Cough reflex testing with inhaled capsaicin in the study of chronic cough

L. Nieto, A. de Diego, M. Perpiñá, L. Compte, V. Garrigues, E. Martínez and J. Ponce

Service of Pneumology and Service of Gastroenterology, Hospital Universitario La Fe, Valencia, Spain

Abstract *Objectives:* To assess the utility of capsaicin test in the differential diagnosis of non-productive causes of chronic cough and to examine the effects of treatment on this reflex. *Participants:* 86 healthy volunteers and 101 patients with chronic cough: asthma (*n*: 54), gastroesophageal reflux (*n*: 35) and post-nasal drip syndrome (*n*: 12). *Design:* Prospective intervention trial. Spirometry, bronchoprovocation test with histamine (PC_{20}), and cough challenge with ascending concentrations of capsaicin (0.49–500 μM) were initially performed in all subjects. Patients were treated for 3 months according to the origin of the cough. Concentrations that elicited two (C_2) and five or more coughs (C_5) were determined before and after treatment. *Results:* In healthy subjects, cough sensitivity to capsaicin was not influenced by gender or smoking status; however, women with chronic cough were more sensitive to cough challenge than men. C_2 and C_5 were significantly lower in patients with asthma or gastroesophageal reflux than in post-nasal drip syndrome. No significant correlation was observed between the capsaicin cough threshold and PC_{20}. Cough sensitivity did not improve significantly in most patients with asthma or gastroesophageal reflux despite adequate medical treatment during 3 months. Discriminative value of capsaicin test to differentiate healthy subjects from patients with asthma or reflux was poor. *Conclusions:* Cough sensitivity to inhaled capsaicin is a safe and reproducible tool in the study of chronic cough. However, its usefulness for the management and differential diagnosis is limited.

doi:10.1053/rmed.2002.1460, available online at http://www.sciencedirect.com

Keywords cough sensitivity; non-productive cough; asthma; capsaicin; gastroesophageal reflux; post-nasal drip syndrome.

INTRODUCTION

Cough is a common symptom in respiratory disease. It has been estimated that 10–40% of office visits per year in general practice are due to this symptom(1). Asthma, gastroesophageal reflux, and post-nasal drip syndrome, alone or in combination, account for the majority of cases of persistent cough in patients who are referred to pulmonary specialists (2,3). In these cases, cough usually results from the stimulation of sensory nerves in the airway with activation of irritant receptors (myelinated rapidly adapting stretch receptors) or C-fiber endings in the larynx or tracheobronchial tree (4). It has been suggested that chronic airway irritation, irrespective of the original cause (inflammation or aspiration), may lead to the development of a sensory hyperresponsiveness of cough receptors (5,6). According to this hypothesis, the mechanism by which gastroesophageal reflux, asthma or post-nasal drip syndrome induces cough may be similar: enhanced sensitization of airway sensory nerves.

Capsaicin, the active ingredient of red pepper, is a commonly used cough stimulant in the study of cough reactivity and for the evaluation of antitussive agents in humans (7–9). It has been shown to produce cough mainly by stimulating C-fiber endings and also by stimulating some rapidly adapting receptors with myelinated fibers (10). Although different studies(11–13)have demonstrated that capsaicin is safe to use and produces a dose-dependent and reproducible cough response in a variety of conditions, there are scarce studies reporting reference values of cough threshold to inhaled capsaicin in healthy subjects. Moreover, usefulness of capsaicin challenge in the diagnostic algorithm of chronic cough has not been determined (14).

The present study was designed with the following purposes: (1) to determine our normal range of cough threshold to inhaled capsaicin, (2) to assess the sensitivity of capsaicin test in the differential diagnosis of non-productive persistent cough, and (3) to examine the effects of treatment of the underlying condition on cough sensitivity.

Received 28 May 2002, accepted in revised form 19 September 2002.
Correspondence should be addressed to: Dr Alfredo de Diego, MD, Service of Pneumology, Hospital Universitario La Fe, Avda. Campanar 21, E-46009 Valencia, Spain. Tel.: +34-96-3862764. Fax: +34-96-3868789; E-mail: alfr591@separ.es

MATERIALS AND METHODS

Study subjects

Patients referred to our Service of Pneumology for study and treatment of unexplained cough of more than 4 weeks duration were included in the study. None of them had been previously diagnosed of any chronic respiratory disease or were taking bronchodilators and/or inhaled corticosteroids. Additional exclusion criteria were the presence of acute respiratory infections during the previous 4 weeks. Active smokers (patients who smoked in the last month) and patients receiving angiotensin-converting enzyme inhibitors or drugs that may influence airway reactivity or lower esophageal sphincter tone were also excluded. In order to obtain our own reference values, a group of healthy individuals free from respiratory disease (normal physical examination and lung function) participated in the study. The study was approved by the institutional review board, and written informed consent was obtained from all subjects after the purpose of the diagnostic evaluation and the capsaicin challenge test had been explained.

Study design

Patients with chronic cough underwent a thorough diagnostic evaluation (14) that consisted of medical history (cough characteristics, respiratory, and digestive symptoms), physical examination, chest radiography, and pulmonary function testing including spirometry and bronchodilator test (Fig. 1). When a diagnosis was initially suspected, a specific diagnostic test was indicated (first step in the algorithm). In this sense, sinus X-ray films, histamine challenge, or 24-h esophageal monitoring were performed on an individual basis. If the diagnosis was established by the tests, specific treatment was instituted. One or more final diagnosis were only established after successful treatment response to adequate 3-month therapy. (Final step of the algorithm). When initial clinical findings did not suggest a specific cause, a bronchoprovocation histamine challenge was first ordered. If it was negative, a 24-h pH-monitoring was indicated. Unsuccessful treatment at 1 month was followed by additional tests in the order which is indicated in the algorithm and treatment response was newly reevaluated.

Asthma was diagnosed in the presence of symptoms of episodic wheezing, cough, and shortness of breath with positive challenge tests (PC_{20} histamine <8 mg/dl) or increased forced expiratory volume in 1s (FEV_1) after bronchodilator inhalation ((([postFEV_1 − preFEV_1]/predicted FEV_1) $>$ 12% and 200 ml). Treatment included inhaled steroids (fluticasone 500 μg/day) and salbutamol as needed. Gastroesophageal reflux was diagnosed when the percentage of time with **a** pH below 4 was >8% for the upright, >4% for recumbent, and >5% for the total time. Patients with gastroesophageal reflux were treated with lifestyle changes and omeprazole (40 mg/day).

Post-nasal drip syndrome was suspected when patients complained of having something dripping into the throat, nasal discharge, mucus drainage in the posterior oropharynx or hoarseness**.** Laryngitis was diagnosed by indirect laryngoscopy taking into consideration the presence of mucosal integrity and erythema. Patients with post-nasal drip syndrome were treated with nasal steroids (fluticasone 250 μg/day) antihistamines (loratadine 10 mg/day), and topical decongestants (d-isoephredine).

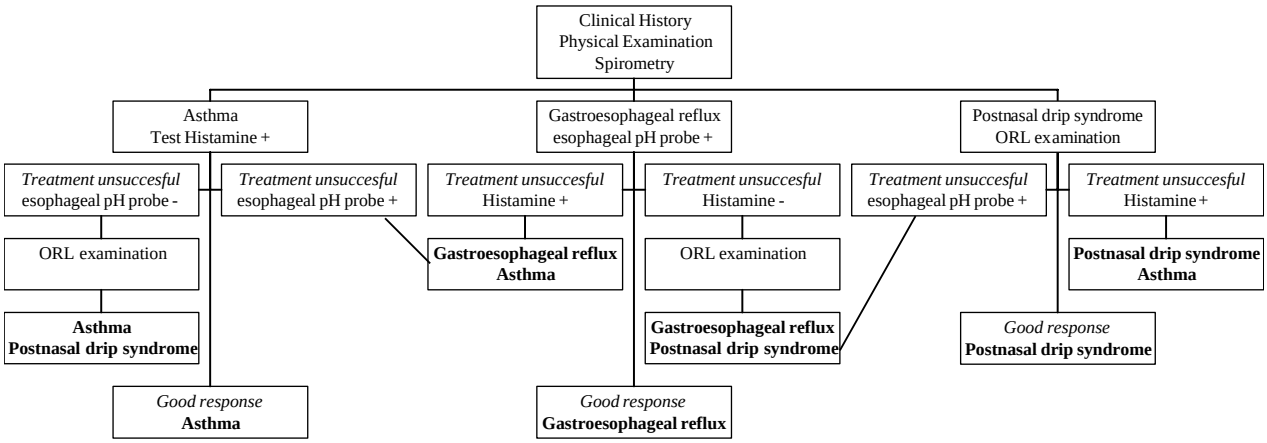

Fig. 1. Diagnostic algorithm for chronic cough. Specific diagnostic tests (sinus X-ray films, histamine challenge, or 24-h esophageal monitoring) were performed on an individual clinical basis (first step). One or more final diagnosis were only established after successful treatment response to adequate 3-month therapy (final step of the algorithm). When initial clinical findings did not suggest a specific cause, a bronchoprovocation histamine challenge was first ordered. If it was negative, a 24-h pH-monitoring was indicated. Unsuccessful treatment at 1 month was followed by additional tests in the order which is indicated and treatment response was reevaluated.

Histamine challenge test was performed according to the method described by Cockcroft *et al*. (15). Histamine doses were inhaled from a Hudson Up Draft II nebulizer (output 0.13 $\pm$ 0.002 ml/min) operated by an electric compressor (Pari Therapiegerät Privat). FEV_1 before and after each inhalation was measured on a dry wedge spirometer (Vitalograph, Vitalograph Ltd., U.K.) and bronchial hyperresponsiveness was expressed as the histamine provocation concentration producing a 20% fall in the FEV_1 (PC_{20} FEV_1).

Gastroesophageal reflux was measured by 24-h esophageal and gastric pH monitoring using antimony pH electrodes (Monocrystant 91-0011, Synectics Medical, Stockholm, Sweden) connected with a dual-channel pH-meter (Digitrapper Mark III, Synectics). Probes were introduced transnasally and the distal electrode were located 5 cm above the upper limit of manometrically defined lower esophageal sphincter. The proximal electrode was placed immediately under the upper esophageal sphincter. Recorded data were evaluated with an ambulatory pH software package (Gastrosoft,WI, U.S.A.). Patients were instructed to lead their normal lives. Esophagoscopy was also performed in patients with gastroesophageal reflux or severe digestive symptoms to exclude the presence of esophagitis.

Following the diagnostic protocol and before specific treatment was indicated, patients and healthy individuals underwent the capsaicin challenge. Maximum FEV_1 and FVC, (Vitalograph LTD, Buckingham, U.K.) were determined before and after the capsaicin challenge to investigate the bronchoconstrictor effect of capsaicin. Cough intensity was measured using a visual decimal scale (0–10, where 0: no cough and 10: maximal cough) initially and after treatment.

In order to evaluate the effects of treatment, capsaicin cough challenge was repeated after a 3-month treatment period. Likewise, reproducibility of the test was assessed in healthy subjects at 3 months.

Capsaicin Challenge

Capsaicin (Sigma Chemical, Poole, U.K.) was prepared by dissolving 30.5 mg of the substance in 1 ml of 96% ethanol and 1 ml of polyoxyethylenesorbitan monooleate 80 (Tween 80) and then dissolved in 8 ml physiologic saline solution to make a stock solution of 10^{-2} M. This solution was further diluted with saline solution to make serial doubling concentrations ranging from 0.49 to 500 μM. Fresh dilutions were prepared each day of testing. Aerosol was delivered through a breath-activated nebulizer controlled by a dosimeter (Optineb Air Liquid), set to nebulize for 0.9 s. The challenge was performed by single-breath inhalation of doses increasing from 0.49 to 500 μM. Subjects were asked to inhale once deeply over 2 s at 2-min intervals. The number of coughs during the first 30 s after each dose was counted. The challenge was terminated when the subject cough five or more times (C_5) or the maximal dose of capsaicin was attained. In subjects who did not have cough, a value of C_5 of 500 μM was assumed. The concentration of capsaicin inducing two coughs (C_2) was also recorded. Subjects were unaware that cough was the specific point of research interest.

Statistical analysis

Capsaicin log C_2 and log C_5 values were expressed as geometric means and 95% confidence intervals (CIs). To test reproducibility, an intraclass correlation coefficient was calculated. In assessing the relationship of cough threshold to age, gender, histamine PC_{20}, FEV_1, and duration of cough, a Pearson's product–moment correlation analysis was performed. Differences in clinical variables in each group were assessed using the Chi-squared test. In patients with gastroesophageal reflux, the association between cough threshold and reflux severity or the presence of esophagitis and laryngitis was also investigated. The differences in mean values for log C_2 and log C_5 for the patient groups were evaluated by analysis of variance. A value of $P < 0.05$ was considered to be significant. Changes in capsaicin concentrations after treatment were expressed in doubling concentrations [(log capsaicin after treatment $-$ log capsaicin before treatment)/log 2) and patients divided into responders (doubling concentrations $>$1.2 or the cutoff point for the 95th percentile in healthy subjects) and non-responders. Differences between responders and non-responders were evaluated by t test for independent samples. Finally, the ability of the capsaicin challenge test in the diagnosis of chronic cough was assessed by calculating the sensitivity, specificity, false-positive rate, and false-negative rate for each inhaled concentration value. The best cut-off point that separates healthy and the different groups was obtained with a graphical ROC curve, constructed by plotting sensitivity against false-positive rate for each value. Area under curve of the different ROC curves were compared. Other cutoff values were obtained corresponding to the values with greatest specificity.

RESULTS

A total of 113 patients (41 men and 72 women) were initially studied. In 12 of the 113 cases, a definitive diagnosis was not established and they were excluded from the analysis. Baseline characteristics of patients in the study are shown in Table 1. The underlying causes of chronic cough included asthma in 54 patients, gastroesophageal reflux in 35 (in association with bronchial hyperrespon-

Table I. Baseline characteristics in 101 patients with chronic cough

Data	Asthma	GER	GER and BHR	PND	*P* value
Age, (years)	41 (17)	47 (14)	52 (15)	38 (18)	
Gender, (male/female)	19/35	3/20	3/9	8/4	NS
Cough duration, (months)	30 (38)	49 (44)	30 (28)	24 (20)	NS
Cough characteristics, (%)					
Productive	20	7	25	18	NS
Paroxysmal	57	41	35	46	NS
Irritative	19	20	40	20	NS
Shortness of Breath, (%)	52	24	57	37	NS
Wheezing, (%)	61*	27	21	33	<0.05 *vs.* GER and PND groups
Rhinitis, (%)	21	14	20	55*	<0.05 *vs.* Asthma and GER group
Pirosis/Disphagia, (%)	27	78*	48	29	<0.05 *vs.* Asthma and PND groups
Laryngitis, (%)	7	21	38	10	NS
Esophagitis, (%)	NA	36	37	NA	NS

GER: gastroesophageal reflux; BHR: bronchial hyperresponsiveness; PND: post-nasal drip; NA: not applicable. NS: not significant.

*$P<0.05$

Data expressed as mean (SD) or percentage of patients.

siveness in 12), and post-nasal drip syndrome in 12. Intergroup differences in relation to demographic, clinical characteristics, and duration of cough were not significant. As expected, the frequency of digestive symptoms and shortness of breath was significantly different in patients with gastroesophageal reflux or asthma group.

Eighty-six healthy volunteers (36 men and 50 women) participated in the study to obtain the reference values. Results of spirometry and capsaicin challenge of this group are shown in Table 2. There were no differences between capsaicin cough thresholds between smokers and non-smokers. Likewise they were not influenced by gender. However, cough sensitivity was related with age ($r=-0.27$, $P<0.05$).

Reproducibility of the capsaicin challenge performed in this group was high (intraclass correlation coefficient: 0.75 (95% CI 0.53–0.87) and 0.88 (95% CI 0.76–0.94) for C_2 and C_5 values, respectively].

There were not any changes in spirometric values following capsaicin challenge.

Results of capsaicin cough challenge in subjects with chronic cough (Table 2) revealed that patients with asthma or gastroesophageal reflux showed significantly lower pre-treatment cough threshold concentrations than healthy subjects or patients with post-nasal drip syndrome. In patients with chronic cough, cough threshold was significantly lower in women than in men and there was a statistically significant inverse relationship between cough sensitivity and age ($r=-0.33$, $P<0.05$). Capsaicin cough sensitivity was significantly related to previous duration of cough, especially in asthmatic patients ($r=-0.43$, $P<0.01$). As a whole, no relationship was found between histamine PC_{20} and cough sensitivity in patients with chronic cough. This absence of correlation was also confirmed in the asthmatic group.

In patients with gastroesophageal reflux, log C_2 and log C_5 values were not influenced by the presence of laryngitis or esophagitis. Capsaicin sensitivity was also not significantly different between patients with proximal or distal reflux.

In all patients, cough decreased progressively and almost disappeared after 90 days of treatment. However, in the majority of patients with asthma or gastroesophageal reflux, cough reflex sensitivity did not vary after 3 months of treatment as shown in Fig. 2. There were no differences in demographic and clinical characteristics, duration of cough, and bronchial hyperresponsiveness between responders ($n=31$) and non-responders ($n=70$).

As indicated by the ROC curves (Fig. 3) the optimal cutoff point to differentiate asthma patients from healthy subjects was 31 μM (sensitivity 94%, specificity 50%) and the optimal cutoff point to distinguish patients with gastroesophageal reflux from healthy subjects was 15 μM (sensitivity 98%, specificity 66%). The area under the ROC curve was slightly higher in patients with gastroesophageal reflux (0.88, SD = 0.41) than in patients with asthma (0.82, SD = 0.36). The lowest cutoff point in healthy subjects was 125 μM. Capsaicin threshold higher than this value showed a 100% specificity for excluding sensory hyperresponsiveness as the cause of cough.

DISCUSSION

We have demonstrated that testing cough sensitivity to inhaled capsaicin is a safe and reproducible tool in the

Table 2 Results of capsaicin cough challenge in 86 healthy subjects and in 101 patients with chronic cough

Data	Healthy subjects	Patients with chronic cough					*P*-value
		Total	Asthma	GER	GER and BHR	PND	
FEV_1 (l/min)							
Baseline	3.40 (0.78)	2.57(0.73)	2.56 (0.82)	2.59 (0.75)	2.50 (0.78)	2.58 (0.75)	NS
Post-capsaicin	3.59 (0.92)	2.58(0.71)	2.55 (0.81)	2.59 (0.69)	2.48 (0.79)	2.58 (0.76)	NS
FVC (l/min)							
Baseline	4.22 (0.87)	3.30(0.85)	3.38 (0.91)	3.39 (0.85)	3.14 (0.96)	3.49 (0.69)	NS
Post-capsaicin	4.54 (1.05)	3.33(0.79)	3.42 (0.94)	3.31 (0.81)	3.18 (1.06)	3.43 (0.92)	NS
Histamine PC_{20}, (mg/ml)		8.48(5.8)	3.63 (2.02) *	11.9 (2.23)	3.68 (2.42)*	11.1 (3.01)	<0.05 *vs.* GER and PND groups
C_2 before treatment (μM)	32.3 (23.4–44.6)		13.8 (6.3–29.5)*	4.8 (2.2–10.7) *	5.4 (2.4–12.6) *	77.6 (32.3–186)	<0.05 *vs.* healthy and PND groups
Men	33 (21 to 56)	24 (15–41)					
Women	32 (21 to 49)	7 (5–10) *					<0.05 *vs.* male gender
Smokers	33 (22 to 45)						
Non-smokers	31 (20 to 57)						
C_5 before treatment (μM)	151 (123 to 186)		33.3 (22.1–48) *	14.3 (6.3–29.5) *	12.3 (4.6–32.3)*	117 (57–239)	<0.05 *vs.* healthy and PND groups
Men	141 (104 to 190)	55 (34–87)					
Women	158 (120 to 209)	29 (13–30) *					<0.05 *vs.* male gender
Smokers	131 (105 to 189)						
Non-smokers	144 (103 to 192)						
ΔC_2, (doubling doses)	0.14 (1.6)	0.62 (1.9)	0.81 (1.92)	0.29 (2.4)	0.01 (0.01)	0.57 (1.27)	NS
ΔC_5, (doubling doses)	0.01 (0.75)	0.67 (2.1)	0.63 (1.5)	0.52 (2.16)	0.13 (2.4)	1.29 (2.29)	NS

*GER: gastroesophageal reflux; BHR: bronchial hyperresponsiveness; PND: post-nasal drip; NS: notsignificant.
$P<0.05$.
Data expressed as mean (SD) or geometric mean (95% CI).

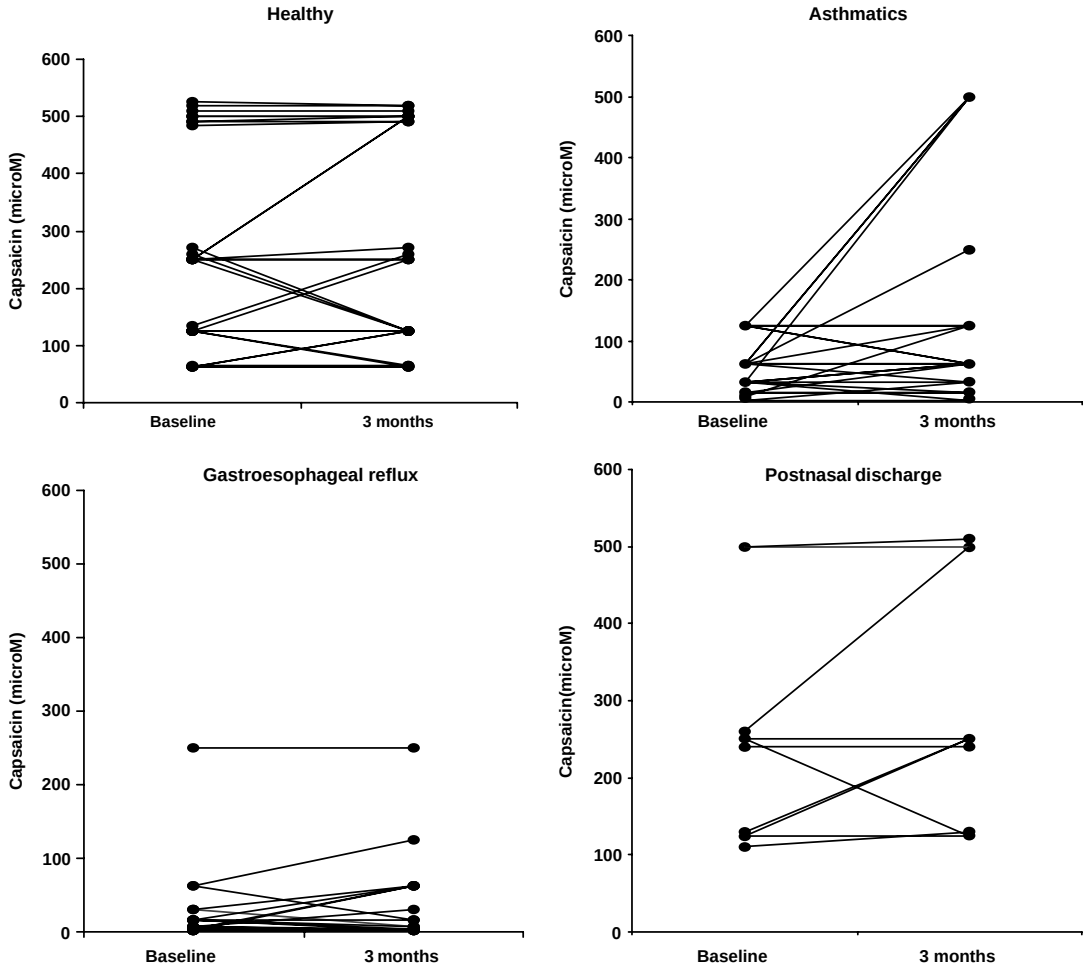

Fig. 2. Individual values of capsaicin concentration before and after 3 months of treatment of the underlying disease in patients with chronic cough and in untreated healthy subjects.

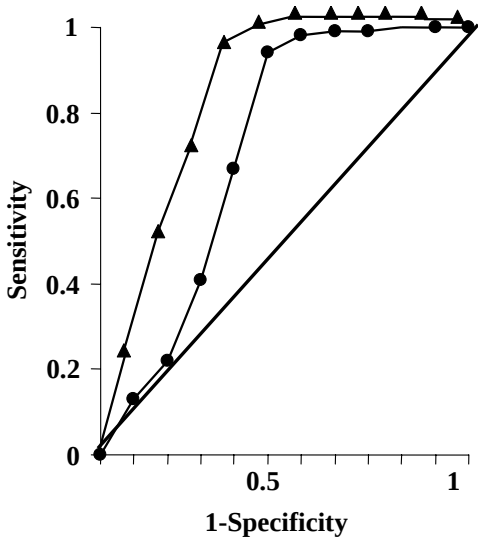

Fig. 3. ROC curves of optimal capsaicin cut-off points to distinguish patients with asthma (●) or gastroesophageal reflux (▲) from healthy subjects.

study of non-productive chronic cough. However, its usefulness for the management and differential diagnosis is limited.

Sensory hyperresponsiveness to capsaicin was only seen in patients with asthma or gastroesophageal reflux. Patients with post-nasal drip syndrome were found to have normal sensitivity of the cough reflex and, therefore, were different from the remaining patients with chronic cough. The absence of this response is important because it could serve to exclude this condition in clinical practice. Cough sensitivity, which was independent of bronchial hyperresponsiveness even in asthmatic patients, was only related to the duration of the symptom and gender. A heightened sensitivity of the cough reflex to inhaled capsaicin in women compared with men was found.

Reference values in healthy subjects have not been clearly established due to the lack of standardization of assessment of cough sensitivity to inhaled capsaicin and the relatively small number of healthy subjects included in the different studies. C_2 and C_5 were arbitrarily chosen

for comparison with international literature. Our mean C_2 and C_5 values of 32 (23–44) and 151 (125–186) μM, respectively, in normals are similar than findings reported by Choudry and Fuller (12). In contrast to sex differences reported by Fujimura *et al*. (16) and by Dicpinigaitis and Rauf (17), cough threshold in our series was similar in males than in females. In the former studies, gender difference was a significant predictive factor for cough sensitivity in either age group. Likewise, gender influence has been documented in other modalities of evoked cough (citric acid, tartaric acid). Reproducibility of the test in 28 healthy subjects who took part in both the first and second experiments (3 months apart) was good, with a high intraclass correlation coefficient, i.e., equal to or less than 1 as expressed in doubling concentrations. Reproducible cough responses to capsaicin challenge in both adults and children have been previously found by others (11,16,18).

Little has been reported on the usefulness of capsaicin test in studies of the cough reflex in disease. Choudry and Fuller[12] found that patients with non-productive cough had a higher sensitivity of the cough reflex (log C_5:1.16) than patients with productive cough (log C_5:1.54) or healthy subjects (log C_5:1.78). Increased cough sensitivity was also found in patients given angiotensin-converting enzyme inhibitors, in patients with previous viral infections, and in patients with gastroesophageal reflux. In the latter, tussive response to capsaicin was enhanced even in patients without cough (13,19). The reason for this response is not clear. Any parameter of esophageal function or reflux is associated with the reduced cough threshold. It has been hypothesized that long-lasting exposure of the mucosa to gastric juices could produce enhancement of the cough reflex.

Airway inflammation in patients with asthma is probably the cause of the increased sensitivity to capsaicin. However, mechanisms underlying cough production are not always related to those determining bronchial reactivity. In the present study, sensory hyperresponsiveness to capsaicin in patients with asthma was independent of bronchial hyperresponsiveness to histamine.

In healthy individuals, several studies have not found differences between smokers and non-smokers (12). These findings are in agreement with the present results. Cough is a frequent symptom in smokers and patients with COPD. Previous studies showed that patients with chronic bronchitis had normal responses to capsaicin (20); however, Doherty *et al*. (21) have demonstrated that cough reflex is also increased in patients with COPD and it is independent of airflow limitation. Upper respiratory infection may cause cough as a result of increased sensitivity of capsaicin-sensitivity afferent airway nerves without affecting airway calibre or responsiveness. O'Connell *et al*. (22) have shown that in subjects with dry cough C_5 was lower during infection than both baseline and recovery.

No previous data have been reported in relation to the diagnostic accuracy of capsaicin cough challenge in terms of sensitivity and specificity. With a cutoff point of 15 μM, the predictive capacity of the test for differentiating patients with chronic cough from those with gastroesophageal reflux was higher than for differentiating patients with chronic cough from asthmatics or patients with post-nasal drip syndrome. In case of using results of capsaicin challenge in diagnostic algorithms, they would seem particularly useful to exclude the diagnosis of post-nasal drip syndrome in a patient with chronic cough.

On the other hand, studies of capsaicin sensitivity after successful treatment of cough are limited. In patients with gastroesophageal reflux, some authors (19) have shown that omeprazol improves not only digestive symptoms but also the cough threshold to capsaicin. This improvement was significant after 5 days in most of the patients. In the present study, cough sensitivity remains increased despite favorable clinical response to treatment both in asthma and gastroesophageal reflux. The inclusion of patients without cough in the former study could explain some of the differences. Likewise, several studies have documented that multiple causes may be present in the individual patient. This feature could modify the results of the capsaicin test. To avoid it, we choose only patients who responded successfully to specific treatment. We prefer this stepping approach instead that every patient has had all examinations. The persistence of cough reflex sensitivity may add a valuable objective parameter to continue with treatment until a normal sensitivity has been attained.

In summary, capsaicin challenge has a limited usefulness in the differential diagnosis and management of diseases causing an increase in the sensitivity of cough reflex.

Acknowledgements

This study was supported by grants from Fondo de Investigación Sanitaria (FIS 99/0739) and Sociedad Española de Patología Respiratoria (SEPAR'99).

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