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Respirology (2008) ••, ••–•• doi: 10.1111/j.1440-1843.2008.01273.x

ORIGINAL ARTICLE

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FUJIMURA M, OHKURA N, ABO M, FURUSHO S, WASEDA Y, ICHIKAWA Y, HARA J. *Respirology* 2008; ••: ••–••

Background and objective: Atopic cough (AC) is an established clinical entity in Japan, in which patients present with a chronic persistent non-productive cough. Exhaled nitric oxide (NO) is a biomarker of eosinophilic airway inflammation. The present study examined whether exhaled NO levels were increased in AC in comparison with cough variant asthma (CVA) and bronchial asthma (BA).

Methods: Consecutive patients presenting with an isolated cough lasting at least 8 weeks were enrolled in the study. The aetiology of the chronic cough was determined according to the Japanese Respiratory Society guidelines for management of cough. Exhaled NO, capsaicin cough sensitivity (C5) and bronchial reversibility were measured at the patients' first visit. Bronchial responsiveness (PC20 to methacholine) was measured at their second visit following a 6-day course of bronchodilator therapy.

Results: There were 58 patients recruited and fully investigated; of these 9 and 11 patients were diagnosed with AC and CVA, respectively, as single causes of chronic cough. Ten patients with BA who had not received corticosteroid therapy in the previous 4 weeks and who attended the same clinic in the same time period acted as controls. Exhaled NO levels in patients with AC were significantly lower than those in patients with CVA and BA. There was no significant difference in the exhaled NO levels between patients with CVA and BA.

Conclusions: Exhaled NO may reflect eosinophilic inflammation of peripheral airways and its measurement may be useful in differentiating CVA from AC and other causes of chronic non-productive cough.

Key words: atopic cough, bronchial asthma, cough variant asthma, exhaled nitric oxide, non-asthmatic eosinophilic bronchitis.

INTRODUCTION

Cough variant asthma (CVA) and atopic cough (AC) are major causes of chronic non-productive cough.[1] AC is a new clinical entity in Japan, in which patients present with a bronchodilator-resistant non-productive cough.[2] Bronchodilator therapy has antitussive efficacy only in CVA. The fundamental features of AC include eosinophilic inflammation of the central airways and increased cough reflex sensitivity,[3] while those of CVA are eosinophilic inflammation of the central to peripheral airways[4] and mildly increased bronchial responsiveness.[5] CVA is recognized as a precursor of asthma, which develops in nearly 30% of patients with CVA.[6,7] AC is not a precursor of asthma.[7]

Exhaled nitric oxide (NO) levels are an index of eosinophilic airway inflammation.[8] De Diego *et al.* reported that exhaled NO levels were similar in CVA and bronchial asthma (BA) patients;[9] however, exhaled NO levels have not been reported in AC

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Received: 18 March 2007; invited to revise: 8 May 2007; revised: 21 June 2007; accepted: 17 July 2007 (Associate Editor: Takahide Nagase).

patients. The present study was conducted to elucidate whether exhaled NO levels are increased in AC in comparison with CVA and BA.

METHODS

Study design

A cross-sectional observational study was used to compare patients with AC, CVA and BA. Consecutive patients presenting in the 2-year period from October 2004 to September 2006, with an isolated cough lasting at least 8 weeks, were enrolled. At the first clinic visit, patients underwent the following sequence of tests: measurement of exhaled NO concentration, capsaicin cough sensitivity and bronchial reversibility, sputum induction by inhalation of 5% saline solution, and assessment of atopic characteristics. Each patient then received bronchodilator therapy (oral clenbuterol, 40 μg/day + inhaled salbutamol sulphate, 200 μg, on demand) for 6 days until the day before the second clinic visit. At the second clinic visit, efficacy of the bronchodilator therapy on cough was assessed as described below and bronchial responsiveness to methacholine was measured. Each patient then received appropriate treatment according to the Japanese Respiratory Society (JRS) guidelines for the management of cough.[2] The study protocol was approved by the ethics committee of the Kanazawa University Hospital and all participants gave informed consent.

Control values for exhaled levels of NO were determined as the levels measured in patients with BA, who also attended the clinic during the period of study recruitment and had not been treated with inhaled or systemic corticosteroids during the previous 4 weeks. The diagnosis of BA was based on the following three criteria: (i) a history of recurrent episodes of wheezing; (ii) reversible airway obstruction documented by a physician; and (iii) an improvement of 12% or more and 200 mL or more in FEV_1 after inhalation of 300 μg of salbutamol sulphate.

Assessment of efficacy of treatment on cough

Efficacy of the treatment on cough was assessed using a visual analogue scale from 0 to 10 cm. At each clinic visit, each patient was asked to indicate which point on the scale represented their current cough experience. Response to bronchodilator therapy, which is the most important criterion for the diagnosis of CVA, was judged to be effective when the point on the visual analogue scale at the second visit, after 6-day therapy with bronchodilator, was 7 cm or less.

Diagnosis of AC and CVA

Atopic cough and CVA as sole causes of chronic cough were diagnosed according to the following criteria for the selection of subjects in clinical studies, as recommended by JRS.[2] Patients with a probable diagnosis of AC or CVA, based on the brief JRS diagnostic criteria,[2] were excluded from this study.

The diagnosis of AC was made according to the following criteria:[2]

- Non-productive cough lasting more than 8 weeks without wheezing or dyspnoea
- Presence of one or more findings indicative of an atopic constitution, including a past history and/or complications of allergic diseases excluding asthma, peripheral blood eosinophilia (>5% or >400 cells/μL), elevated total serum IgE (>150 IU/mL), positive specific IgE antibody to aeroallergens and/or induced sputum eosinophilia (≥2.5%)
- No bronchial reversibility, defined as less than a 10% increase in FEV_1 after inhalation of 300 μg of salbutamol sulphate
- Bronchial responsiveness within normal limits (PC20 ≥ 10 mg/mL)
- Increased cough reflex sensitivity (capsaicin concentration eliciting five or more coughs (C5) ≤ 3.9 μM)
- Cough resistant to bronchodilator therapy (oral clenbuterol 40 μg/day plus inhaled salbutamol 200 μg at bedtime and on demand)
- No abnormal findings indicative of cough aetiology on CXR
- Normal FEV_1 (≥80% of predicted value), FVC (≥80% of predicted value) and FEV_1/FVC ratio (≥70%)

When all criteria were satisfied, a definite diagnosis of AC was applied. All patients diagnosed with AC were successfully treated with histamine H1-antagonists alone and/or with inhaled and/or oral corticosteroids.

The diagnosis of CVA was made according to the following criteria:[2]

- Isolated chronic non-productive cough lasting more than 8 weeks
- Absence of a history of wheezing or dyspnoea, and no adventitious lung sounds on physical examination
- Absence of postnasal drip accounting for the cough
- FEV_1, FVC and FEV_1/FVC ratio within normal limits
- Presence of BHR (PC20 < 10 mg/mL)
- Relief of cough with bronchodilator therapy
- No abnormal findings indicative of cough aetiology on CXR

When all criteria were satisfied, a definite diagnosis of CVA was applied. All patients with CVA had been successfully treated with bronchodilators, the leukotriene antagonist, montelukast and/or inhaled corticosteroids.

Measurement of exhaled NO concentration

Exhaled NO concentrations were measured by the online method using a chemiluminescence analyser (model 280, Sievers Instruments, Boulder, CO, USA) according to the American Thoracic Society (ATS) guidelines,[10] between 9 AM and 1 PM at the patient's first visit. Expiratory flow rate was 0.05 L/s as recommended by the guidelines and exhalation pressure was 16 cm H_2O. Measurement of exhaled NO was repeated until three reproducible NO plateau values were achieved. Exhaled NO was then calculated as the

Table 1 Characteristics of patients with atopic cough (AC), cough variant asthma (CVA) and typical bronchial asthma (BA)

	AC	CVA	BA
Number of subjects	9	11	10
Gender (male : female)	2:7	2:9	3:7
Age (years)	42.8 ± 13.1	44.8 ± 16.1	43.0 ± 18.9
Height (cm)	164.3 ± 7.1	160.0 ± 8.8	157.9 ± 5.2
Body weight (kg)	62.7 ± 13.1	55.7 ± 8.4	55.1 ± 10.8
FVC, % predicted	119.2 ± 9.0	111.3 ± 19.6	102.4 ± 14.2
FEV_1, % predicted	103.3 ± 10.1	98.1 ± 10.7	83.6 ± 12.5***$^{\$\$}$
FEV_1/FVC (%)	80.1 ± 7.6	79.4 ± 9.9	72.1 ± 13.6
C5 (μM)	1.0 (1.20)	18.9 (1.4)****	7.8 (2.2)**
PC20 (mg/mL)	39.7 (1.52)	2.4 (1.22)****	0.81 (1.60)****$^{\$}$

**$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ compared with AC; $^{\$}P < 0.05$ and $^{\$\$}P < 0.01$ compared with CVA.
Data are presented as mean ± SD. Data in C5 are presented as geometric mean (geometric standard error of the mean).
C5, capsaicin concentration eliciting five or more coughs.

mean of these three values and exhaled NO values in 10 patients with BA were used as control values.

Pulmonary function testing

Routine pulmonary function, cough reflex sensitivity and bronchial reversibility were measured in that order at the first visit, and bronchial responsiveness was determined at the second visit, 1 week after the first visit. FVC, FEV_1 and flow–volume curves were measured using a dry wedge spirometer (Chestac 11, Chest Co. Ltd, Tokyo, Japan). Spirometry was performed and evaluated according to the ATS criteria.[11] Capsaicin cough threshold (C5) was measured as an index of cough reflex sensitivity.[12,13] To assess bronchial reversibility, spirometry was performed before and 30 min after inhalation of 300 μg of salbutamol sulphate. The provocative concentration of methacholine causing a 20% fall in FEV_1 from pre-challenge values (PC20) was measured as an index of nonspecific bronchial responsiveness.[14]

Statistical analysis

Data values for exhaled NO, capsaicin cough threshold (C5) and bronchial responsiveness (PC20) were expressed as geometric mean with geometric standard error of the mean (GSEM). Other data values were expressed as mean and SD. Differences in data values between patients with AC, CVA and BA were analysed by one-way analysis of variance (ANOVA) followed by Fisher's partial least squares difference. Data for exhaled NO, PC20 and C5 were logarithmically transformed. *P*-values < 0.05 were considered statistically significant.

RESULTS

The diagnostic procedures were completed in 58 of the 80 patients with chronic cough who were enrolled in the study. Of the 58 patients, 9 and 11 patients, respectively, met the diagnostic criteria for AC and CVA as the sole causes of chronic cough. Patient characteristics are summarized in Table 1. Duration of cough before the initial visit, successful treatment for relief of cough and time required after the first visit for relief of cough are summarized for individual patients with AC and CVA in Table 2. The atopic characteristics of patients with AC and CVA are shown in Table 3.

Exhaled NO levels in patients with AC (13.0 p.p.b., GSEM 1.14) were significantly lower than those in patients with CVA (39.4 p.p.b., GSEM 1.25, $P = 0.0007$) or BA (36.1 p.p.b., GSEM 1.26, $P = 0.0020$) (Fig. 1). There was no significant difference in the exhaled NO levels between patients with CVA and BA ($P = 0.7618$).

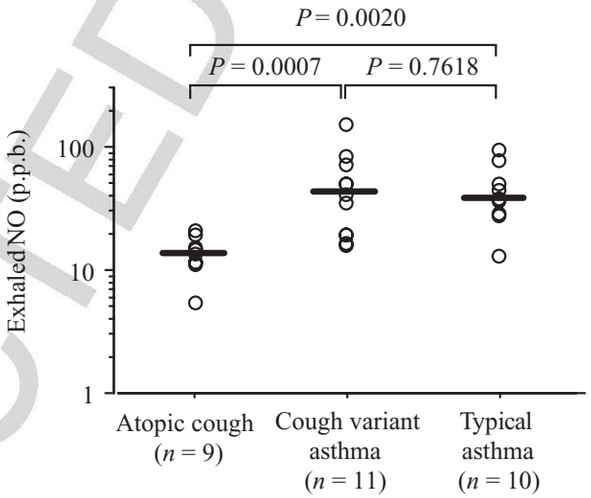

Figure 1 Exhaled nitric oxide (NO) levels in patients with atopic cough, cough variant asthma and typical bronchial asthma. Horizontal bars represent geometric mean values. *P*-values as determined by Fisher's partial least squares difference are shown.

Table 2 Results of treatment in patients with atopic cough (AC) and cough variant asthma (CVA)

Patient	Age (years)	Sex	Symptom duration (months)	Successful treatment	Time† for relief of cough (weeks)
AC					
1	52	F	8	Azelastine, FP, PSL	9
2	39	F	2	Azelastine	4
3	63	F	2	Azelastine	4
4	41	F	7	Azelastine, FP	5
5	21	M	2	FP, PSL	10
6	40	F	2	Azelastine	4
7	51	F	2	Azelastine, FP	4
8	51	M	24	Azelastine, FP	6
9	27	F	2	Azelastine	3
CVA					
1	37	F	4	Clenbuterol	1
2	41	F	2	Clenbuterol	8
3	52	F	2	Clenbuterol, montelukast	4
4	69	F	120	Procaterol, theophylline	3
5	46	F	4	Clenbuterol, salbutamol inhaled	4
6	17	F	2	Clenbuterol	6
7	30	F	5	Clenbuterol, montelukast, salmeterol inhaled	2
8	55	M	3	BDP	4
9	64	M	2	Clenbuterol, BUD, salmeterol inhaled	4
10	27	F	3	Montelukast, BUD	5
11	55	F	5	Clenbuterol, montelukast, BUD	8

†Time required to relive cough after the first visit.

BDP, beclomethasone dipropionate inhalation (200–400 μg bd); BUD, budesonide inhalation (200–400 μg bd); F, female; FP, fluticasone dipropionate inhalation (200–400 μg bd); M, male; PSL, oral prednisolone (20 mg once daily) for less than 3 weeks.

Table 3 Characteristics of atopic constitution in patients with atopic cough (AC) and cough variant asthma (CVA)

Case number	Past history	Complication	Family history	Eosinophils in peripheral blood (%)	Eosinophils in peripheral blood (/μL)	Eosiniphils in sputum (%)†	Total IgE in serum (IU/mL)	Specific IgE in serum
AC								
1	—	—	—	7.6	251	NG	11	—
2	—	—	—	1.0	42	0	32	JC
3	PO	UR	—	3.7	218	0	138	—
4	UR	—	—	0.7	43	NG	94	—
5	CBA	PO	—	3.7	283	NG	2200	HD, D, RW, JC
6	—	—	AR	2.0	138	NG	220	HD
7	—	—	—	3.3	158	NG	68	RW, JC
8	—	UR	—	4.0	176	NG	86	HD, D
9	—	PO, AD	—	8.2	558	19.5	175	JC, HD, D, CD
CVA								
1	UR	—	—	1.5	72	0	40	—
2	—	—	—	3.0	255	NG	0	—
3	—	—	—	1.0	63	NG	56	HD, RW
4	—	—	—	1.5	60	NG	58	—
5	—	AR	—	0	0	1.0	9	—
6	—	AR	BA	3.0	177	NG	0	—
7	—	—	—	6.0	360	NG	320	HD, D
8	—	—	—	10.8	756	3.6	187	JC, RW
9	AR, UR	—	—	1.0	69	NG	32	—
10	CBA	PO	AR	4.8	262	0.8	504	HD, D
11	—	—	—	1.9	110	NG	61	JC

†Per cent of nucleated cells.

AD, atopic dermatitis; AR, allergic rhinitis; BA, bronchial asthma; CBA, ••; CD, cat dander; D, Dermatophagoides; HD, house dust; JC, Japanese cedar; NG, inadequate sputum sample; PO, pollinsis; RW, ragweed; UR, urticaria.

Table 4 Comparison between atopic cough, non-asthmatic eosinophilic bronchitis and cough variant asthma

	Cough variant asthma	Atopic cough	Eosinophilic bronchitis without asthma
Physiology			
Cough reflex sensitivity	Not increased	Normal	Increased
Bronchial responsiveness	Increased but less than asthma	Not increased	Not increased
Pathology			
Eosinophils in			
Induced sputum	Increased	Increased	Increased
Biopsied bronchi	Increased same as asthma	Increased but less than asthma	Increased same as asthma
BAL fluid	Increased same as asthma	***Not increased***	***Increased same as asthma***†
Inflammatory markers			
Exhaled NO	Increased	***No increased***	***Increased***
Outcome			
Asthma onset without ICS	30%	***No***	Not investigated
with ICS	6%	Not investigated	***9%***

†Asthmatics treated with inhaled corticosteroids.
Underlined findings were from the present study.
ICS, ••; NO, nitric oxide.

DISCUSSION

The present study showed that exhaled NO levels were significantly lower in patients with AC compared with patients with CVA or BA. Normal values of exhaled NO could not be determined in this study because it was difficult to recruit gender- and age-matched, non-atopic, never-smoking normal subjects. Exhaled NO is a biomarker of eosinophilic airway inflammation, which is increased in steroid-naive asthmatic patients.[8] Two diagnostic criteria for each cause of chronic cough are recommended by the JRS.[2] One set of criteria is for the selection of subjects for clinical studies (research use) and the second set comprises brief criteria for clinical use. In the present study, only patients with AC and CVA, diagnosed according to the criteria for research use,[2] were included. The more strict diagnostic criteria for research use are applied to standardize the selection of subjects for clinical studies so that data are available to all research groups. It is difficult to satisfy all the required criteria for research use, which accounts for the small proportion of enrolled patients who actually completed the present study. Further studies using the brief diagnostic criteria for AC and CVA may be of value in assessing whether measurement of exhaled NO is useful for making the diagnosis of CVA in clinical practice.

Chronic cough is defined as isolated persistent cough lasting ≥8 weeks. CVA is strictly diagnosed when a patient with an isolated chronic cough, who has no history of wheezing or dyspnoea indicative of BA and no wheezes on lung auscultation, responds to bronchodilator therapy.[5] Although non-specific bronchial responsiveness is mildly increased in this group of patients,[5,7] there is considerable overlap with normal subjects.[15] In addition, Irwin and coworkers have shown that measurement of bronchial responsiveness cannot predict the efficacy of bronchodilator therapy in patients with isolated chronic cough.[16] Efficacy of bronchodilator therapy in preventing cough is the most important criterion for the diagnosis of CVA, and increased bronchial responsiveness is the second. This is however disputed by some chest physicians who believe that chronic cough with increased bronchial responsiveness is CVA. Therefore in the present study, patients with CVA were selected only if their cough responded to bronchodilator therapy and bronchial responsiveness was increased.

Atopic cough is a recently described clinical entity in Japan, in which patients present with a chronic isolated bronchodilator-resistant non-productive cough.[2] The fundamental features are increased cough reflex sensitivity,[3,7,15] with normal bronchial responsiveness and eosinophilic inflammation of central airways.[3] These features differ from those of CVA: increased bronchial responsiveness[5,15] and eosinophilic inflammation of central to peripheral airways.[4] CVA is a precursor of typical asthma but AC is not.[7]

In many countries non-asthmatic eosinophilic bronchitis (EB) is being accepted as a clinical entity in patients presenting with chronic cough that responds to corticosteroid therapy.[17] The diagnostic criteria are sputum eosinophilia and normal bronchial responsiveness.[17] It has been reported that in such patients with EB, cough reflex sensitivity is increased,[18] eosinophils in BAL fluid are increased[19,20] and onset of asthma is typical.[21] Two studies have shown that exhaled NO levels in patients with EB are increased to the same levels as those in patients with BA.[20,22] It appears that there are differences between AC and EB (Table 4) and that AC is different from EB. The findings that exhaled NO and eosinophils in BAL fluid are increased in BA and EB, but not in AC, suggest that exhaled NO reflects eosinophilic inflammation of peripheral airways, which have a much larger surface area than the central airways.

De Diego *et al.* compared airway inflammatory markers in patients with typical asthma and those with CVA, which was diagnosed by both increased bronchial responsiveness and relief of cough with bronchodilator therapy, as in the present study.[9] The authors showed that there was no difference in exhaled NO, cough reflex sensitivity or bronchial responsiveness in typical asthma compared with CVA. Similar levels of exhaled NO and cough reflex sensitivity were confirmed in the present study, while bronchial responsiveness was increased in typical asthma compared with CVA. It is likely that typical asthma was more severe in the present study compared with the study of De Diego and coworkers. Based on this, it is concluded that exhaled NO levels are increased in CVA to the same levels seen in steroid-naive typical asthma.

In conclusion, this study confirmed that the pathogenesis of AC is different from that of CVA or EB, and suggests that exhaled NO may reflect eosinophilic inflammation of peripheral airways. The lower levels of exhaled NO in AC may be useful in differentiating it from CVA, EB and other causes of chronic nonproductive cough.[23]

ACKNOWLEDGEMENT

This study was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture (17607003) by the Japanese Government.

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