

Safety of sputum induction with isotonic saline in adults with acute severe asthma

P. A. B. WARK, J. L. SIMPSON, M. J. HENSLEY* and P. G. GIBSON

Airways Research Centre, John Hunter Hospital, and *Faculty of Medicine, University of Newcastle, Newcastle, New South Wales, Australia

Summary

Background Sputum induction is a safe and effective technique to study airway inflammation in stable asthma. However, it has the potential to induce bronchospasm and the safety and efficacy of the technique in acute asthma has not been determined.

Objective The objective of this study was to evaluate the safety and efficacy of a protocol to induce sputum using isotonic saline in adults with acute exacerbations of asthma.

Methods Adults ($n = 47$) presenting to the emergency room with acute asthma and an $FEV_1 > 1.0$ L underwent supervised sputum induction with 0.9% saline delivered by an ultrasonic nebuliser. Induction was ceased if there was a fall of 20% or greater from baseline FEV_1 .

Results Subjects had moderate to severe exacerbations of acute asthma. An adequate sputum sample was obtained in 87% of subjects. Four subjects ceased induction because of symptom distress. There was a fall $\geq 20\%$ in 28% of subjects. Bronchoconstriction was successfully reversed by salbutamol in all subjects. Predictors of significant bronchoconstriction were older age, use of ingested corticosteroids, and a requirement for high-dose nebulized salbutamol for the exacerbation. Maintenance long-acting β_2 -agonist therapy protected against bronchoconstriction during sputum induction.

Conclusions Sputum induction in acute asthma using isotonic saline is highly efficacious in obtaining an adequate sputum sample. There is the potential for significant bronchoconstriction to occur but this can be managed safely with minimal discomfort to subjects.

Keywords: induced sputum, acute asthma, safety

Clinical and Experimental Allergy, Vol. 31, pp. 1745–1753. Submitted 5 March 2001; revised 21 May 2001; accepted 20 June 2001.

Introduction

Sputum induction has emerged as a useful non-invasive technique to assess airway inflammation in subjects with asthma and has been shown to return reproducible data with regard to cellular and soluble markers of inflammation [1,2]. Since the method was first described in subjects with stable asthma [3] it has been established that the inhalation of hypertonic saline from an ultrasonic nebuliser results in an adequate sputum sample from at least 70% of subjects

[4,5]. While induction was less successful if normal saline was used, sputum cell counts were unaffected by the tonicity of saline [6]. The main safety concern with sputum induction has been the risk of bronchoconstriction, as hypertonic saline is a potent stimulus to airway narrowing in asthmatics. Pre-treatment with salbutamol has been used to alleviate this [6,7] but significant airflow obstruction can still occur in subjects with severe asthma [8].

It is important to evaluate airway inflammation in acute severe asthma, especially as the inflammatory cell pattern, cytokine profile [9] and treatment response may differ from stable asthma. Bronchoscopy remains contraindicated in acute severe asthma, and sputum induction with hypertonic

Correspondence: Peter Gibson, Department of Respiratory and Sleep Medicine, John Hunter Hospital, Locked Bag 1, Hunter Region Mail Centre, NSW 2310, Australia. E-mail: mdpgg@mail.newcastle.edu.au

saline may also be contraindicated because of the risk of severe bronchoconstriction. Recently the use of isotonic saline to induce sputum in acute exacerbations of asthma has been reported [9–11]. The safety of this technique has not previously been evaluated in detail and is the subject of this report. In a study of the causes and characteristics of airway inflammation in acute severe asthma [12] additional information was collected to systematically assess the safety of sputum induction using isotonic saline in acute asthma. In this paper we aim to evaluate the safety and efficacy of sputum induction in adults with acute exacerbations of asthma, using an inhalation protocol with nebulised 0.9% saline after pre-treatment with inhaled salbutamol.

Methods

Design

Subjects who presented spontaneously to the emergency room with an acute exacerbation of asthma were recruited. After triage, medical assessment and initial bronchodilator treatment, a clinical history was taken defining medications used for asthma when stable and since symptoms deteriorated, and the time course of the exacerbation. Spirometry was performed (Vitalograph Ltd, Buckingham, UK) and subjects were pre-treated with salbutamol and sputum induced using 0.9% saline. Background asthma severity was assessed using the Aas score, which is based on clinical symptoms, frequency and severity of exacerbations and medication use [13]. Subjects returned 4 weeks after presentation when they had clinically returned to baseline; they were then assessed with allergy skin test and bronchial provocation challenge using hypertonic saline. The study was approved by the Hunter Area Research Ethics Committee and all subjects gave written informed consent.

Subjects

Adults ($n = 47$), aged 16–81 years, who presented to the emergency room of John Hunter Hospital with an acute exacerbation of asthma, who were unable to produce spontaneous sputum, and who had no contraindication to sputum induction, were included. Asthma was defined based upon a previous physician diagnosis and ATS criteria of a compatible history, together with either bronchial hyper-responsiveness (BHR; $PD_{20 \text{ saline}} < 15 \text{ mL}$) or an improvement in FEV_1 of $> 15\%$ after salbutamol 200 μg . An acute exacerbation of asthma was defined as: a deterioration in symptoms of wheeze, breathlessness or cough; an increase in use of short-acting β_2 -agonist for relief of symptoms; or a deterioration in self-monitored peak expiratory flow (PEF) leading to presentation to the emergency room for treatment

of acute asthma. Subjects were excluded if they did not fulfill the inclusion criteria, if they were on daily maintenance oral corticosteroids for asthma, if they had a coexisting physician diagnosis of chronic obstructive pulmonary disease ($n = 9$), if they had an infiltrate on chest radiograph suggesting pneumonia ($n = 1$), if their FEV_1 was less than 1 L after salbutamol 200 μg ($n = 33$), or if they had another contraindication to sputum induction in acute asthma (pneumothorax $n = 1$, pregnancy $n = 3$).

Sputum induction

Sputum induction was performed by a medical practitioner in the emergency department and after pre-treatment with salbutamol 200 μg delivered via a Volumatic spacer (Allen and Hanbury's, Melbourne, Victoria, Australia). All subjects received supplemental oxygen at 2 L/min via nasal prongs during sputum induction, and inhaled sterile normal saline (0.9%) from a DeVilbiss 2000 ultrasonic nebuliser (Oregon, Pike, PA, USA) with 23 cm corrugated tubing and a Hans Rudolph 2700, 2-way non-rebreathing valve box (Hans Rudolph Inc, Kansas City, KS, USA) with a rubber mouthpiece. The nebuliser output was 1.8 mL/min and particle size (MMAD) $< 5 \mu\text{m}$. Saline was nebulised for 30 s, 1 min and then for 2×2 -min periods. Spirometry was performed before and after salbutamol and then 1 min after each nebulisation period. Sputum induction was ceased when an adequate sample was obtained after nebulisation, if FEV_1 fell below 20% of pre-induction baseline, or at the subject's request. Salbutamol 200 μg was given following the induction if there had been a fall in FEV_1 of at least 15% from baseline or if the subject requested it for symptoms. Salbutamol 200 μg was then repeated at 5-min intervals until the FEV_1 had returned to within 10% of baseline. Sputum was processed as described [10,12]. Nebuliser output was measured by weighing the nebuliser cup and tubing before and after induction. At the time of collection a sample was judged to be adequate if there was estimated to be at least 400 μL of lower airway secretions present. In the laboratory the sample was judged adequate upon the presence of intact, classifiable cells on a cytospin slide (countable cytospin). The protocol for sputum induction is summarized in Fig. 1.

Statistical methods

Statistical analysis was carried out using STATA (STATA Corporation, College Station, TX, USA). Most variables were not normally distributed and values have been expressed as medians and the interquartile range. Differences between groups have been analysed using Wilcoxon's rank sum test. Differences in proportions between groups were analysed by Fisher's exact test or chi-square. Univariate relationships between continuous variables were analysed by Spearman's

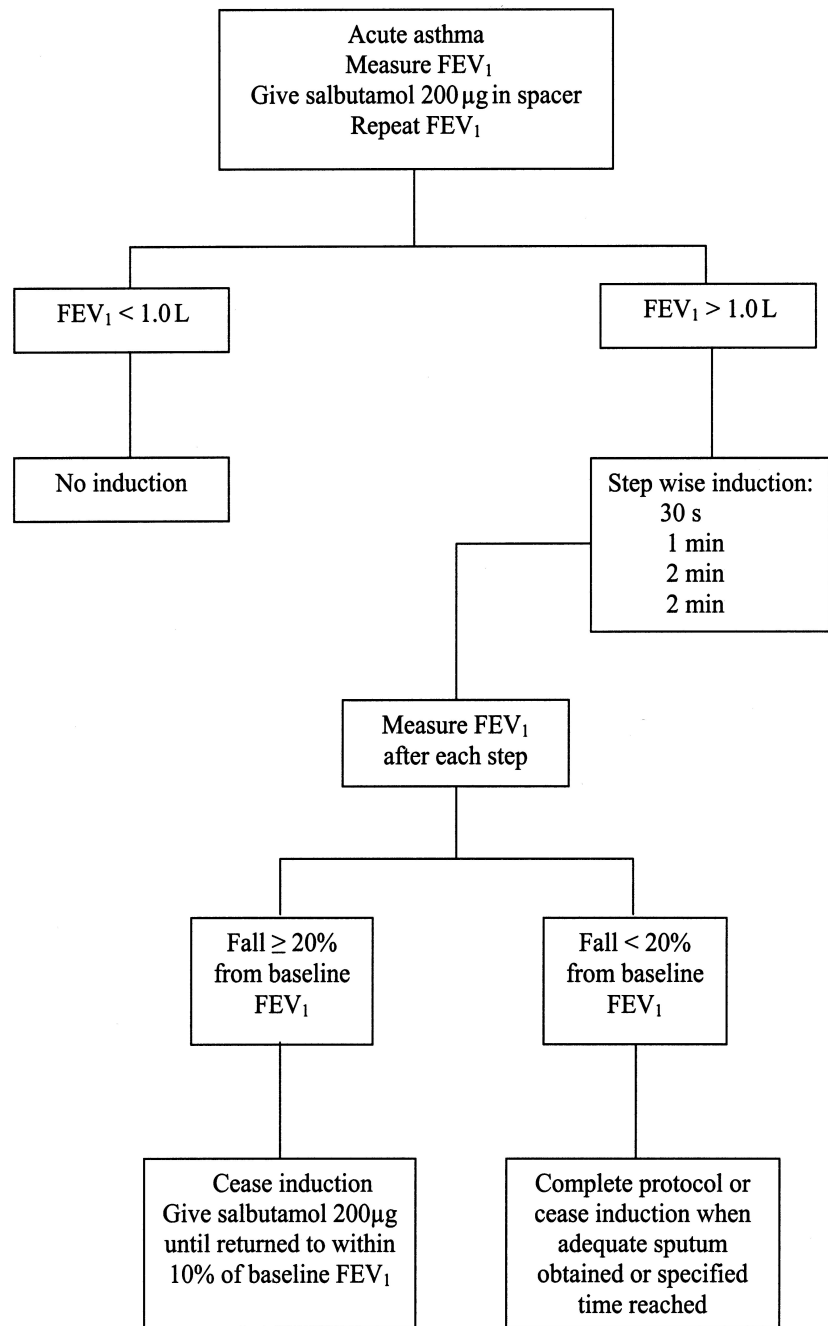


Fig. 1. Protocol for sputum induction in acute asthma using nebulised isotonic saline.

rank correlation coefficient, r . Multivariate analysis of variables was carried out after log transformation. Significance was accepted when P was < 0.05 .

Results

Sputum induction

The 47 subjects (14 male) had moderate airway obstruction

at presentation with a median (IQR) baseline FEV₁ after salbutamol of 57.6% (31.3–97.3) predicted (the FEV₁ measured after salbutamol 200 µL is referred to as the baseline FEV₁). Most subjects (31, 66%) required admission to hospital for management of the exacerbation. The background asthma severity gave an Aas score of 4 (1–5). At recovery 4 weeks later, FEV₁ improved to 74.6% (42.3–110.1) predicted. An adequate sputum sample was obtained from 87% ($n = 41$) of subjects (Fig. 1). In most subjects

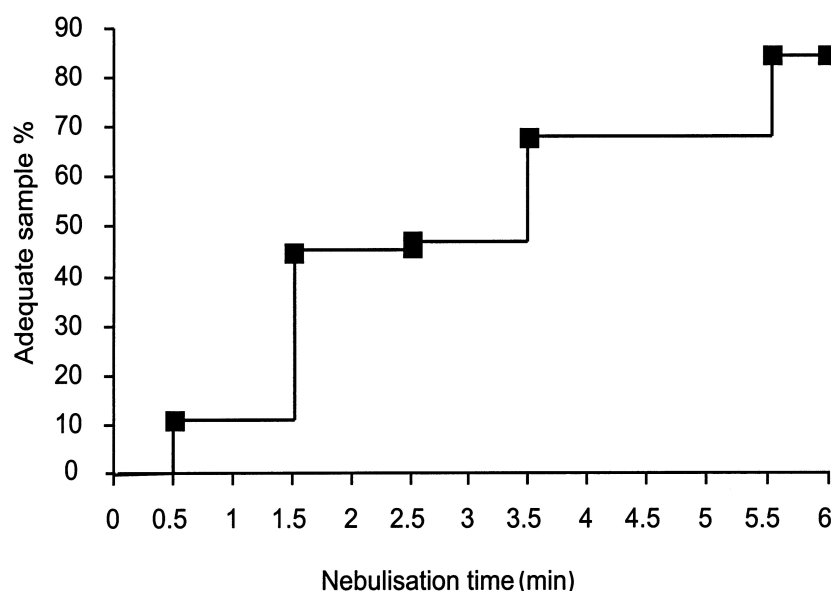


Fig. 2. Nebulisation time required to induce sputum with 0.9% saline in acute asthma.

(68%) an adequate sample was obtained after 3.5 min nebulisation time, corresponding to a dose of 6.3 mL (Fig. 2).

The median fall from baseline FEV₁ during sputum induction was 9% and 62% of subjects required rescue β_2 -agonist during induction (Table 1). Most subjects had adequate reversal of bronchoconstriction with 200 μ g salbutamol, with post-induction FEV₁ improving to 99% of baseline values. All subjects had returned to within 10% of their baseline FEV₁ within 15 min of completing the induction. Nineteen subjects had achieved 90% of their baseline FEV₁ by 5 min after one dose of salbutamol 200 μ g, eight took 10 min and two doses of salbutamol 200 μ g, while two subjects took 15 min and three doses of

salbutamol 200 μ g. The induction was ceased because of significant airway obstruction (fall in FEV₁ > 20% baseline) in 13 (28%) subjects. Four subjects (9%) requested that the induction cease because of symptomatic distress, three due to coughing and one due to breathlessness. The sputum sample was inadequate in two of these subjects. Only one subject was unable to produce adequate sputum after completing the full protocol.

Characteristics of subjects who fell $\geq 20\%$ with sputum induction

A significant fall in FEV₁ of $\geq 20\%$ from baseline occurred following induction in 13 subjects (Fig. 3) and these subjects were compared with those who fell less than 20% (Table 2). Both groups had similar lung function before and after the induction and similar asthma severity. Those who fell by 20% or more were more likely to have commenced oral prednisone prior to presentation and to have used a higher dose of nebulized salbutamol in the previous 12 h (Fig. 4). Subjects using a long-acting β_2 -agonist were less likely to have a significant fall in FEV₁ (Fig. 4).

Predictors for a fall in FEV₁ with sputum induction

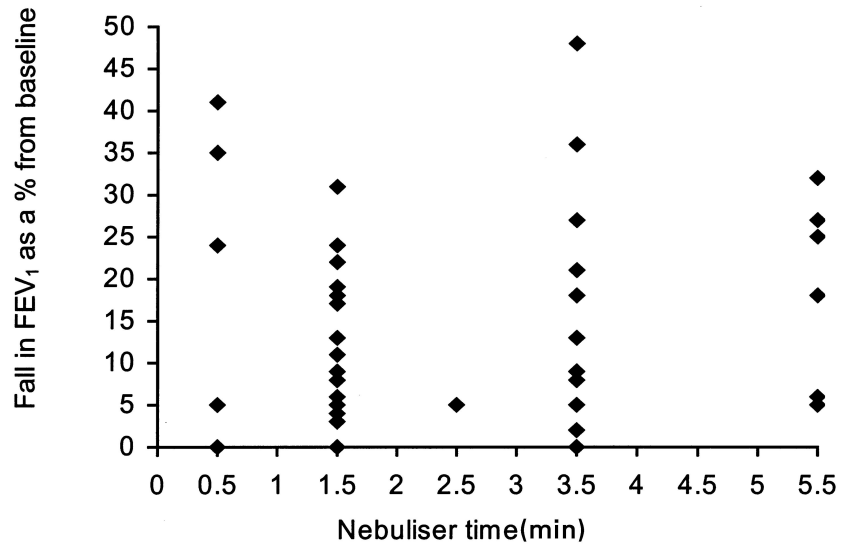
There was no relationship between the maximum fall in FEV₁ during sputum induction and the baseline FEV₁ prior to induction ($r = -0.14$, $P = 0.3$) or the degree of airway hyper-responsiveness measured as PD₂₀ saline at recovery, 4 weeks post-exacerbation ($r = -0.1$, $P = 0.7$). Similarly, there was no relationship between the maximum fall in FEV₁ and the time of nebulization ($r = 0.1$, $P = 0.8$) or

Table 1. Characteristics of sputum induction in acute asthma, median (interquartile range)

Nebuliser time (min)	1.5 (0.5–5.5)
Total nebuliser dose (mL)	2.3 (0.8–8.2)
Adequate sputum obtained, <i>n</i> (%)	41 (87%)
Baseline FEV ₁ (L)*	1.9 (1.1–3.0)
Maximum fall in FEV ₁ , % baseline	9 (2–35)
Rescue β_2 required, <i>n</i> (%)	29 (62%)
Post-induction FEV ₁ as percentage baseline FEV ₁	99.1 (92–100)
Adverse effects leading to cessation of induction, <i>n</i> (%)	
Fall FEV ₁ $\geq 20\%$	13 (28%)
Patient discomfort	4 (9%)

*Recorded after inhaled salbutamol 200 μ g.

Fig. 3. Distribution of fall in FEV₁ by nebulised saline time. This graph demonstrates the time when the maximum fall in FEV₁ occurred in individuals during sputum induction. Falls in FEV₁ occurred at all time-points and were not more common with longer nebulisation time.



the dose of saline delivered ($r = 0.02$, $P = 0.9$). There was, however, a relationship between the fall in FEV₁ and the amount of nebulized salbutamol used in the 12 h prior to presentation to the emergency room ($r = 0.54$, $P = 0.01$). There were weaker correlations with the number of puffs of salbutamol used in the 12 h prior to presentation, and a greater fall in FEV₁ ($r = 0.33$, $P = 0.06$), and a higher dose of prednisone used being associated with less of a fall in FEV₁ during the induction ($r = -0.41$, $P = 0.07$).

A multivariate linear regression model was used to determine which variables were independently associated with a fall in FEV₁, after adjustment for the dose of saline

delivered during sputum induction (fall in FEV₁/mL saline delivered). The model with the best fit explained 95.2% of the variation in the magnitude of the fall in the adjusted FEV₁ during sputum induction (Table 3). In this model increased age (coefficient 0.03, $P = 0.03$) was associated with a greater fall in FEV₁ during induction, while the use of long acting β_2 -agonists (-0.95 , $P = 0.04$) was associated with a significantly lesser fall in FEV₁ during induction.

Discussion

This study establishes the efficacy and safety of a protocol

Table 2. Characteristics of subjects whose FEV₁ fell $\geq 20\%$ with sputum induction, median (interquartile ranges)

	< 20% fall in FEV ₁	$\geq 20\%$ fall in FEV ₁	Analysis
<i>n</i>	34	13	NA
FEV ₁ at baseline* (L)	1.9 (1.2–2.8)	1.7 (1.3–2.3)	$P = 0.3$
FEV ₁ % predicted* at baseline	64.2 (35.3)	56.3 (42.3–69.7)	$P = 0.2$
Nebuliser time (min)	1.5 (1.5–5.5)	3.5 (1.5–3.5)	$P = 0.7$
Total nebuliser dose (mL)	2.2 (2.19–7.7)	5.2 (1.9–5.8)	$P = 0.9$
Post-induction FEV ₁ , % baseline	99.1 (94.7–100)	98.9 (95.8–100)	$P = 0.9$
Time for FEV ₁ to return to 10% of baseline FEV ₁ (min)	0 (0–5)	10 (5–10)	$P < 0.001$
Asthma severity score†	4 (1–5)	4 (4–5)	$P = 0.4$
Dose of nebulized salbutamol used (mg)‡	30 (30–35)	60 (40–60)	$P = 0.01$
Inhaled salbutamol, puffs§	12 (8–24)	18 (16–20)	$P = 0.2$
Inhaled corticosteroids BDP/μg day¶	800 (750–2000)	500 (500–1500)	$P = 0.4$

*Recorded after inhaled salbutamol 200 μg. †The amount of inhaled salbutamol (100 μg per metered dose puff) measured in puffs in the 12 h prior to presentation with acute asthma. ‡Dose of inhaled corticosteroids used measured in beclomethasone μg used per day. This is where 1 μg of beclomethasone = 1 μg of budesonide and 0.5 μg fluticasone. §Aas severity score. ¶Dose of nebulized salbutamol used in the 12 h prior to emergency room presentation (mg).

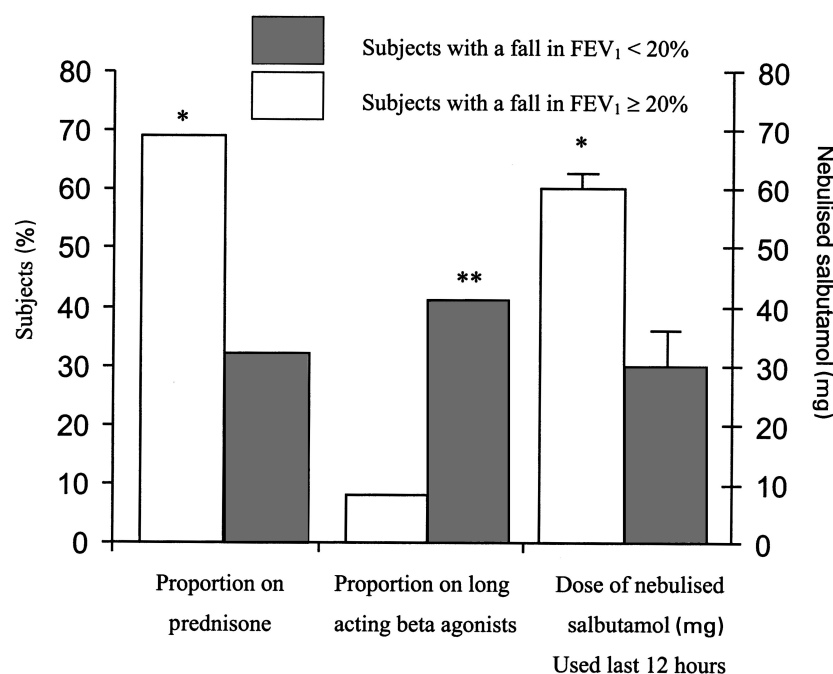


Fig. 4. Use of medication in subjects, comparing those whose FEV₁ fell 20% or more with those whose FEV₁ did not. Salbutamol dose is expressed as the median dose, mg used in the 12 h prior to presentation; the 75th quartile is represented by the error bar.

*Significantly higher than subjects who did not fall. **Significantly higher proportion than those who did have a fall in FEV₁.

to induce sputum using isotonic saline in adults with acute exacerbations of asthma. We found that we were able to obtain an adequate sputum sample in 87% of subjects and that there were no serious complications. Despite β_2 -agonist pre-treatment, and despite the use of isotonic saline, 28% of subjects had a significant fall in FEV₁. However, all subjects could be rescued with salbutamol and had returned to within 10% of their baseline FEV₁ by 15 min after induction. The predictors of a fall in FEV₁ during sputum induction in acute asthma were an older age, an exacerbation requiring the use of prednisone and the use of high-dose salbutamol for the exacerbation. The prior chronic use

of long-acting β_2 -agonists seemed to protect against a fall in FEV₁ during sputum induction.

The recent widespread use of sputum induction to assess airway inflammation in asthma and more recently COPD has occurred because it is relatively well tolerated, easy to perform, safe and provides valid and reproducible results [2,6]. The success of the technique relies upon the subject producing a technically adequate sputum sample, which is possible to obtain in more than 75% of subjects from as young as 8 years of age [3–5]. These results have been obtained using an ultrasonic nebuliser with concentrations of saline of 3% or greater.

Table 3. Multivariate regression model to predict the maximum fall in FEV₁ during sputum induction

Maximum fall in FEV ₁ as percentage from baseline/mL saline	Coefficient	Standard error	P > (t)
Log nebulized salbutamol dose (1 mg)	0.32	0.18	0.2
Log prednisone dose (1 mg)	1.33	0.35	0.06
Long-acting β_2 -agonists or theophylline used	− 0.95	0.19	0.02
Admitted to hospital	0.30	0.26	0.4
Length of stay in hospital (days)	− 0.06	0.04	0.3
FEV ₁ % predicted, prior to salbutamol	− 0.01	0.002	0.3
Aas severity score	− 0.15	0.07	0.2
Age (yr)	0.03	0.01	0.03
Sex (male)	0.03	0.19	0.9
Constant	1.63	0.34	0.01

Prob > F, $P = 0.04$. Adjusted R-squared = 0.9524.

Popov *et al.* reported that using isotonic saline was better tolerated than hypertonic saline [2,6]. Although the sputum quality was no different to hypertonic saline [2,6], an adequate sputum was obtained less often, in only 36% of inductions compared with 79% with 5% hypertonic saline [6]. In view of the unstable nature of subjects with acute asthma, we considered that the risks from hypertonic saline might be excessive. We therefore evaluated the safety and success of induction using isotonic saline for sputum induction in acute severe asthma. We found that isotonic saline gave a good success rate in acute asthma and that only a brief period of nebulisation was required. Normal saline was nebulised for a median period of 1.5 min and at a median dose of 2.3 mL of saline delivered. This success rate is likely to be due to the fact that subjects with acute asthma have more intense airway inflammation and consequently more sputum production. It may be argued that an adequate sputum specimen could be obtained by spontaneous expectoration alone. Our previous experience is that spontaneous sputum alone in acute asthma will yield an adequate sample in only 50% of subjects [9] and that the viability and quality of samples is poorer [14]. Of the samples collected, 87% had readable cytopins and the proportion of the total cell count that was viable was a median 65.2% (interquartile range 20.3–91.9). These results are comparable with inductions obtained using hypertonic saline [6] and are superior to spontaneous sputum alone [14]. Within the time-frame that inductions were carried out there was no relationship between longer nebuliser time and poorer cell viability ($r = -0.28$). This is an important observation as it demonstrates that even though investigators may be concerned by bronchoconstriction from sputum induction in acute asthma, relatively less time is needed to induce a technically adequate sample and therefore samples can be obtained before excessive bronchoconstriction occurs, with better quality samples than would be obtained by spontaneous expectoration alone.

The other attraction of using induced sputum to investigate airway inflammation is its tolerability and safety profile. The main concern with hypertonic saline is that it can induce bronchoconstriction. Initial studies were performed in mild stable asthmatics and found only small falls in FEV₁ (1.3–5.3%) with induction [3,15]. Later Wong *et al.* [4] studied a more heterogeneous population of asthmatics with an FEV₁ 26–123% of predicted. They had a mean fall in FEV₁ of 7.4% but one subject fell by 47% from baseline.

De la Fuente *et al.* [8] specifically assessed the safety of sputum induction in subjects with severe asthma, using salbutamol pre-treatment and hypertonic saline in increasing concentrations as tolerated from 3% to 5%. They assessed safety firstly in eight subjects with stable but

severe airflow limitation and an FEV₁ < 1.0 L and found that seven were unable to tolerate their protocol, two because of severe bronchoconstriction and five because of severe cough and breathlessness. For this reason we elected not to perform inductions on subjects whose FEV₁ did not increase above 1.0 L after salbutamol pre-treatment. De la Fuente *et al.* then studied 43 subjects with severe but stable asthma, Aas scores between 4 and 5 and a baseline FEV₁ of 60–80% predicted. This group had a mean fall in FEV₁ of 6% from baseline, with none falling below 20%, but those with more severe obstruction had a greater fall in FEV₁.

Only one other paper has looked at the safety of sputum induction in acute asthma [11]. This was in 10 subjects with exacerbations of asthma who were managed as outpatients and had serial inductions during their exacerbation. If spontaneous sputum could not be obtained then sputum induction was performed initially with isotonic saline and then increasing concentrations of hypertonic saline. They recorded a fall in FEV₁ of greater than 10% on 10 occasions.

We have extended these data by adapting sputum induction to subjects with acute severe asthma who presented to the emergency room, with the majority requiring inpatient management. Subjects were pre-treated with salbutamol, and incremental doses of 0.9% saline were used. In an earlier study of children with acute asthma, there were no serious side-effects and the median fall in FEV₁ was 5–8% [9,10]. In the setting of an acute asthma exacerbation our protocol was well tolerated with only four subjects halting the induction due to symptoms of cough or breathlessness. Although a fall of 20% or greater in FEV₁ from baseline occurred in 28% of subjects, there was prompt recovery after rescue salbutamol. Nebuliser output may also influence safety and we used an ultrasonic nebuliser with an output of 1.8 mL/min. Good induction results have been achieved using lower output nebulisers (0.9 mL/min) [16] and this could be considered as an alternative to minimize bronchoconstriction in this setting.

It must be stressed that we used a number of additional procedures to ensure the safety of subjects during sputum induction in acute asthma. In addition to β_2 -agonist pre-treatment and pre-specified criteria for stopping the induction and reversing the effects with β_2 -agonist, the inductions were performed in the emergency department by an experienced physician and with subjects receiving supplemental oxygen. This ensured careful supervision and prompt access to resuscitation facilities if required. Without these precautions patient safety may be compromised. Furthermore, the dose of saline was given in doubling time periods, starting at 30 s, and never longer than 2 min. This is a significant difference to induction protocols in stable asthma where the initial dose may be nebulised over 4 or 5 min.

Given the significant bronchoconstriction that occurs with even small doses of saline in acute asthma (Fig. 2), we believe the incremental dose protocol to be preferable for use with sputum induction in acute asthma. A potential limitation of this approach is that it may alter sputum cell counts. The cellular and mediator profile of induced sputum can change with increasing doses of hypertonic saline [17,18]. Using hypertonic saline 3%, the proportion of sputum neutrophils is significantly lower and the proportion of macrophages significantly higher after 8 min of nebulisation compared with 4 min, while sputum eosinophils also fall by 20 min of nebulisation compared with 4 min [18]. These changes are important then to keep in mind when making comparisons between samples. A stepwise approach to nebulisation remains preferable for safety reasons but nebuliser time for the collection of samples will need to be kept consistent in order to make comparisons.

We sought to determine what factors might be associated with an increased risk of bronchoconstriction during sputum induction in acute asthma. Subjects who developed more severe airflow obstruction had used more nebulised salbutamol and were more likely to have commenced oral prednisone for the exacerbation. These may be markers of an exacerbation that is either more severe or of longer duration, suggesting that under these circumstances caution should be exercised during sputum induction. In the multivariate analysis the dose of nebulised salbutamol or prednisone did not appear to have an independent effect, presumably those who commenced prednisone and are using higher doses of nebulised salbutamol have more severe disease. We also found that chronic use of a long-acting β_2 -agonist appeared to protect against significant airflow obstruction during sputum induction in acute asthma, despite all subjects receiving pre-treatment with salbutamol. The mechanism of this interesting and unexpected effect is not clear. It is known that long-acting β_2 -agonists reduce exacerbations [19] and may reduce levels of IL-8 [20]. Since IL-8 is a key mediator in acute severe asthma [9] that promotes neutrophil recruitment and vascular leakage, use of a long-acting β_2 -agonist may protect against these events.

In conclusion, we have demonstrated that a sputum induction protocol conducted under direct medical supervision, with salbutamol pre-treatment and using graded inhalation of 0.9% saline is both safe and efficacious in obtaining an adequate sputum sample in adults with acute asthma. Significant falls in lung function can still occur but are readily reversed by β_2 -agonist therapy. When conducted by experienced personnel, who are able to administer bronchodilators appropriately, sputum induction can be carried out safely with minimal discomfort to subjects in the setting of acute asthma. The technique may be of

importance in assessing causes, pathogenesis and treatment responses of airway inflammation in acute asthma.

Acknowledgements

Funded by Asthma NSW, National Health and Medical Research Council (Australia) and the John Hunter Hospital Charitable Trust.

References

- 1 in-'t-Veen JCHW, de-Gouw HW, Smits HH et al. Repeatability of cellular and soluble markers of inflammation in induced sputum from patients with asthma. *Eur Respir J* 1996; 9:2441–7.
- 2 Pizzichini E, Pizzichini MMM, Efthimiadis A et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid phase measurements. *Am J Respir Crit Care Med* 1996; 154:308–17.
- 3 Pin I, Gibson PG, Kolendowicz R et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax* 1992; 47:25–9.
- 4 Fahy JV, Liu J, Wong H, Boushey HA. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. *J Allergy Clin Immunol* 1994; 93:1031–9.
- 5 Gibson PG, Wlodarczyk J, Hensley MJ et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998; 158:136–45.
- 6 Popov TA, Pizzichini MMM, Pizzichini E et al. Some technical factors influencing the induction of sputum for cell analysis. *Eur Respir J* 1995; 8:559–65.
- 7 Wong HH, Fahy JV. Safety of one method of sputum induction in asthmatic subjects. *Am J Respir Crit Care Med* 1997; 156:299–303.
- 8 de la Fuente PT, Romagnoli M, Godard P, Bousquet J, Chanez P. Safety of inducing sputum in patients with asthma of varying severity. *Am J Respir Crit Care Med* 1998; 157:1127–30.
- 9 Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. *Am J Respir Crit Care Med* 2000; 161:769–74.
- 10 Twaddell SH, Gibson PG, Carty K, Wolley KL, Henry RL. Assessment of airway inflammation in children with acute asthma using induced sputum. *Eur Respir J* 1996; 9:2104–8.
- 11 Pizzichini MMM, Pizzichini E, Clelland L et al. Sputum in severe exacerbations of asthma. *Am J Respir Crit Care Med* 1997; 155:1501–8.
- 12 Wark PAB, Johnston SL, Moric I, Hensley MJ, Gibson PG. Viral infection induces IL-8, eosinophil activation and neutrophil influx in acute severe asthma. *Am J Respir Crit Care Med* 2000; 161:A605.
- 13 Aas K. Heterogeneity of bronchial asthma. *Allergy* 1981; 36:3–14.
- 14 Pizzichini MMM, Popov T, Efthimiadis A et al. Spontaneous

- and induced sputum to measures indices of airway inflammation. *Am J Respir Crit Care Med* 1996; 154:866–9.
- 15 Pin I, Freitag AP, Girgis-Gabardo A et al. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. *Am J Respir Crit Care Med* 1992; 145:1265–9.
- 16 Hunter CJ, Ward R, Woltmann G, Wardlaw AJ, Pavord ID. The safety and success rate of sputum induction using a low output ultrasonic nebuliser. *Respir Med* 1999; 93:345–8.
- 17 Holz O, Jorres RA, Koschyk S et al. Changes in sputum composition during sputum induction in healthy and asthmatic subjects. *Clin Exp Allergy* 1998; 28:284–92.
- 18 Gerschman NH, Liu H, Wong HH, Liu JT, Fahy JV. Fractional analysis of sequential induced sputum samples during sputum induction: evidence that different lung compartments are sampled at different time points. *J Allergy Clin Immunol* 1999; 104:322–8.
- 19 Pauwels RA, Lofdahl CG, Postma DS et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *N Engl J Med* 1997; 337:1405–11.
- 20 Li X, Ward C, Thien F et al. An antiinflammatory effect of salmeterol, a long-acting beta (2) agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. *Am J Respir Crit Care Med* 1999; 160:1493–9.