Relationship between the perception of dyspnoea and airway inflammatory markers

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**Abstract** Poor dyspnoea perception in asthmatic patients seems to be associated with increased risk of asthma exacerbation. We have studied the relationship between baseline dyspnoea perception and inflammatory markers in sputum in eight patients with mild asthma and in 13 patients with moderate to severe asthma. The perception of dyspnoea was scored on the Borg scale. Eosinophilic cationic protein (ECP) was measured by fluoroenzymoimmunoassay and by an interleukin (IL)-5 sandwich ELISA. The baseline Borg score was significantly higher in patients with severe asthma than in patients with mild to moderate asthma (4 ± 0.29 vs. 2.28 ± 0.28, P < 0.05). The proportion of eosinophils and ECP levels in the sputum were significantly higher in patients with moderate to severe asthma. IL-5 in sputum was significantly increased in moderate to severe asthmatic patients compared to mild asthmatic patients. A significant relationship was found between the baseline perception score and FEV1/FVC (r = −0.53, P < 0.01), sputum eosinophils (r = 0.70, P < 0.01) and sputum ECP (r = 0.62, P < 0.01). These findings suggest that the baseline perception score is related to inflammatory markers in sputum, and that the perception of dyspnoea as well as airway inflammatory markers may be considered to evaluate asthma severity.

INTRODUCTION

Bronchial asthma is defined as a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. In particular, mast cells, eosinophils, T lymphocytes, neutrophils and epithelial cells have been implicated. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and in the early morning (1).

Patients with severe asthma seem to have a reduced ability to perceive airflow obstruction. Perception of dyspnoea during breathing through a series of tubes with increasing resistance was significantly decreased in patients with a history of near-fatal asthma attacks, as compared with patients who had asthma of similar severity but no history of such attacks (2,3). The authors reported that inhalation of a short-acting β2-agonist decreased dyspnoea, but increased the perception of dyspnoea induced by a resistive load in patients with asthma (4). Markers of airway inflammation in induced sputum were correlated with clinical and physiological variables (5,6).

In the present study we hypothesized that airway inflammation in patients with asthma may influence perception of dyspnoea. The aim of this study was therefore to evaluate the relationship between the perception of dyspnoea and inflammatory markers in sputum.

METHODS

Subjects

Twenty-one patients with asthma were enrolled for this study (Table I). The diagnoses of asthma were established in the patients by their symptoms of recurrent episodic wheezing, cough and/or dyspnoea, accompanied either by methacholine airway hyper-responsiveness or by a significant improvement of forced expiratory volume in 1 sec (FEV1) (15%) following anti-asthma therapy. The severity of asthma (8) was classified according to current symptoms (cough, wheeze, dyspnoea), prescribed asthma medication and results of methacholine provocation test. Sixteen patients were hyper-responsive to inhaled methacholine as shown by a provocative concentration
to cause a 20% fall in FEV₁ (PC₂₀). The patients with severe asthma consisted of six patients who had two or more exacerbations during the preceding year requiring treatment with oral corticosteroids despite high-dose inhaled corticosteroid therapy (budesonide 800 μg twice daily or fluticasone propionate 500 μg twice daily). The patients with moderate asthma consisted of seven patients who had inhaled high dose corticosteroid therapy (budesonide 800 μg twice daily or fluticasone propionate 250 μg twice daily). The patients were recruited from our outpatient allergy department. Most of them had visited our hospital for many years, mostly on a 3–6 month basis and during exacerbations. Inhaled corticosteroids were routinely tapered to lowest possible doses. All subjects had inhaled short-acting β₂-agonists on demand as rescue medication. The subjects were studied prior to corticosteroid treatment during an exacerbation. No subject had respiratory infection for 4 weeks prior to the study. All subjects were informed and gave their consent before starting the study. The ethics committee of our hospital approved the study protocol.

**Scoring of dyspnoea**

The scoring of dyspnoea perception on admission was measured using a modified Borg scale (9). This is a linear scale of the numbers ranking the magnitude of difficulty in breathing, ranging from 0 (none) to 10 (maximal) (Table I).

**Bronchial hyper-responsiveness**

Methacholine challenge tests were carried out by a modified method described by Chai et al. (10). Concentrations of 0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5.0 and 25 mg ml⁻¹ methacholine were prepared by dilution with buffered saline. A Micro-dosimeter (S&M Instrument Co, Doylestown, PA, U.S.A.) was used to deliver the aerosol generated by a DeVilbiss 646 nebulizer. Subjects inhaled five breaths of increasing concentrations of methacholine until FEV₁ fell by more than 20% of its basal value or the highest concentration was reached. The higher value of these FEV₁ at (30 or 90 and 180 sec after each inhalation) was adopted for analysis.

**Sputum induction and processing**

Sputum was induced only when it could not be produced spontaneously. The sputum induction was performed as a modification of the method described by Fahy et al. (11). All subjects were premedicated with two puffs of inhaled salbutamol (200 μg). Subjects inhaled 3% hypertonic saline solution aerosols generated by a ultrasonic nebulizer (NE-U03, OMRON Co., Tokyo, Japan) with maximum output of 0.15–0.3 ml min⁻¹ and mass median aerodynamic diameter of 4.5 μm. Hypertonic saline was inhaled for 25–30 min according to the severity of asthma until adequate volume of sputum was expectorated. They were instructed to cough the sputum into a sterile plastic container. The volumes of samples and duration of sputum induction were recorded. FEV₁ was measured before, during and after induction of sputum. Sputum induction was stopped in subject with a fall of the FEV₁ ≥15%.

Sputum was selected from saliva and processed within 2 h. The method of sputum examination described by Popov et al. (12) was modified. Sputum was treated by adding equal volumes of 0.1% dithiothreitol (Sputalysin 10%; Gibco BRL, U.S.A.) followed by equal volumes of Dulbecco’s phosphate-buffered saline (D-PBS). The sample was then mixed gently and placed in a shaking water bath at 37°C for 15 min to ensure complete homogenization. The sample was removed from the water-bath periodically for further brief gentle mixing. The suspension was filtered through a gauze (1 mm pore size), the filtrate was centrifuged at 1500 rpm for 10 min and the supernatant was aspirated and stored in Eppendorf tubes at −70°C for later assay. The cell pellet was resuspended in D-PBS, 1000 μl and total non-squamous cells were counted in a modified Neubauer haemocytometer. The cell suspension was adjusted to 0.5 × 10⁶ ml⁻¹ and then 50 μl of cell suspension was placed into cups of Sakura cytocentrifuge (Model CF-127, Tokyo, Japan). Two coded cytospins were prepared at 600 rpm for 5 min, air-dried and stained by Diff-Quick (Kookje Scientific Products, Japan) stain. Cell differentials of 400 non-squamous cells were performed in Diff-Quick stain slides by two investigators who did not know the subject’s history, and results were expressed as a percentage of the total non-squamous cell count.

**ECP and IL-5 measurement**

The concentration of ECP in 400 μl in the supernatant of induced sputum was determined using fluoroimmunoas-
say (UniCAP system). IL-5 was measured by quantitative sandwich enzyme immunoassay (QuantikineTM; R&D Systems, Inc., MN, U.S.A.) as described by Dickason (13). Samples were analysed in duplicate. The limits of detection for ECP and IL-5 assays were 2.0 µg l⁻¹ and 3 pg ml⁻¹, respectively.

**Blood sampling**

Venous blood was collected into tubes containing 5·0 ml ethylenediaminetetraacetic acid (K3 Vacutainer BD, Rutherford, NJ, U.S.A.) before sputum induction. Differential white blood cell count was obtained with use of a Coulter STKS instrument (Coulter Corp., Hialeah, FL, U.S.A.)

**Statistical analysis**

All data were analysed using the SPSS version 7.5 for Windows. Data are expressed as mean ± SEM. We used the Mann–Whitney U-test for unpaired data to perform statistical analysis. Spearman’s correlations with non-normal distributions were used to assess relationships between variables. A P-value of <0.05 was considered significant.

**RESULTS**

The characteristics of the subjects including demographics, asthma severity are shown in Table 2. The baseline Borg score was significantly higher in patients with severe asthma than patients with mild to moderate asthma (2.28 ± 0.28 vs. 4.1 ± 0.29, P < 0.05; Fig 1).

The proportion of eosinophil and ECP level in sputum were significantly higher in patients with moderate to severe asthma compared to those with mild asthma (5·4 ± 1·3% vs. 38·5 ± 5·6%, P < 0.01; 208·5 ± 52·0 µg l⁻¹ vs. 152·0 ± 253·5 µg l⁻¹, P < 0.01). IL-5 in sputum was significantly higher in patients with moderate to severe asthma compared to patients with mild asthma (32·7 ± 6·5 pg ml⁻¹ vs. 0 pg ml⁻¹). A significant relationship was found between the baseline perception score and FEV₁/FVC (r = 0·53, P < 0.01; Fig. 2) as well as between baseline perception score and both sputum eosinophil percentage (r = 0·70, P < 0.01; Fig. 3) and sputum ECP (r = 0·62, P < 0.01). However, no relationship was found between baseline perception score and serum eosinophils or serum ECP.

**DISCUSSION**

This study has demonstrated that the dyspnoea perception score is related to inflammatory markers in sputum, which suggests that the perception of dyspnoea as well as airway inflammatory markers may be considered to evaluate the clinical severity of asthma.

Poor perception in asthma may lead to a delay in starting appropriate asthma treatment, which is probably one of the key factors contributing to death from asthma.
Recent studies have reported that blunted perception of acutely induced bronchoconstriction (15), and less severe breathlessness induced by inspiratory loads, was detected in near fatal attack patients (16). We have also reported that inhalation of a short-acting $\beta_2$-agonist treatment decreases dyspnoea, but increases perception of dyspnoea induced by a resistive load in patients with asthma (4).

The severity of dyspnoea is shown to be greater in response to hypertonic saline as compared with methacholine at the same reduction of FEV₁ (17), which might be explained by differences in perception due to inflammatory activity within the airways (17,18). An association between perception of breathlessness and airway inflammation has previously been reported, as measured by eosinophilic infiltration and epithelial shedding in bronchial biopsies from patients with asthma (19). However, there have been no published data comparing baseline perception score with airway inflammation markers in sputum. In the present study, the baseline Borg score was significantly higher in patients with severe asthma compared with patients with mild to moderate asthma. According to asthma severity, the proportion of eosinophil and ECP levels in sputum were significantly increased in patients with moderate to severe asthma than in those of mild asthma. IL-5 in sputum was also significantly increased in moderate to severe asthmatic patients compared to mild asthmatic patients. These results support the idea that awareness of perception of dyspnoea is correlated with sputum inflammatory markers, and that increased eosinophilic inflammation is associated with more pronounced dyspnoea score. Further studies are needed to evaluate the relationship between perception score and inflammatory marker changes after long-term anti-inflammatory control medication.

In't Veen et al. (20) reported that increased sputum eosinophilia is an indicator of clinical instability, and that eosinophilic airways inflammation might affect dyspnoea perception in severe asthma. In this study the proportion of eosinophil in sputum was increased in patients with severe asthma, suggesting that sputum eosinophils might be a valuable marker of clinical severity in asthma. Roisman et al. (19) reported that perception of bronchoconstriction in asthmatic patients is related to eosinophilic inflammation and to epithelial damage in airways, and that corticosteroid treatment is associated with improved perception of bronchoconstriction induced by bradykinin. The sensation of dyspnoea was also found to be significantly related to the FEV₁ and the specific inspiratory resistance in the asthmatics (21). This study shows a significant relationship between the baseline perception score and FEV₁/FVC, sputum eosinophils and sputum ECP, which is in agreement with the results of Roisman et al. (19). This study shows that perception of breathlessness associated with bronchoconstriction may be influenced by the degree of eosinophilic infiltration, which is related to the clinical severity of asthma.

Low baseline FEV₁ and high bronchial responsiveness are associated with a low degree of ‘perceptiveness’ for bronchoconstriction, suggesting that patients with a more severe degree of asthma either show adaptation of ‘perceptiveness’ for airway obstruction or that low perceptiveness leads to more severe asthma. Although asthmatic patients poorly perceive dyspnoea, dyspnoea perception is related to inflammatory markers (22). Therefore, we suggest that the perception of dyspnoea may be considered in evaluating of clinical severity of asthma.

The degree of inflammation in airways as well as asthma severity depends on the anti-inflammatory load. Anti-leukotrienes and inhaled corticosteroid might affect the perception differently depending on different targeting and mechanism of action, and possibly side-effects. In this study the effect of sputum on anti-inflammatory therapy was small because sputum induction was studied before corticosteroid therapy.

In conclusion, we found that the baseline perception score is related to inflammatory markers in sputum, suggesting that the perception of dyspnoea as well as airway inflammatory markers may be considered to evaluate the clinical severity of asthma.
Acknowledgements

We thank Mrs Park for technical advice.

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