first event provides more precision [29].

The results are unlikely to have been influenced by investigator bias or differences in asthma management in the CS. The study was planned to ensure that the only difference between strategies was the temporary adjustment of inhaled steroid dose when sputum eosinophils were >2% in the SS arm. As a consequence, the present study has a number of strengths specifically related to the randomised controlled design that lend weight to the results. First, a similar number of patients with asthma of different severity were included in both groups. Second, the minimum maintenance dose of inhaled corticosteroid was identified in the first phase of the study, ensuring that exacerbations counted in the second phase of the study were not an artefact of further reductions in steroid treatment. Third, the treatment was directed by eight physicians involved in both clinical and sputum strategies within four university centres to minimise investigator bias. Fourth, the exacerbations were patient defined by symptoms and the use of a rescue bronchodilator, reducing the risk of investigator bias in determining the presence or absence of an exacerbation and hence the need for treatment. Finally, patients were seen at the time of exacerbations to identify their severity and type. This allowed treatment to be made appropriate for the type of inflammation in the sputum arm or for clinical variables in the clinical arm.

Alternatively, it could be argued that there were factors in the study that potentially could have biased the results in favour of the sputum arm. These include the variable duration for Phase 2 of the study, the definition of an eosinophilic exacerbation and undertreatment with inhaled steroid in the clinical arm. The type of analysis performed and the results of the study do not support these assumptions.

First, the variable duration of Phases 1 and 2 was unlikely to affect the study outcome. The analyses of exacerbation rates were based on multiple event analysis (where all exacerbations in Phase 2 were counted) and the time to the first event analysis (where only the first exacerbation was counted). In both of these analyses, daily rates were computed and compared between groups. Also, it is not expected that the risk of an exacerbation on a particular day of Phase 2 would be affected by the time it took to reach the maintenance dose, thus excluding the variable length of Phase 1 as a potential bias. However, it is possible that there would be a loss in efficacy in patients whose Phase 1 was long and data over Phase 2 was short, but this has power rather than bias implications.

Second, the decision to define sputum eosinophilia as $\geqslant$3% and symptomatic plus eosinophilic exacerbations as >3%, rather than >2%, was not a factor in the results. There was only one exacerbation between 2 and 3% (at 2.3% in the sputum group) emphasising the lack of impact this decision had on the results.

Finally, the possibility of systematic mistreatment in one of the study arms also seems unlikely when reviewing the study results. The current authors analysed this in four ways. First the algorithms for treatments strategies were designed to ensure that the only difference between arms was the adjustment of inhaled steroids by clinical variables in the clinical arm and by sputum eosinophils in the sputum arm. The results clearly show that the amount of inhaled steroids adjusted for the duration of Phase 2 was similar in both strategies in patients on or not on LABA, thereby excluding the possibility of systematic undertreatment in the clinical arm as the cause of the higher number of exacerbations. However, at the time of analysis it was identified that one-third of the patients in the clinical arm were indeed undertreated as judged by sputum eosinophilia but this was not recognised from symptoms and spirometry. In contrast, patients in the sputum arm received sufficient corticosteroid to control their sputum eosinophilia and potentially they could have been overtreated. This situation was reversed at the time of exacerbations. Patients in the clinical arm were overtreated because they received an increase in the steroid dose whether the exacerbations were eosinophilic or not. In contrast, there was potentially less overtreatment in the sputum arm because, in general, corticosteroid treatment was only increased if there was an eosinophilia present.

A second consideration regarding mistreatment is the approach to treatment that was as similar as possible to the authors' current clinical practice [1]. Thus, the minimum treatment to maintain control was established. Compliance was not formally examined but stressed at each study visit in both strategies. If control existed for a substantial period, down-titration of inhaled steroid was usually not tried. However, while permanent increases in maintenance treatment were similar in both strategies down-titration was higher in the sputum strategy, again indicating potentially less treatment in the sputum arm.

A third consideration is the possibility that undertreatment could occur at times of seasonal allergen exposure if treatment was insufficient to prevent a worsening in eosinophilic inflammation. In practice this would be handled by advising the patient to step-up corticosteroid treatment if symptoms began to increase during the season. However, the design of the study did not allow the current authors to do this because the number and type of exacerbations needed to be identified. Hence, instead of the patients increasing treatment themselves the current authors promptly saw the patients and adjusted the treatment appropriately in both groups. This would not explain the higher number of noneosinophilic exacerbations in both groups, or the similar rate of eosinophilic and noneosinophilic exacerbations among seasons in both groups.

A final consideration is the influence of the regular use of LABA. This was more likely to be appropriate in the sputum strategy because, ideally, LABA is needed when symptoms are associated with variable airflow limitation in spite of the control of eosinophilic inflammation. Therefore, the lack of cell counts in the clinical arm meant that the physician had to guess whether continuing symptoms required an increase in steroid dose or the addition of LABA. As the use of LABA was the same in both arms and steroid treatment was underused in the clinical arm (as judged by sputum eosinophilia) LABA was more likely to have been misused in this arm.

The observation that treatment to control sputum eosinophilia reduced eosinophilic exacerbations may not be a surprise, since the treatment was designed to prevent these, but it has not been demonstrated before. The more common occurrence of noneosinophilic exacerbations in both groups unaffected by the control of eosinophilia might have been suspected from some previous observations [6, 33, 34]. For example, DOULL *et al.* [35] reported that exacerbations of symptoms in children with asthma were not reduced by prophylactic treatment with inhaled steroids. REDDEL *et al.* [36] controlled asthma with inhaled corticosteroids but, while continuing treatment, could not prevent exacerbations which were considered, by the group, to be of viral cause. WARK *et al.* [6] observed that amongst adults presenting at the emergency department 70% had viral exacerbations which were noneosinophilic. Overall, their noneosinophilic group had a neutrophilia with a modest increase in total cell count and an increase in the percentage of neutrophils to <80%. The sputum cellular observations have been made by others during viral respiratory infections [6, 37] and were observed in the noneosinophilic exacerbations in the present study, suggesting that these were mainly of viral cause. Some of the exacerbations with a more intense neutrophilia may have been bacterial.

The importance of noneosinophilic exacerbations relates to how they should be treated. In this study they were not prevented by corticosteroid treatment. The effect of an increase in corticosteroid dose on them has not been reported. However, from the vast majority of studies of uncontrolled asthma [10], moderate-to-severe COPD [7, 8] and chronic cough [11], the lack of eosinophilia has indicated corticosteroid resistance. There is only one study which reports contrary results and this was open and uncontrolled [38]. Overall, the implication of these studies' results is that the treatment of noneosinophilic exacerbations is palliative until the exacerbations resolve spontaneously, or with an antibiotic if a bacterial infection is present. This is how they were managed in the sputum arm of the present study. Some support for palliative treatment was also observed in the present study by a reduction of noneosinophilic exacerbations and the length of time free of exacerbation by treatment with a LABA, only in the sputum strategy arm. As this was not observed in patients on a LABA in the clinical strategy it is possible that, when eosinophilic inflammation is under control, a LABA may prevent a deterioration of asthma caused by a viral or bacterial infection. However, this was a secondary analysis and can only be interpreted as exploratory or hypothesis generating because the study was not powered to detect if treatment effects were different between patients on LABA or not.

To summarise, the current study has three main messages with clinical implications. First, the majority of asthma exacerbations in optimally treated patients are mild and noneosinophilic. Second, not all exacerbations can be prevented by treatment according to current guidelines, even with measurements of airway inflammation. However, the use of sputum cell counts greatly reduces the risk for an eosinophilic exacerbation and prolongs the time for patients to be free of an exacerbation. Third, patients who are more likely to benefit from monitoring of sputum cell counts are those with moderate-to-severe asthma and those who require and are maintained on long-acting β_2-agonists. These observations support the role of sputum cell counts in the management of moderate-to-severe asthma and confirm different patterns of airway inflammatory response at exacerbations that have different causes and therapeutic implications.

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