

Sputum eosinophilia in cough-variant asthma as a predictor of the subsequent development of classic asthma

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Summary

Background A significant proportion of patients diagnosed with cough-variant asthma eventually manifest classic asthma signs, such as wheezing and dyspnoea. The aim of this study was to investigate whether the percentage of eosinophils and/or concentration of eosinophilic cationic protein (ECP) in sputum induced from patients with cough-variant asthma can predict the development of classic asthma.

Methods Sixty-two children with cough-variant asthma were prospectively studied for 4 years. At the initiation of the study, sputum was induced with hypertonic saline, and the sputum samples were analysed for total and differential cell counts, and for ECP. Each subject was checked clinically at least every 3 months, and details of classic asthma signs experienced during the intervening periods were taken.

Results Twenty-four (47.1%) of the 51 subjects available for follow-up developed signs of classic asthma, while 27 did not. The only significant difference in the sputum parameters between these two groups was a higher percentage of sputum eosinophils in subjects who developed classic asthma. A significant association was found between sputum eosinophil percentage and classic asthma development, but not between the concentration of sputum ECP and classic asthma development.

Conclusion Sputum eosinophilia in cough-variant asthma may be a correlate of the later development of classic asthma. This suggests that sputum differential cell counts may be useful in the clinical management of patients with cough-variant asthma, as they may enable the prediction of the subsequent classic asthma development.

Keywords classic asthma, cough-variant asthma, ECP, eosinophil, induced sputum

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Introduction

Cough-variant asthma is an occult form of asthma, in which the only sign or symptom is a chronic cough [1]. This type of asthma was initially described as uncommon, but it now appears that cough-variant asthma is one of the most common causes of chronic cough in children [2], as well as in adults [3].

Some studies have indicated that cough-variant asthma may be a forerunner of classic asthma, i.e. many patients who initially present with cough-variant asthma eventually manifest the classic signs of asthma [4–6]. The follow-up of such patients has shown that between one-third [4] and two-thirds [6] go on to develop episodic wheeze and dyspnoea. However, it is not known as to which patients with cough-variant asthma are at a greater risk of developing these signs.

Asthma is a chronic inflammatory airway disease, in which eosinophils play a central role [7]. Studies of airway inflammation in cough-variant asthma have shown that the percentage of eosinophils and the concentrations of eosinophilic cationic protein (ECP), one of the activation markers of

eosinophils, are increased in the sputum or bronchoalveolar lavage fluid (BALF) [8, 9], as reported in classic asthma [10, 11]. It is well recognized that the intensity of eosinophilic inflammation correlates with the clinical severity of asthma [10]. The number of eosinophils and their activation markers are increased during asthmatic exacerbation [12]. In addition, asthmatic subjects who developed subsequent exacerbation have been shown to have significantly higher sputum eosinophil counts at baseline [13]. Based on these results, we hypothesized that the intensity of eosinophilic inflammation in patients with cough-variant asthma may serve as a marker of the subsequent development of classic asthma.

The aim of this study was to determine whether the percentage of eosinophils and/or the concentration of ECP in induced sputum from patients with cough-variant asthma can predict the subsequent development of classic asthma.

Methods

Subjects

Children diagnosed as having cough-variant asthma were enrolled in this study. Initially, patients were referred to our

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clinics for their cough, which had persisted for a minimum of 2 months (range: 9 weeks to 2 years). The cough was usually dry or productive with a scant amount of clear sputum and was mostly nocturnal. None of the patients had a history of wheezing or dyspnoea, nor was any wheeze or prolonged expiratory phase detected on physical examination. Cough-variant asthma was diagnosed when the patients met the following three criteria: (1) bronchial hyper-responsiveness (BHR), (2) an increased peak expiratory flow (PEF) variability, and (3) a favourable response of the cough to asthma therapy. A methacholine inhalation test was performed using a modification of the method described by Chai et al. [14]. Lung function was measured with a computerized spirometer (Microspiro-HI 298; Chest, Tokyo, Japan). To be eligible for the study, forced expiratory volume in 1 s (FEV₁) had to be 80% or more of the predicted value [15]. A Rosenthal-French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA), triggered by a solenoid value set to remain open for 0.6 s, was used to generate the aerosol from a DeVilbiss 646 nebulizer (DeVilbiss Health Care, Somerset, PA, USA), with pressurized air at 20 psi. Each subject inhaled 5 inspiratory capacity breaths of buffered saline and increasing concentrations of methacholine until the FEV₁ fell by more than 20% of its post-saline value. The provocative concentration causing a 20% fall in FEV₁ (PC₂₀) was obtained from the log concentration–percent fall in FEV₁ curve by linear interpolation of the last two points. Subjects were considered to have BHR if their PC₂₀ was less than 18 mg/mL. Each participant was asked to record PEF mornings and evenings for 14 consecutive days using a Mini-Wright adult-type peak-flow meter (Clement Clarke Ltd, London, England). Morning values were obtained just after rising and evening values between 17:00 and 19:00 hours. In subjects using bronchodilators, premedication PEFs were recorded. Before starting the measurements, subjects were carefully trained by a nurse, and the manoeuvre was rehearsed until reproducibility, within 20 L/min, was obtained. The amplitude percent mean was used to summarize diurnal PEF variation for each subject, and a mean of 14 days was then calculated. An increased PEF variability was defined as >9.26% [16]. Bronchodilators (inhaled β_2 -agonists and/or oral theophylline) were effective against their coughs, but these recurred when the medication was stopped. Normal results were obtained for the following tests: chest radiographs, sinus films, and tuberculin skin tests. No other apparent causes of cough were identified in the subjects. A skin prick test was performed, and atopy was defined by the presence of at least one positive reaction (>3 mm weal diameter) to a battery of 12 common airborne allergens.

Study design

At the initiation of the study, sputum was induced. For comparison, a separate group of 15 healthy subjects with no history of asthma or other allergic diseases, and no identifiable airway infections for a 4-week period prior to the study, served as controls. These control subjects underwent sputum induction at the time of their sole visit and were not asked to return for further assessment. Subjects with cough-variant asthma were followed at our clinics for 4 years. For the follow-up, they were given peak-flow meters and

inhaled bronchodilator (salbutamol), and were asked to record symptoms of asthma (wheeze at rest or on exertion, shortness of breath, or chest tightness) and to measure the PEF rate before and after salbutamol (200 µg) inhalation, when these symptoms occurred. Subjects attended clinics every 3 months, whenever possible, for clinical assessment and medication adjustment. At each attendance, subjects were evaluated by one of the authors, who had no knowledge of sputum results, concerning their symptoms and signs experienced in the interim and given a physical examination, if appropriate. The development of classic asthma was accepted according to a modification of the criteria described by Prieto et al. [17], that is, when a subject had typical symptoms (episodic wheezing or dyspnoea) and showed at least a 15% increase in PEF after inhaled salbutamol.

Parents gave written informed consent for their children to participate in the study, and the study protocol was approved by the hospital ethics committee.

Sputum induction and processing

Sputum was induced with an aerosol of hypertonic saline using a slight modification of the method described by Pizzichini et al. [18]. Inhaled salbutamol at 200 µg was given via MDI 15 min before sputum induction. Hypertonic saline (3%, 4%, and 5%) generated by an ultrasonic nebulizer (Ultraneb 2000, DeVilbiss, Somerset, PA, USA) was nebulized at each concentration for 7 min. The output of the nebulizer was calibrated at 2.5 mL/min. At the start and after each period of inhalation, FEV₁ was measured for safety reasons. After inhalation of the hypertonic saline, subjects were asked to blow their nose, rinse their mouth, and swallow the water to minimize contamination from post-nasal drip and saliva. The subjects were then instructed to cough sputum into a sterile container. The volume of the entire sputum sample was measured, and an equal volume of 0.1% dithiothreitol (DDT) (Sputolysin 10%; Calbiochem Corp., San Diego, CA, USA) was added. The samples were then mixed gently using a vortex mixer and placed in a shaking water bath at 37 °C for 15 min to ensure complete homogenization. The resulting suspension was filtered through a 48-µm nylon gauze, and a total leucocyte cell count was performed, and cell viability (trypan blue exclusion method) was determined. The filtrate was centrifuged at 2000 × *g* for 4 min, and the supernatant was aspirated and stored in Eppendorf tubes at –70 °C for later ECP assay. The cell suspension was adjusted to 1.0 × 10⁶/mL, and cytocentrifuge preparations were made with 60 µL of cell suspension (Shandon III cytocentrifuge; Shandon Southern Instruments, Sewickley, PA, USA). The slides were stained with the May–Grunwald–Giemsa stain, and a 400 differential non-squamous cell count was performed. Results were expressed as a percentage of the total non-squamous cell count. Samples containing more than 30% squamous cells were excluded from the analysis.

Measurement of ECP

The concentration of ECP in the sputum supernatant was measured by a radioimmunosorbent assay (RIA) using an ECP radioimmunoassay kit (Pharmacia Diagnostics, Uppsala-

la, Sweden). The detection limit of this kit was 2.0 µg/L, and the assay was run in duplicate; the mean values were used for the statistical analysis. Levels of ECP were expressed as weight per volume of sputum.

Statistical analysis

Results are expressed as median (range), unless otherwise stated. The demographic or clinical data of two groups were compared with the Student's *t*-test or the Wilcoxon rank sum test. Proportional differences between two groups were analysed by the χ^2 -test. Comparisons of cell counts, cell type percentages, and ECP levels between two groups were performed with the Mann-Whitney test. The score test for trend was used to evaluate the potential relationship between the development of classic asthma and either eosinophil percentage or the ECP level. All analyses were carried out using Stat View II (Abacus Concept Inc., Berkeley, CA, USA) on a Macintosh computer (Apple Computer Inc., Cupertino, CA, USA). A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Sixty-two subjects with cough-variant asthma and 15 healthy controls successfully underwent sputum induction procedure. Of the 62 enrolled subjects with cough-variant asthma, 11 were lost to follow-up, despite every effort being made to continue the regular check-ups. The clinical characteristics of the subjects who completed the follow-up, and of the subjects who dropped out, are summarized in Table 1. Completing and non-completing participants were similar regarding all measured clinical characteristics and sputum results (data not shown), thus suggesting a low risk of dropout bias in the present study. Those who developed classic asthma during the course of follow-up (*n* = 24) were designated as Group 1, and those who did not (*n* = 27) as Group 2. Among the Group 1 patients, seven of the 24 developed classic asthma in the first year, seven in the second year, six in the third year, and four in the fourth year. Among the Group 2 patients, 14 subjects continued to suffer from a persistent cough for at least 2 weeks every year, 10 subjects alternated between 'cough' year and 'symptom-free' year during the follow-up, and three subjects were symptom-free during the last 2 or 3 years of the study. The two groups did not exhibit any significant difference with respect to age, sex, frequency of atopy, methacholine PC₂₀, or PEF variability.

Sputum data of the two cough-variant asthma groups and of the control group are shown in Table 2. Groups 1 and 2 had a higher percentage of eosinophils, and higher levels of ECP than the controls. No differences in the total cell count or in all other cell types were found. On comparing the two cough-variant asthma groups, Group 1 was found to have a higher percentage of sputum eosinophils than Group 2. The total cell count and all other cell types showed no significant differences between the two groups. Sputum ECP levels in Group 1 were higher than those in Group 2, although this did not reach statistical significance (*P* = 0.084).

To further evaluate the association between eosinophil percentages or ECP levels and the development of classic asthma, the risk of development of classic asthma was assessed in subjects classified according to eosinophil percentage or ECP levels at the time of entry into this study. As the eosinophil percentage and ECP concentration distributions were not normal, the medians and the upper quartiles of the parameters were used as cut-off points. In the analysis of the groups about a median eosinophil percentage of 4.8% (Fig. 1), seven (28%) of 25 subjects with less than 4.8% of eosinophils developed classic asthma, compared with 17 (65.4%) of 26 subjects with eosinophils above 4.8% (*P* = 0.008). Similarly, 14 (36.8%) of 38 subjects with less than 8.3% of eosinophils (the upper quartile) developed classic asthma, compared with 10 (76.9%) of 13 subjects with eosinophils above 8.3% (*P* = 0.012). Using a cut-off point of 2.5%, which is considered as the upper limit of normal [19], similar figures were obtained (2/13 [15.4%] vs. 22/38 [57.9%], *P* = 0.008). The score test for trend in the four groups formed by the above cut-off points showed a significant association between sputum eosinophil percentage and the development of classic asthma (*P* = 0.002, Fig. 1). Table 3 shows the results of sensitivity, specificity, predictive values, and relative risks of the three cut-off points of eosinophil percentage in predicting the development of classic asthma. Obviously, as the cut-off point of eosinophil percentage increases, specificity improves, but sensitivity becomes low. The probability of development of classic asthma increased with increasing eosinophil percentage values. Examination of the eosinophil percentage by receiver operating characteristic (ROC) curve revealed that the area under the curve was 0.752 (95% CI: 0.618–0.885) and that 10.4% had the best sensitivity/1 – specificity ratio to predict the development of classic asthma.

In the analysis of the groups about prescribed ECP levels, the frequency of subjects who developed classic asthma was no different between the two groups based on a median value

Table 1. Baseline characteristics of cough-variant asthma groups

	Group 1	Group 2	Dropped out
Number	24	27	11
Age (years)*	11.4 ± 2.3	11.0 ± 2.5	11.6 ± 2.9
Sex (M/F)	14/10	15/12	6/5
Atopy, <i>n</i> (%)	15 (62.5%)	15 (55.6%)	6 (54.5%)
Methacholine PC ₂₀ †	5.79 (2.70 – 12.42)	5.64 (2.66 – 11.94)	5.61 (2.55 – 12.33)
PEF variability*	11.2 ± 1.8	11.0 ± 1.8	11.5 ± 1.8

*Mean ± SD. †Geometric mean (range of 1 SD).

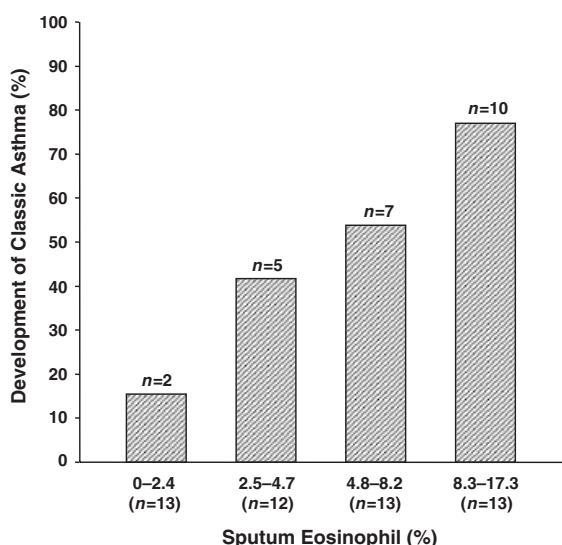


Fig. 1. Risk for development of classic asthma according to sputum eosinophil percentage at the initiation of study. The eosinophil percentage and the development of classic asthma were significantly associated (score test for trend: $P = 0.002$).

division ($28.5 \mu\text{g/L}$; 10/25 [40%] vs. 14/25 [56%], $P = 0.258$) or on an upper quartile division ($46.1 \mu\text{g/L}$; 16/38 [42.1%] vs. 8/13 [61.5%], $P = 0.226$). The score test for trend showed that the development of classic asthma was not predicted by the ECP level ($P = 0.223$; data not shown).

Discussion

Our results have shown that the percentage of eosinophils and the concentrations of ECP in induced sputum were signifi-

cantly increased in patients with cough-variant asthma as compared with normal controls. In addition, a high percentage of sputum eosinophils in patients with cough-variant asthma was found to be a risk factor for the development of classic asthma, but a high concentration of ECP in induced sputum was not predictive in this regard.

Previous investigators have suggested that many patients who initially present with cough-variant asthma eventually develop mild to moderately severe manifestations of classic asthma. Corrao et al. [4] were the first to appreciate the subsequent development of classic asthma in two adult subjects with cough-variant asthma. Hannaway and Hopper [6], and König [5] found that typical attacks of wheezing developed in a significant number of children (80% and 75%, respectively) when they were followed for several months to 8 years. In a previous study of a group of different subjects, we also observed that 16 of 29 children with cough-variant asthma began to wheeze within 4 years of follow-up [20]. The proportion of subjects who developed the classic signs of asthma in the current study, 47.1%, cannot be compared directly with those of the above-mentioned studies, because of differences in subject recruitment, documentation of classic signs of asthma, and the follow-up period.

Our patients were carefully screened at the onset of this study for any current or past history of wheezing or dyspnoea, whether reported or auscultated, to eliminate hidden or unrecognized cases of classic asthma. The diagnosis of cough-variant asthma was accepted only when positive results on bronchial challenge and documentation of increased PEF variability were followed by a favourable response of cough to asthma therapy. The principal weakness of our study is the potential for misclassifying subjects. The development of classic asthma was accepted if the subject had typical symptoms (wheezing or dyspnoea) and at least a 15%

Table 2. Sputum total and differential cell counts, and eosinophil cationic protein (ECP) concentrations in the studied groups

	Group 1 ($n = 24$)	Group 2 ($n = 27$)	Control group ($n = 15$)
Total non-squamous cells ($\times 10^6/\text{cm}^3$)	1.24 (0.14–4.32)	1.16 (0.19–5.13)	0.94 (0.11–3.23)
% Macrophages	50.4 (28.3–84.0)	53.8 (30.5–81.3)	64.5 (30.3–85.0)
% Neutrophils	17.2 (3.8–41.8)	20.0 (4.3–43.3)	16.5 (1.3–52.5)
% Lymphocytes	2.8 (0.3–5.8)	3.5 (0–12.3)	1.8 (0.5–7.8)
% Eosinophils	7.3 (0.8–17.3)*†	3.0 (0–12.8)*	0.3 (0–2.3)
% Epithelial cells	6.4 (0.8–19.5)	8.3 (1.3–20.5)	8.8 (0.5–23.0)
ECP ($\mu\text{g/L}$)	34.9 (2.8–83.2)*	25.8 (2.0–66.2)*	4.9 (2.0–17.3)

Results are expressed as median (range). * $P < 0.01$ compared to the control group. † $P < 0.01$ compared to Group 2.

Table 3. Discriminative properties of sputum eosinophil percentage at different cut-off points for predicting the development of classic asthma

Eosinophil percentage	Sensitivity (95% CI)	Specificity (95% CI)	+PV	–PV	Relative risk (95% CI)
2.5% ≤	91.7 (73.0–99.0)	40.7 (22.4–61.2)	57.9	84.6	1.55 (1.11–2.16)
4.8% ≤	70.8 (48.9–87.4)	66.7 (46.0–83.5)	65.4	72.0	2.13 (1.18–3.84)
8.3% ≤	41.7 (22.1–63.4)	88.9 (70.8–97.6)	76.9	63.2	3.75 (1.17–12.05)

CI: confidence interval; +PV: positive predictive value; –PV: negative predictive value.

increase in PEF after inhaled salbutamol [17]. This strict criterion undoubtedly excluded some mild episodes of classic asthma, since such episodes could have been affected by the circumstances of the event, the perceptions of patients or parents, or by their enthusiasm to document. However, using a conservative criterion reduces the chance of misidentifying non-specific respiratory symptoms or doubtful occasions as being the development of classic asthma. A 4-year period was chosen for the follow-up. It is possible that follow-up beyond the chosen 4-year period may have revealed additional cases and that other prognostic factors could have been identified.

There have been several studies characterizing the nature of inflammation in the airways of patients with cough-variant asthma. Carney et al. [21] reported that an increased eosinophil count in induced sputum was observed in three of six patients with a chronic cough and BHR, who might be given a diagnosis of cough-variant asthma. In a comparative study, Niimi et al. [8] found that the serum ECP level and the percentage of eosinophils in BALF and in biopsy specimens were elevated and comparable to those found in patients with classic asthma associated with wheeze. In accordance with these studies [8, 21], we also found that eosinophil percentages and ECP levels are raised in the induced sputum of patients with cough-variant asthma as compared with those found in normal children. The eosinophil percentages and ECP levels reported in the present study are of the same order as those in subjects with mild classic asthma, as previously reported [22].

In the present study, the eosinophil percentage in sputum was higher among those who developed classic asthma than those who did not. Furthermore, a significant association was found between the eosinophil percentage and the development of classic asthma at follow-up, when the median and the upper quartile of the eosinophil percentage were used as cut-off points. On the contrary, ECP levels in sputum did not predict as to which patients with cough-variant asthma would develop the classic signs of asthma. This discrepancy between the eosinophil percentage and the ECP levels in sputum, in terms of the association with the future development of classic asthma, may be due to the reduced accuracy of the sputum ECP assay as compared with sputum eosinophils [23]. We did not repeat sputum induction when the subjects developed classic asthma. Therefore, we cannot answer the question as to whether the occurrence of wheezing or dyspnoea is linked or not to an increase in eosinophil influx into the airways. Further studies are needed to resolve this issue.

In addition to sputum parameters, we examined various clinical features that we believed might be important in predicting the development of classic asthma. BHR is known to be an important risk factor for the development of asthma [24]. In our previous longitudinal study [20], the development of wheezing in patients with cough-variant asthma was found to be associated with an increase in BHR. It is tempting, therefore, to speculate that the degree of BHR may be a reliable marker of the subsequent development of classic asthma. Studies have shown a relation between asthma and PEF variability [25], and changes in PEF variation during long-term treatment with inhaled corticosteroids correlated with an improvement in symptoms [26]. In the present study, however, neither the baseline methacholine PC₂₀ level nor PEF variability was statistically different between those who developed classic asthma and those who did not. This might

have been due to the narrow ranges of both parameters in our study population. There is a close association between atopic status and the presence of asthma [27], and atopy can heighten BHR when this is present [28]. However, no significant difference in the frequency of atopy was found between the two studied groups.

An increased number of eosinophils in the bronchi may reflect an overall increase in inflammatory activity, resulting in the increased production of cytokines that activate mast cells or other inflammatory cells [29]. We speculate that subjects with marked eosinophilia have an increased risk of developing wheezing or dyspnoea, if they experience a degree of airflow obstruction that is sufficient to induce these symptoms, on exposure to allergic or non-allergic stimuli. Under-treatment of asthma may result not only in the poor control of symptoms but also in irreversible pathological airway changes, which are assumed to be due to persistent inflammation [30]. Recently, increased thickness of the bronchial basement membrane was reported in patients with cough-variant asthma [31], indicating that a similar process of airway remodelling, as is observed in asthma, is present in cough-variant asthma. Early anti-inflammatory treatment may therefore be indicated in patients with cough-variant asthma, in order to prevent not only progression into classic asthma but also a long-term sequela. This issue should be investigated in future studies.

In conclusion, sputum eosinophilia in cough-variant asthma may be a correlate of the later development of classic asthma. This suggests that sputum differential cell counts may be useful in the clinical management of patients with cough-variant asthma, as they may enable the prediction of the subsequent classic asthma development.

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