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REPORT OF WORKING GROUP 2

Safety of sputum induction

Leader of the Working Group: E. Pizzichini*

Members of the Working Group: M.M.M. Pizzichini*, R. Leigh#, R. Djukanović¶, P.J. Sterk+

One of the most important recent advances in the investigation of airway inflammation has been the introduction of sputum induction by inhalation of an aerosol of hypertonic saline, by PIN *et al.* [1] in 1992, to directly obtain airway secretions in asthma. This method has a number of advantages over invasive methods. Safety and practicality are the most obvious. The method of obtaining induced sputum is relatively noninvasive and can be carried out at random [2] and repeatedly in subjects with varying disease severity [3–13]. Therefore, it is not surprising that the examination of induced sputum has become the most clinically applicable method for the assessment of airway inflammation.

The induction procedure is simple and safe. The risks in patients with stable asthma or chronic obstructive pulmonary disease (COPD) with mild-to-moderate airflow limitation are acceptable [1, 3–5, 8, 12–14]. It can also be safe in patients with more severe airflow limitation [6, 10, 11] provided that the induction is performed with caution using a modified procedure [6]. The safety of sputum induction has been specifically addressed in several recent publications [3–5, 12, 14]. To date, there have been no reports of death or need for hospital admission in patients undergoing sputum induction for the assessment of airway inflammation; the airway constriction caused by sputum induction with hypertonic saline is quickly reversed by treatment with an inhaled short-acting β_2-agonist.

It is well known, however, that inhalation of hypertonic or even isotonic saline can cause airway constriction in asthmatic subjects and in COPD, particularly in those with associated airway hyperresponsiveness (AHR). In 1958, BICKERMAN *et al.* [15], using aerosols of saline generated by jet nebuliser with concentrations ranging 3.0–15%, observed that "it was necessary to limit the saline aerosol to 3.0% in some patients with bronchial asthma and pulmonary emphysema since the more hypertonic solution appeared to accentuate obstructive dyspnoea". Later, in 1981, SCHOEFFEL *et al.* [16] demonstrated progressive bronchoconstriction in response to inhalation of nonisotonic saline as the osmolarity was increased or decreased relative to that of normal saline. The airway constrictive response to hypertonic saline has been used as a measure of AHR [17]; however, it does not always correlate with AHR to methacholine [18].

The mechanism whereby inhalation of hypertonic saline causes airway constriction is unknown, but may involve activation of airway mast cells [19] or sensory nerve endings [20]. Aerosols of distilled water can also cause airway constriction which can be severe, as evidenced by the report of death in one asthmatic subject undergoing distilled water challenge [21]. Salbutamol is known to inhibit AHR to hypertonic saline aerosol [22], and pretreatment with an inhaled short-acting β_2-agonist may prevent airway constriction caused by sputum induction [15, 23]. However, it is also clear that pretreatment with a short-acting β_2-agonist does not completely prevent airway constriction in all subjects [3–6, 10, 13].

Difficulties in determining which factors might affect the safety of spututm induction are compounded by variations between studies in methods of induction and subject populations (tables 1 and 2). Therefore, there are several factors related to subjects or methodology which may interact and thus influence the safety of sputum induction. These need to be taken into account. Some of these factors have been reported previously, but their relevance still requires investigation. The factors that may affect safety of sputum induction include pretreatment with short-acting β_2-agonist [33], degree of airflow limitation present before induction [4], overuse of short-acting β_2-agonist [6, 32, 34], degree of asthma control, nebuliser output, concentration of inhaled saline, duration of saline inhalation, and frequency and timing of safety assessment by forced expiratory volume in one second (FEV_1) or peak expiratory flow (PEF) measurement (tables 3 and 4). These factors are addressed in the present article.

Pretreatment with salbutamol

Despite great variation between methods of sputum induction, for safety purposes these methods can be

*NUPAIVA (Asthma Research Centre), University Hospital, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil. #Firestone Institute for Respiratory Health, Hamilton, Ontario, Canada. ¶Southampton University General Hospital, Southampton, UK. +Leiden University Medical Centre, Leiden, the Netherlands.

Correspondence: E.Pizzichini, NUPAIVA (Asthma Research Centre), Hospital Universitário, Universidade Federal de Santa Catarina, 88040-390 Florianópolis, Santa Catarina, Brazil. Fax: 55 48 2347711. E-mail: pizzich@mcmaster.ca

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Table 1. – Methods of sputum induction for measurement of inflammatory indices in stable mild-to-moderate asthma or chronic obstructive pulmonary disease

First author [Ref.]	Ultrasonic nebuliser characteristics			Induction procedure				
	Nebuliser® (manufacturer)	Output mL·min^{-1}	Particle size μm	Bronchodilator[#] pretreatment μg	Saline inhalation			
					Concentration %	Duration of inhalation min		
						Each period	Total	Mean
PIN [1]	Fisoneb (Fisons, Pickering, Ontario, Canada)	0.6	5.9	200	3.0, 4.0, 5.0	5	30	30.0
GIBSON [2]	Timeter MP500 (Oregon Scientific, Pike, PA, USA)	2.5	2.6	None	4.5	0.5, 1, 2, 4, 8	⩽16	NA
FAHY [13]	DeVilbiss 65 (DeVilbiss Corp., Somerset, PA, USA)	2.4	4.5	200	3.0	20	20	20.0
IREDALE [18]	Mistogen (Mistogen Equipment Co., Lancaster, PA, USA)	1.2	1.9	None	0.9, 4.5	0.5, 1, 2, 4, 8, 16	⩽30	15.7
POPOV [23]	Fisoneb (Fisons)	0.87	5.6	200	3.0, 4.0, 5.0	7.0	21	21.0
BACCI [24]	Sirius (Technomed, Florence, Italy)	1.1	2.8	None	3.0, 4.0, 5.0	5.0	⩽30	10.0[+]
MAESTRELLI [25]	Mistogen EN 145 Electronic Nebulizer (Mistogen Equipment Co., Oakland, CA, USA)	1.2	1.9	200	3.0, 4.0	10	⩽20	NA
IN'T VEEN [26]	DeVilbiss UN 2000 (DeVilbiss Corp.)	2.5	4.5	400	4.5	5.0	⩽30	NA
KEATINGS [27]	DeVilbiss UN 99 (DeVilbiss Corp.)	6.2	4.5	200	3.0	5.0	⩽25	15
HOLZ [28]	OMRON NE-U06 (Omron Corp., Tokyo, Japan)	1.7	4.9	200	3.0, 4.0, 5.0	10	30	30.0
SPANEVELLO [29]	DeVilbiss 65 (DeVilbiss Corp.)	2.4	4.5	400	4.5	1, 2, 4, 8, 16	31	31.0
LOUIS [30]	DeVilbiss UN 99 (DeVilbiss Corp.)	3.0	4.5	200	4.5	5.0	⩽20	NA
BRIGHTLING [31]	Medix (Clement Clarke International Ltd, Harlow, UK)	0.9	5.5	200	3.0, 4.0, 5.0	5.0	15	15.0

NA: not available. [#]: salbutamol; [+]: median.

Table 2.–Methods of sputum induction for measurement of inflammatory indices in moderate-to-severe, exacerbated or stable, asthma or chronic obstructive pulmonary disease (COPD)

First author [Ref.]	Ultrasonic nebuliser characteristics			Induction procedure			
	Nebuliser (manufacturer)	Output mL·min^{-1}	Particle size µm	Bronchodilator# pretreatment µg	Saline inhalation		
					Concentration %	Duration of inhalation min	
						Each period	Total	Mean
Stable asthma								
GROOTENDORST [12]	DeVilbiss UN 2000 (DeVilbiss, Corp., Somerset, PA, USA)	2.5	4.5	200	4.5	5.0	⩽15	11.7
TEN BRINKE [32]	DeVilbiss UN 2000 (DeVilbiss, Corp.)	2.5	4.5	400	0.9, 3.0, 4.5	5.0	⩽17	NA
Exacerbated or stable asthma or COPD								
TWADDELL [8]	OMRON NE-U06 (Omron Corp., Tokyo, Japan)	2.5	4.5	If PEF fell >10%	4.5	0.5, 1, 2, 4, 8	⩽16	7.8
PIZZICHINI [6]	Fisoneb (Canadian Medical Products Ltd., Makham, Ontario, Canada)	0.87	5.6	200	0.9, 3.0, 4.0, 5.0	1, 2, 4, 7	⩽21	8.4
DE LA FUENTE [4]	DP-100 (Syst'Am, Paris, France)	2.4	4.5	200	3.0, 4.0, 5.0	5.0	⩽30	NA

NA: not available; PEF: peak expiratory flow. #: salbutamol.

broadly divided into two types: induction with or without pretreatment with a short-acting β_2-agonist. In the first method, the patient is pretreated with an inhaled short-acting β_2-agonist to inhibit airway constriction and an aerosol generated by an ultrasonic nebuliser is then inhaled. The concentration of saline and duration of inhalation that have been used in studies published to date are shown in tables 1 and 2. In the second method, hypertonic saline is given without pretreatment and inhaled for increasing periods ranging 30 s–16 min. This method also permits measurement of airway responsiveness to hypertonic saline at the same time. In both methods, before and after each period of inhalation, or whenever the subject becomes symptomatic, FEV1 (or PEF) is measured and any fall in FEV1 (or PEF) is recorded. If the FEV1 (or PEF) falls by $\geqslant$20%, inhalation should be discontinued (tables 3 and 4). Although useful in terms of providing concomitant information about AHR, the latter approach is more complicated than that of pretreatment with a short-acting β_2-agonist. In addition, if the subject has severe AHR and develops bronchospasm before sufficient sputum has been collected, salbutamol rescue medication may be required in order to allow additional hypertonic saline to be administered. No studies have been conducted to assess when it is safe to recommence the induction. Usually, if the value is within 5% of baseline after waiting 15 min, it is considered safe to continue saline inhalation.

The dose of salbutamol used in pretreatment has not been standardised. Some authors have chosen to give 400 µg, arguing that breakthrough bronchospasm develops frequently with 200 µg, but this has not been formally examined to date. In a small study of 10 subjects with mild stable asthma, the effects of pretreatment with placebo or salbutamol 200 µg were investigated [23]. Saline concentrations of 3 followed by 4 and then 5% were each inhaled for 7 min. The mean fall in FEV1 recorded during induction was significantly greater after pretreatment with placebo (20.7%) than after salbutamol pretreatment (8.4 %) (table 3). The degree of protection against saline-induced bronchoconstriction that was achieved with salbutamol varied between subjects.

Tables 3 and 4 review studies in which the safety of sputum induction after pretreatment with salbutamol was examined by repeated measurement of FEV1. This review enabled calculation of the mean of the reported mean falls in FEV1 after the procedure, regardless of which method of induction was used. Thus, 692 sputum inductions performed in subjects with mild stable asthma caused a mean (95% confidence interval) fall in FEV1 of 5.7% (4.1–7.2%) [1, 3–5, 7, 13, 14], whereas, in 143 sputum inductions performed in subjects with moderate-to-severe but stable asthma, it was 5.6% (95% confidence interval not calculated) [4, 14]. In 94 sputum inductions in subjects with uncontrolled or exacerbated asthma, the fall was 7.2% (4.1–10.3%), regardless of the severity of airflow limitation before or after treatment with salbutamol [4, 6, 11, 34]. It is important to point out, however, that, when severe airflow limitation was present before commencing the procedure, the

Table 3. – Safety of sputum induction for measurement of inflammatory indices in mild-to-moderate stable asthma or chronic obstructive pulmonary disease (COPD)[#]

First author [Ref.]	Subject characteristics							Sputum induction					
	Subjects n	Sp ind n	Condition	Age yrs	IS n	FEV1 % pred		Method	Safety assessment			Fall %	
						Mean	Min	[Ref.]	Type	Interval min	Interruption	Mean	Max
With bronchodilator pretreatment[¶]													
PIN [1]	17	17	Asthma	27.3	16	88.9	NA	[1]	FEV1	5	fall $\geqslant$20%[+]	5.3	30.0
PIZZICHINI [7]	19	19	Asthma	43.2	13	74.9	57.0	[1][§]	FEV1	7	fall $\geqslant$20%[+]	5.7	22.7
	10	10	Smoker	33.0	0	104.0	99.0	[1][§]	FEV1	7	fall $\geqslant$20%[+]	4.2	7.2
WONG [3]	78	351	Asthma	31.5	NA	86.0	60.0	[14]	FEV1	20	[##]	5.9	69.0
DE LA FUENTE [4]	13	13	Asthma	29.0	4	95	90[ƒ]	[1][§]	FEV1	5	fall $\geqslant$10%[+,##]	2.0	4.0
HUNTER [5]	37	53	Asthma	45.6	17	82.6	NA	[1][§]	FEV1	5	fall $\geqslant$20%[+]	5.4	23.0
	29	29	misc.	50.2	2	90.9	NA	[1][§]	FEV1	5	fall $\geqslant$20%[+]	4.3	NA
FAHY [13]	18	18	Asthma	38.7	9	88.9	26.0	14	FEV1	20	[##]	7.4	47.0
VLACHOS-MAYER [14]	211	211	Asthma	44.8	119	89.9	69.0	[1][§]	FEV1	7	fall $\geqslant$20%[+]	5.4	32.0
	6	6	COPD	59.7	2	73.2	69.8	[1][§]	FEV1	7	fall $\geqslant$20%[+]	5.4	9.4
POPOV [33]	10	10	Asthma	40.0	5	90.0	68.0	[1][§]	FEV1	7	fall $\geqslant$20%[+]	8.4	NA
LOUIS [30]	15	15	Asthma	29.0	6	84.0	35.0	[27]	PEF	5	fall $\geqslant$45%	27.0	49
Without bronchodilator pretreatment													
IREDALE [18]	24	24	Asthma	NA	17	84.3	76.2	[21]	FEV1	1	fall $\geqslant$20%[+]	24.7	26.7
POPOV [33]	10	10	Asthma	40.0	5	90.0	68.0	[1][§]	FEV1	7	fall $\geqslant$20%[+]	20.7	73.0
BACCI [24]	21	21	Asthma	36.0	0	91.3	53.0	[1][§]	FEV1	5	fall $\geqslant$20%[+]	29.4	NA

Sp ind: sputum inductions; IS: inhaled steroids; FEV1: forced expiratory volume in one second; % pred: percentage of the predicted value; Min: minimum; Max: maximum; NA: not available; misc.: miscellaneous; PEF: peak expiratory flow. [#]: control subjects not included; [¶]: 180 or 200 μg salbutamol; [+]: or specimen obtained; [§]: modified; [ƒ]: 25th percentiled; [##]: or patient experienced discomfort.

Table 4. – Safety of sputum induction for measurement of inflammatory indices in moderate-to-severe stable or exacerbated asthma or chronic obstructive pulmonary disease (COPD)[#,¶]

First author [Ref.]	Subject characteristics							Sputum induction				
	Subject n	Sp ind n	Condition	Age yrs	IS n	FEV1 % pred		Method [Ref.]	Safety Assessment			Fall %	
						Mean	Min		Type	Interval min	Interruption	Mean	Max
Stable asthma or COPD													
DE LA FUENTE [4]	30	30	Asthma	55.0	30	71.0	55.0[+]	[1][§]	FEV1	5	fall $\geqslant$20%[f,##]	3.0	4.0
PIZZICHINI [10]	18	21	COPD	67.1	15	29.0	13.0	[5]	FEV1	1–2	fall $\geqslant$20[f,##]	16.5	29.7
GROOTENDORST [12]	20	20	Asthma	14.9	20	85.4	60.5	[12]	FEV1	20	fall $\geqslant$20[f,##]	NA	>20.0
VLACHOS-MAYER [14]	93	93	Asthma	55.8	93	43.9	18.4	[5]	FEV1	1–2	fall $\geqslant$20[f,##]	8.2	32.3
	19	19	COPD	63.9	14	35.2	20.5	[5]	FEV1	1–2	fall $\geqslant$20[f,##]	9.7	29.5
LOUIS [30]	22	22	Asthma	29.0	6	84.0	35.0	[27]	PEF	5	fall $\geqslant$20[f,##]	20.0	48.0
Exacerbated or uncontrolled asthma													
TWADDELL [8]	8	12	exac asthma	12.0	6	62.0	25.0	[4]	PEF	0.5, 1, 2, 4, 8	fall $\geqslant$20,[##]	NA	10.0
PIZZICHINI [6]	10	30	exac asthma	41.8	7	48.1	37.0	[1][§]	FEV1	1–2	fall $\geqslant$20[f,##]	8.2	42.0
DE LA FUENTE [4]	8	8	Mild asthma	48.0	4	95.0	90.0[+]	[1][§]	FEV1	5	fall $\geqslant$20[f,##]	4.0	8.0
	13	13	Severe asthma	52.0	13	61.0	50.0[+]	[1][§]	FEV1	5	fall $\geqslant$20[f,##]	6.0	10.0
Pizzichini [11]	8	12	PRED-dep asthma	54.3	8[++]	55.0	45.0	[5]	FEV1	1–2	fall $\geqslant$20[f,##]	7.0	19.5
Pizzichini [34]	31	31	Uncontrd asthma	30.8	None	73.9	19.0	[1] or [6][§]	FEV1	7 or 1–2	fall $\geqslant$20[f,##]	10.7	53.0

Sp ind: sputum induction; IS: inhaled steroids; FEV1: forced expiratory volume in one second; % pred: percentage of the predicted value; Min: minimum; Max: maximum; NA: not available; PEF: peak expiratory flow; exac: exacerbation; PRED-dep: prednisone dependent; Uncontrd: uncontrolled. [#]: control subjects not included; [¶]: all subjects pretreated with bronchodilator (180 or 200 μg salbutamol); [+]: 25th percentile; [§]: modified; [f]: or specimen obtained; [##]: or patient experienced discomfort; [++]: plus prednisone.

induction procedure was carried out more carefully and FEV1 (or PEF) was measured more frequently. This accounts for the safety of the procedure when performed in these subjects. Excessive airway constriction, as defined by a fall in FEV1 of >20%, has been reported in asthmatics [1, 3, 5, 6, 14, 34] and patients with COPD [10, 14]. It occurred in six studies in which almost 500 inductions were performed in mild stable [1, 3, 5] or mild-to-severe asthma [14, 34] and in severe exacerbations of asthma [6]. A mean (minimum to maximum) of 11.1% (5.8–32%) of asthmatics studied (table 5) showed a fall in FEV1 of >20%. Importantly, the highest fall reported in each study occurred in subjects with mild airflow limitation after bronchodilator administration (baseline FEV1 63–93% of the predicted value). Therefore, it should be emphasised that research subjects without any clear risk factors may occasionally develop sudden and severe bronchospasm during sputum induction even after pretreatment with β_2-agonist. This can happen quickly; in one study, a subject developed a >20% fall in FEV1 within 2 min of starting induction [6].

Predictive factors for induction-related bronchospasm

Predictors of excessive bronchoconstriction, *i.e.* the degree of baseline airflow limitation and the degree of AHR to methacholine or histamine, have so far been reported in two studies [4, 32]. However, two recent studies [5, 14] have failed to confirm their predictive value. Two other studies have reported a strong correlation between recent overuse of a short acting β_2-agonist and magnitude of fall in FEV1 after sputum induction [6, 34]. There is increasing evidence that continuous β_2-agonist use can result in a reduction in its bronchoprotective effect against a variety of both specific and nonspecific bronchoconstrictor stimuli [35, 36]. It has also been demonstrated that 200 or 400 μg salbutamol does not protect against excessive bronchoconstriction if exposure to a relatively strong stimulus occurs [37]. Whether excessive bronchoconstriction during sputum induction occurs due to loss of bronchoprotective effect resulting from overuse of β_2-agonists [36] or lack of protection against excessive airway constriction [37] after exposure to hypertonic saline needs to be formally investigated.

Monitoring pulmonary function during induction and duration of procedure

No standardised approach to the monitoring of pulmonary function during sputum induction has been adopted, although a protocol has been proposed [3]. The method used to measure pulmonary function varies; most investigators use FEV1 as a measure of safety (tables 2 and 3). The duration of monitoring interval ranges 1–>10 min. However, considering the potential danger of excessive bronchoconstriction caused by inhalation of hypertonic saline, it is important that all subjects be monitored closely and throughout the procedure for changes in symptoms during sputum induction and that airflow measurements be

Table 5. – Reported excessive bronchoconstriction[#] (EB) caused by sputum induction in asthmatic subjects[¶]

First author [Ref.]	Subjects n	Asthma chars	EB[#] n (%)	Induction procedure				Maximal fall in FEV1				
				Baseline	Safety assessment			FEV1 % pred		sal %	Time min	Fall %
				FEV1 % pred	Type	Interval min	Interruption	Pre-BD	Post-BD			
PIN [1]	17	Mild, stable	1 (5.8)	88.9	FEV1	5.0	fall $\geq$20%[+]	NA	NA	NA	NA	30
PIZZICHINI [6]	10	exac	4 (13.0)	74.3	FEV1	1–2	fall $\geq$20%[+,§]	NA	93.0	3.0	3.0	42
WONG [3]	78	Mild-to-moderate	11 (14.0)	>60.0	FEV1	20	fall $\geq$20%[+]	51.0	63.0	3.0	20	69
HUNTER [5]	36	Mild, stable	3 (8.3)	82.6	FEV1	5.0	fall $\geq$20%[+]	NA	NA	NA	NA	23
VLACHOS-MAYER [14]	304	Mild-to-severe	24 (8.0)	73.1	FEV1	7 or 1–2	fall $\geq$20%[+,§]	75.0	NA	5.0	21	50
PIZZICHINI [34]	31	Mild-to-severe[f]	10 (32)	73.9	FEV1	7 or 1–2	fall $\geq$20%[+,§]	67.5	77.5	3.0	7.0	53

chars: characteristics; EB: excessive bronchoconstriction; FEV1: forced expiratory volume in one second; % pred: percentage of the predicted value; BD: bronchodilator; PT: pretreatment; sal: saline; NA: not available; exac: exacerbation. [#]: as defined by a fall in FEV1 of $\geq$20%; [¶]: all subjects pretreated with bronchodilator (200 μg salbutamol); salbutamol); [+]: or specimen obtained; [§]: or patient expiratory discomfort; [f]: steroid-naive.

made more frequently. However, the potential advantages of measuring pulmonary function after shorter time intervals, as opposed to longer time intervals, or only if subjects report bothersome symptoms, have not yet been formally studied.

Opinions differ as to when to stop sputum induction because of safety concerns, probably because most subjects are able to tolerate the entire induction procedure. Opinion ranges from detection of a fall in FEV_1 of >10–20% to a fall in PEF of >10% from baseline or whenever subjects experience bothersome symptoms (tables 3 and 4). Many subjects experience dyspnoea during sputum induction, at which time their pulmonary function should be checked. However, there is concern regarding poor perception of dyspnoea in some patients, who may report it only after large falls in FEV_1 or PEF have occurred. Thus, it is prudent to make the first measurement of pulmonary function shortly after commencing sputum induction (see Task Force recommendations in article entitled "Sputum induction" [38]) in order to identify subjects who are highly sensitive to the bronchoconstrictor effects of hypertonic saline.

Although no study to date has examined the ideal procedure and timing for assessment of the effects of sputum induction on lung function or at which point to interrupt the procedure, there are some indications in the published literature that may help in the design of further studies. For example, the safety of sputum induction in more severe asthma is similar to that reported in mild asthma (tables 2 and 3). This is probably due to the use of alternative methods of induction (see recommendations). Differences in methods of induction include the concentration of saline (usually starting with normal saline), repeated measurements of FEV_1 at intervals of 1–2 min and interruption of the procedure as soon as a sputum sample is obtained [6]. In another study of safety of sputum induction in a more severe group of asthmatics (FEV_1>1 lL), DE LA FUENTE *et al.* [4] used a modification of the method described by PIN *et al.* [1]. They started the induction by giving 3% hypertonic saline followed by 4 and 5% saline, each inhaled for two 5-min periods. These authors did not proceed to the next concentration if the FEV_1 had fallen by $\geqslant$10%, and they discontinued inhalation if bothersome symptoms developed. This may explain why none of their subjects developed excessive airway constriction. However, it should be borne in mind that interruption of induction before the end of the procedure may influence cellular and fluid phase measurements [39], especially when repeated inductions are performed in the same individual and the duration of the procedure is neither exact nor similar.

Concentration of physiological saline and nebuliser output

In studies conducted to date, the concentration of saline used for sputum induction has ranged 0.9–5.0% (tables 1 and 2). Some investigators vary the concentration during the procedure, starting with 3% and gradually increasing this [1]. The concentration of saline used for sputum induction and the output of the nebuliser may be expected to influence the safety and tolerability of the procedure. Although the degree of airway constriction caused by hypertonic saline has been shown to be a direct function of the dose of hypertonic saline delivered to the airways [17], there have been no reported clinical studies in stable asthma demonstrating that either saline concentrations of up to 5.0% or the nebuliser output determine the likelihood of airway constriction during sputum induction. It has, however, been shown that the general discomfort experienced by subjects during sputum induction is proportional to the nebuliser output [33].

Key points

It is important to: 1) use experienced personnel and apply standard operating procedures including details of safety and hygiene precautions; 2) be aware of the degree of asthma severity of all volunteers; 3) know whether the subjects′s asthma is currently clinically stable; 4) use a modified protocol for subjects with severe asthma (see Task Force recommendations in article entitled "Sputum induction" [38] and *Task Force conclusions regarding the safety of sputum induction* section below); 5) premedicate with 200 μg salbutamol; 6) record the pre- and post-bronchodilator FEV_1; 7) monitor airflow regularly during induction; and 8) always stop if FEV_1 falls >20% from postbronchodilator baseline value (unless induction has been started without prior premedication with β_2-agonist, in which case induction can be continued after reversal of bronchoconstriction (to within 5% of baseline) has been achieved with salbutamol).

Outstanding questions

Premedication and prediction of excessive bronschospasm

1) What are the predictors of excessive airway constriction following sputum induction? 2) Are there mechanisms which determine excessive airway constriction during sputum induction? 3) Should premedication be standardised, and, if so, at what dose?

Monitoring

1) What is the ideal measurement for assessment of safety in sputum induction? 2) What is the ideal interval between safety measurements? 3) What is the optimal duration of inhalation for both safety and success of sputum induction? 4) Is comparison between methods using different durations of inhalation possible?

Concentration of physiological saline and nebuliser output

1) Does the use of increasing concentrations of saline improve the safety of sputum induction?

2) Does the inhalation of isotonic saline before hypertonic saline improve the safety of sputum induction? 3) Does the approach of pretreatment followed by normal and then hypertonic saline for increasing times ranging 30 s–8 min increase the safety of the procedure in at-risk patients?

Task Force conclusions regarding the safety of sputum induction

Published reports regarding sputum induction highlight the fact that various methods have been used successfully in different laboratories; the following conclusions should serve as guidelines, particularly for those who are inexperienced in performing sputum induction procedures.

1. Sputum induction is safe and the risks of inducing bronchoconstriction are acceptable as long as adequate precautions are taken. The procedure should be performed by technologists or nurses who are adequately trained and have sufficient experience in the procedure. This may require a period of training to be undertaken at another centre where sputum induction is already well established.

2. A physician should be in the vicinity at all times during sputum induction, and should be available immediately in the event of any adverse events.

3. The hygiene and sterility of the equipment must be in line with the official policy of the hospital in which the procedure is conducted. The saline used for sputum induction should be prepared and stored under sterile conditions, based on good manufacturing practice. All tubing and parts that come into contact with the patient should be sterilised. The nebulisers should be cleaned thoroughly between each patient.

4. In areas in which there is a high risk of airborne transmission of tuberculosis or other respiratory pathogens, sputum induction should be performed in an isolated room, preferably one in which there is laminar flow. It is advisable to consult with the local infection control staff when setting up a sputum induction laboratory in such an environment.

5. Bronchodilator (short-acting β_2-agonist) pretreatment should always be given except in rare (research) cases for which the inhalation of hypertonic saline is part of a bronchial challenge protocol to assess airway responsiveness. However, investigators should be aware of the fact that a greater degree of bronchoconstriction is likely to occur in the absence of pretreatment with a short-acting β_2-agonist. It is advised that, after β_2-agonist treatment has been given during induction, the FEV_1 should be allowed to return to within 5% of baseline before recommencing saline inhalation.

6. It is acceptable to use either a standard concentration of hypertonic saline (3 or 4.5%, depending on which is commercially available), given for a standard period of time (15–20 min), or increasing concentrations of hypertonic saline (3, 4.0 and 5%). Both of these protocols can be further modified in subjects with severe airflow limitation (postbronchodilator FEV_1 <60% pred), in patients with uncontrolled symptoms or where there are any other concerns about the safety of the procedure. Such a modification should include initiating the induction procedure using 0.9% saline for shorter periods of time and stopping the induction when a sufficient sample has been obtained, or when there is a fall in either FEV_1 or PEF of >20%. It is not known whether sputum induction can be performed safely in asthmatics in whom FEV_1 is <1 L or <50% pred.

7. For asthmatic volunteers, protocols differ depending on the severity of the disease. Not enough information is available to enable clear definition of severe asthma, and there are no quantifiable measurements that can predict the response to inhalation of either physiological or hypertonic saline. In all cases, those conducting the induction should err on the side of caution.

Details of the induction procedure are given in the article entitled "Sputum induction" [38]. In the majority of instances, salbutamol (200 μg) should be given (check FEV_1 after 10 min), unless measurement of responsiveness to hypertonic saline is a study objective.

For mild-to-moderate asthmatics, either a fixed concentration of saline (*e.g.* 3 or 4.5%) or incremental concentrations of saline solution can be used. Induction can be conducted at 5-min intervals. Alternatively, if there is any concern, induction should be performed for 1, 4 and 5 min, with a further three 5-min periods, measuring FEV_1 at the end of each induction interval. Patients should be encouraged to expectorate after 5, 10 and 15 min of induction.

In high-risk asthmatics (*e.g.* severe asthma, highly reactive airways, exacerbation and using increasing doses of β_2-agonist) 0.9% sterile saline (also referred to as isotonic or normal saline) solution should be used initially. This can be followed cautiously by 3 and 4.5% if the FEV_1 remains >80% of baseline. Induction should be performed for periods of 30 s and 1, 2, 4 and 8 min, and FEV_1 should be measured at the end of each induction interval. The patient should be asked to expectorate after the 4- and 8-min periods.

8. Always stop the induction if there is a fall in FEV_1 of $\geqslant$20% compared with postbronchodilator values or if bothersome symptoms occur.

9. Sputum induction has been used safely in subjects with severe COPD, but there have been no systematic studies addressing safety issues in this patient category.

10. Although both the concentration of hypertonic saline used and the duration of the induction procedure vary in different published reports, sputum induction procedures should be standardised in formal protocols (standard operating procedures) for each clinical study and each laboratory. When the concentration of saline or the duration of induction differ from the original protocol at the first visit, then these differing parameters should be used for that subject at all subsequent visits in that particular study.

11. Although it is acceptable to monitor either FEV_1 or PEF as a measure of safety during the procedure, it is still unclear how often these measurements should be performed throughout the induction procedure. However, it is recommended that assessment of

airway function is made after each inhalation period (*i.e.* at time intervals which depend on the protocol used throughout the procedure (see Task Force recommendations in article entitled "Sputum induction" [38])), or more frequently if there are any safety concerns. It is better to err on the side of caution by performing measurements at shorter time intervals than not measuring them frequently enough.

12. At the end of the induction procedure, patients should be given an inhaled short-acting β_2-agonist, particularly if there has been a fall in forced expiratory volume in one second of >10% from the baseline value. Patients should remain and be monitored in the laboratory until their forced expiratory volume in one second or peak expiratory flow has returned to within 5% of the baseline value.

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