

Letter to the Editor

Patients' characteristics associated with unsuccessful sputum induction in asthma

To the Editor:

Sputum induction has been widely used to assess airway inflammation in patients with asthma.¹ An important limitation of sputum induction is that samples adequate for evaluation are often not obtained, unlike other materials for assessing airway inflammation, such as exhaled breath or serum.² Despite methodology of sputum induction might affect its success rate,³ the association between the characteristics of patients and the results of sputum induction is unknown. We therefore retrospectively compared the characteristics of patients with asthma classified according to the success or failure of sputum induction.

At our outpatient clinic, sputum induction for clinical or research purposes was attempted a total of 1093 times from May 1998 through October 2005, including 576 examinations in 407 patients with asthma. These 407 patients were classified into 2 groups according to the success or failure of the first attempt at sputum induction on presentation and were compared with respect to age, sex, disease duration, ever-smoking and current smoking history, pack-year of smoking, serum total IgE, atopy, FEV₁, FEV₁/forced vital capacity, use and dosage of inhaled corticosteroids (ICSs), and disease severity. The protocol was approved by our Ethics Committee, and written informed consent was obtained from all participants.

Reproducibility of the success or failure of sputum induction was evaluated on the basis of data obtained from 124 patients in whom sputum induction was repeated. Sputum induction was performed twice in 86 patients, 3 times in 32, 4 times in 5, and 5 times in 1. We analyzed data from 66 of these subjects, who underwent 2 consecutive attempts at sputum induction while in a stable condition, as defined by stable disease for at least 1 month while receiving continuous treatment with ICS. This subgroup included 26 men and was age 51.6 (18.6) years at the time of the first attempt at sputum induction. The median (range) interval between the 2 attempts at sputum induction was 6.7 (3.0-62.5) months.

Sputum induction and processing were performed as previously described.^{1,4} Briefly, after premedication with salbutamol (200 µg), the subjects inhaled hypertonic (3%) saline solution delivered with an ultrasonic nebulizer for 15 minutes. The concentration and duration of saline inhalation were in accordance with guidelines.⁵

Induction procedures were considered successful if patients expectorated adequate sputum containing more than 200 non-squamous cells, and unsuccessful if subjects failed to expectorate sputum or produced inadequate samples.⁶ Subjects with FEV₁ values after bronchodilator premedication of less than 60% of the predicted value did not undergo sputum induction.⁶ These methods remained constant throughout the study period.

Variables are expressed as means (SDs) unless otherwise stated. The unpaired *t* test, Mann-Whitney *U* test, and χ^2 test were used to compare the groups. Logistic regression analysis was performed to test for independent effects of disease duration, ever-smoking history, and sex on unsuccessful sputum induction. A *P* value of <.05 was considered statistically significant. The degree of agreement between 2 consecutive attempts at sputum

induction in the same subject was also tested. The absence of a systematic difference (ie, bias) between the 2 attempts for the same patient was assessed with the McNemar test (*P* > .05 meant no bias). The results were then compared by using the κ test for agreement and repeatability; *P* < .05 meant that the test results were reproducible.

Sputum was successfully induced in 297 of 407 subjects (73.0%). Failure to obtain induced sputum was significantly associated with a longer disease duration and a lower rate of ever having smoked and was marginally associated with female sex compared with successful induction of sputum (Table I). On multivariate analysis, failure of sputum induction was significantly associated with long-standing disease and lack of a smoking history (Table II). These results were unchanged when use or dosage of ICS or both were included as additional independent factors (data not shown). The use of ICS was low in both groups (Table I) because the data were collected at first presentation.

In the reproducibility study, 62 (94%) of the 66 subjects had the same results on both attempts at sputum induction. Induced sputum samples were successfully obtained both times in 54 subjects (82%); in 8 (12%) subjects, both attempts resulted in failure. The first attempt was successful and the subsequent attempt was unsuccessful in 1 patient, and the converse was true in 3 patients. The results indicated good reproducibility (*P* = .32 by McNemar test; κ = .76, *P* < .001 by κ test). The dose of ICS and the FEV₁ at the time of the 2 attempts at sputum induction were both similar (data not shown).

To our knowledge, this is the first study to investigate the detailed characteristics of patients with asthma in whom sputum induction was successful or unsuccessful. Failure of sputum induction in patients with asthma was significantly associated with long-standing disease and the absence of a smoking history. The success or failure of sputum induction were reproducible in patients repeatedly examined while in stable condition, suggesting that it is a fixed rather than a variable characteristic of each patient.

A smoking history significantly contributed to the success of sputum induction in our patients. Exposure to cigarette smoke induces increased expression of *MUC5AC* mRNA by activating epidermal growth factor receptors in a human epithelial cell line and in a rat model *in vivo*.⁷ Repeated exposure to cigarette smoke induces goblet cell metaplasia in guinea pigs, which persists after cessation of exposure for 4 months.⁸ These observations indicate that cigarette smoking exaggerates mucus production and that its effects remain even after smoking cessation. This may explain our higher rate of successful sputum induction in patients who had ever smoked than in those who had never smoked. The lack of an association with current smoking may have been related to the small number of current smokers (Table I). Long-standing disease was also associated with failure of sputum induction. The mechanism responsible for this phenomenon is uncertain. In our previous study conducted in patients with exacerbations of asthma, a subset of patients repeatedly showed normal titers of serum eosinophil cationic protein,⁹ a surrogate marker of eosinophilic airway inflammation.² These patients were significantly older and had a longer disease duration than patients who had elevated protein levels. It was concluded that eosinophilic inflammation may contribute relatively less to

TABLE I. Patient characteristics according to success or failure of sputum induction

	Success	Failure	P value
N	297	110	
Age (y)	50.2 (18.2)	48.1 (19.6)	.31
Female (%)	160 (53.8)	69 (62.8)	.09
Disease duration (y)	8.2 (11.9)	11.6 (17.3)	.03
Log IgE (IU/mL)	2.2 (0.68)	2.3 (0.68)	.99
Atopy (%) [*]	197 (66.2)	73 (66.3)	.98
FEV ₁ (% predicted)	85.6 (22.0)	85.1 (25.3)	.85
FEV ₁ /forced vital capacity (%)	73.3 (11.6)	75.2 (11.9)	.17
Use of inhaled steroids (%)	108 (36.4)	48 (43.6)	.20
Dose of inhaled steroids (μg/d) [†]	280 (458)	312 (468)	.54
Severity step, median (range) [‡]	3 (1-4)	3 (1-4)	.26
Smoker, ever (%)	89 (30.0)	20 (18.2)	.02
Smoker, current (%)	22 (7.4)	7 (6.4)	.72
Pack-years [§]	19.4 (21.0)	20.5 (13.6)	.8

Values are given as mean (SD) or n (%), except where noted.

^{*}Defined by at least 1 positive specific IgE antibody against house dust mite, molds, grass pollens, weed pollens, cat dander, and dog dander.

[†]Equivalent to chlorofluorocarbon-beclomethasone dipropionate. The dose of patients untreated with ICSs is 0.

[‡]Defined as step 1 (intermittent), step 2 (mild persistent), step 3 (moderate persistent), or step 4 (severe persistent) in accordance with Global Initiative for Asthma guidelines 2004.

[§]For exsmokers and current smokers only.

TABLE II. Factors associated with unsuccessful sputum induction in asthma: logistic regression analysis

Factor	Odds ratio (95% CI)	P value
Female sex	1.16 (0.71-1.92)	.56
Smoking history	0.52 (0.29-0.90)	.02
Disease duration	7.78 (1.41-44.5)	.02

the pathophysiology of asthma in patients who are older and have a longer disease duration.⁹ Repeated exposure of mice to an antigen initially induces bronchoalveolar eosinophilia and goblet cell hyperplasia, but these findings are attenuated with time.¹⁰ Long-standing asthma thus might be associated with less inflammation and mucus secretion. This remains speculative, however, because the relationship between the duration of asthma and the pathological features of airways remains unknown.

In conclusion, patients with asthma who have long-standing disease and lack a smoking history are less likely to produce

induced sputum successfully. The characteristics of patients should be considered when analyzing the results of induced sputum studies, because potential selection bias may preclude the extrapolation of such results to patients with asthma in general.

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

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doi:10.1016/j.jaci.2007.11.032