

REPORT OF WORKING GROUP 7

The use of induced sputum in clinical trials

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As asthma is considered to be an inflammatory disorder of the airways, it seems logical to include an assessment of this inflammatory process as an outcome measure in clinical trials. Biopsy studies illustrate that clinical or lung function characteristics such as symptoms, peak flow variability or degree of airway responsiveness do not consistently correlate with histological alterations. Therefore, these clinical indices cannot be seen as accurate markers of airway inflammation [1–3]. Conversely, repeated bronchoscopic sampling is not feasible in large-scale clinical studies. Hence there is interest in a relatively non-invasive but direct marker of airway inflammation.

Analysis of induced sputum seems to meet these criteria. Provided proper precautions are taken, induction of sputum is safe, even in patients with more severe asthma [4, 5]. In addition, sputum cell counts, particularly eosinophil counts, have been well validated in terms of responsiveness to intervention. It has been argued that, in comparison with other noninvasive markers of inflammation, induced sputum offers the most balanced assessment of the degree of inflammation, being more responsive to intervention than serum eosinophil cationic protein, yet not as over-sensitive as exhaled nitric oxide [6–8].

As for any outcome measure, when including induced sputum in a clinical trial, specific features of sputum analysis need to be taken into account when designing the study: 1) origin of sputum; 2) methodological aspects; 3) selection of subjects; and 4) power calculations.

Origin of sputum

The induced sputum technique samples the inflammatory cells and soluble markers present in the airway lumen of the bronchial tree, which, although reflective of, does not represent an identical situation to the

local inflammatory process in the mucosa. This probably explains the poor correlation between the cellular composition of biopsy samples and sputum, bronchial wash or bronchoalveolar lavage [9–11]. Therefore, although induced sputum can provide information regarding the ongoing overall inflammation in asthma, it might not be the ideal substrate for studying the exact pathophysiological events that occur within the airway wall.

Methodological aspects

Regarding the use of induced sputum in clinical trials, a few methodological issues should be re-emphasised. Consecutive inductions within a short time interval can cause an increase in the percentage of neutrophils in sputum [12, 13]. It has also been reported that the composition of sputum can change somewhat throughout the duration of the induction procedure and that standardising this variable would seem advisable [14]. Current recommendations are to process sputum samples within 2 h after induction, although a recent study has shown that this can be prolonged to 9 h (A. Efthimiadis, Firestone Institute for Respiratory Health, Hamilton, Ontario, Canada, personal communication). This can be important when deciding on the number and timing of inductions to be performed.

Selection of subjects

It should be remembered that, to date, analysis of induced sputum has mainly been validated with regard to the percentage of eosinophils in the cell pellet. The responsiveness to intervention of other possible outcome measures, be they cells or soluble mediators, has been far less thoroughly established.

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Received: April 4 2002; Accepted: April 16 2002

The use of eosinophil counts as the main outcome measure of the induced sputum technique requires specific consideration, in part related to the overall aim of the study planned, to be given to the screening of patients for study inclusion. If the aim of the study is to evaluate the biological activity of a given compound on eosinophils, it is acceptable to select subjects based on the number of eosinophils in their sputum samples. However, this does not apply if the aim of the study is to conduct a clinical trial in asthma as a disease entity. Although sputum eosinophil counts in samples obtained from the same patient on different occasions are repeatable, the interindividual variability is large, even in samples obtained from patients with very similar clinical characteristics [15, 16].

Therefore, including only those patients with a specific degree of sputum eosinophilia leads to a selection bias that hampers a proper interpretation and generalisation of the results.

Another point that must be considered is that, when subjects are randomised based on lung function or clinical criteria, the wide variability in sputum eosinophilia can cause unexpected differences between the groups, at baseline. An approach to preventing this is to stratify patients at randomisation to ensure that the full range of baseline eosinophilia is equally represented in each study group [17]. It can only be used in proof of concept studies aimed at reducing numbers of sputum eosinophils.

Power calculations

An important consideration in the design of most clinical trials is estimation of the appropriate sample size required to draw adequate conclusions from the study. This depends on the set probability of making a type I (α level) or type II (β level) error, the design of the study, the reliability of the outcome measurement and the desired effect size. The α level or probability of falsely rejecting the null hypothesis is usually set at 5%. The β level represents the probability of falsely accepting the null hypothesis, usually set at 10–20%. Hence, $1-\beta$, the power of the study, expresses the probability of avoiding a type II error. The power of the study relates intimately to the variability in the outcome measurement (either inter- or intrasubject, depending on study design) and the desired effect size. The present authors strongly recommend that researchers determine sample size requirements based on the variability and reproducibility of sputum eosinophil counts in their own population. A less favoured option is to rely on data from the literature, taking into account the induction and processing techniques used. As an example, duplicate measurements were examined from 84 volunteer subjects from multiple centres (table 1). All of these subjects had been diagnosed with asthma and were not currently receiving anti-inflammatory treatment. No intervention took place between the two sputum measurements, which were separated by ~ 1 week. The formula used to determine sample size requirement is $(T_{1-\alpha/2} + T_{1-\beta})\phi/\text{LCID}$,

Table 1.—Eosinophil counts in duplicate sputum samples obtained from asthmatic subjects currently not on maintenance treatment

	Sample 1	Sample 2	Difference
Mean \log_{10} -transformed data	0.674	0.672	0.002
SD \log_{10} -transformed data	0.729	0.743	0.333
Geometric mean	4.722	4.698	

where T is score of the paired t -test corresponding to the desired α and power $(1-\beta)$, ϕ is the standard deviation of the outcome of interest and LCID is the least clinically important difference. When designing a parallel study, ϕ relates to the interindividual variability in eosinophil count, whereas, in a crossover study, the variable of interest is the intrasubject reproducibility. An alternative consists of feeding these data into power tables. This allows the determination of the sample size required to draw conclusions with various levels of confidence regarding the significance of a predefined difference in eosinophil count over the study period within or between groups. An example is given in figure 1. These data are based on the reproducibility of changes in sputum eosinophil count following allergen challenge. From these data, it would appear that, when performing repeated measures analysis of variance on sputum samples obtained at 7 and 24 h after allergen challenge, the estimated sample size required to observe a 50% attenuation of allergen-induced increase in the percentage of eosinophils in a crossover study design is only five subjects. As total cell counts in sputum show poorer reproducibility, using total counts instead of percentage of eosinophils increases the estimated sample size

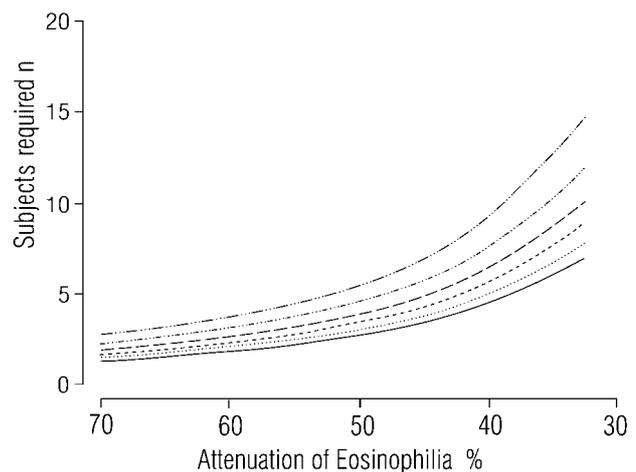


Fig. 1.—Sample size requirements for demonstrating statistically significant attenuation of allergen-induced increases in percentage of sputum eosinophils (power: —: 0.70;: 0.75; ----: 0.80; - · - · -: 0.85; - - - -: 0.90; - · - · -: 0.95). For example, a drug that blocks 50% of the allergen-induced increase in eosinophil number can be demonstrated to be significant using only four subjects (90% power).

Table 2. – The attenuating effect of various steroid formulations, doses and treatment durations on sputum eosinophils. Improvements are expressed as percentage improvement from a pre-treatment baseline

First author [Ref.]	Steroid	Dose µg	Duration weeks	Improvement %
JATAKANON [8]	Bud	100	4	69
	Bud	400	4	71
ALDRIDGE [22]	Bud	800	6	61
TAYLOR [23]	Cicl	100	2	36
	Cicl	400	2	65
	Cicl	1600	2	63
LIM [24]	Bud	1600	4	71
FAHY [25]	BDP	336	4	6
TURNER [26]	BDP	336	4	75
BACCI [27]	Bud	400	3 months	91
VAN RENSEN [28]	FP	1000	4	85
KEATINGS [29]	PRED	30 mg	2	86
CLAMAN [30]	PRED	0.5 mg·kg ⁻¹	6 days	87

Bud: budesonide; Cicl: ciclosporin; BDP: beclomethasone dipropionate; FP: fluticasone propionate; PRED: prednisone.

required from five to 21, for the same level of power, desired effect size and study design [18].

A more difficult element is how to determine the desired effect size. A first possibility is to aim at normalisation of sputum eosinophil counts. Increasing evidence based on studies in healthy volunteers indicates that a normal range of sputum eosinophils in adults and children is <2.5% [19–21]. However, any form of treatment rarely achieves full normalisation of all outcome measures in asthma. To date, it is uncertain whether reducing eosinophil counts, in addition to treating symptoms or lung function characteristics, improves the long-term clinical outcome of the disease. As a consequence, it is equally unclear what constitutes a "least clinically important difference" (LCID) as opposed to a statistically significant change in sputum eosinophil count. What has been shown is that an allergen inhalation challenge that causes a late asthmatic response is associated with an approximately five-fold increase in sputum eosinophil numbers [18]. Conversely, treatment with steroids reduces sputum eosinophil counts. In the various studies reported to date, oral or inhaled steroids were given at different doses for varying time periods. It is therefore difficult to compare these various studies, but, overall, it would seem that inhaled steroids from low doses onwards, offer a $\geq 60\%$ reduction in median sputum eosinophil percentage (table 2). These data indicate that sputum eosinophilia changes substantially in response to intervention. It could therefore be proposed that the LCID should be a $\geq 50\%$ change in sputum eosinophil count.

Of interest is that, from the limited amount of data available, it would seem that, as for clinical outcome measures such as symptom score or peak flow, the dose/response curve for the effect of inhaled steroids on sputum eosinophil counts is rather flat [8]. Importantly, however, the change in eosinophil count does not always correlate with the degree of clinical improvement [24, 25, 28, 31], thus further suggesting that measurement of sputum eosinophil number could offer complementary information to mere clinical follow-up.

Key points

1) Analysis of induced sputum has only been validated for the percentage of eosinophils in the cell pellet; 2) the interindividual variability in sputum eosinophilia is large even in subjects with similar clinical characteristics; and 3) the least clinically important difference in sputum eosinophil counts remains to be established.

Outstanding questions

1) The least clinically important difference in sputum eosinophil counts remains to be established; and 2) the prognostic significance of reductions in sputum eosinophil count on the long-term clinical outcome in asthma needs to be investigated.

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