Dyspnea, respiratory function and sputum profile in asthmatic patients during exacerbations

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Abstract  Dyspnea is often used as a marker of asthma severity although a wide variation in dyspnea perception associated with bronchoconstriction (PB) has been described in asthmatic patients. Our hypothesis is that changes of airway inflammation, airway narrowing and hyperinflation may account for a part of the variability of breathlessness in spontaneous asthma attack. In asthmatic patients with exacerbation of the disease, we evaluated respiratory function, dyspnea (using visual Analogue Scale — VAS) and peak expiratory flow (PEF) values and variability (amplitude % mean), and sputum cellular and biochemical profile before (day I) and after (day II) therapy with i.v. corticosteroids and inhaled β2-agonists, as appropriate. By day II, forced expiratory volume in 1 s (FEV1), inspiratory capacity (IC), PEF or VAS values and variability, sputum eosinophils and eosinophilic cationic protein (ECP) had improved. Improvement of dyspnea expressed as a decrease in VAS and reduction in variability of dyspnea sensation significantly correlated with increase in FEV1 %predicted value (%pv) (P=0.03; r=0.72 and P=0.02; r=0.74, respectively). No significant correlation was found between IC and VAS either in absolute values or as changes from days I and II, nor between sputum outcomes and PEF or VAS, regardless of how they were measured. We conclude that in acute asthmatic patients, dyspnea measurement, functional measurements and sputum analysis may be useful in monitoring disease activity, response to therapy and can provide different information on the state of the disease.

Keywords  asthma; dyspnea; eosinophils; peak expiratory flow; inspiratory capacity.

INTRODUCTION

Dyspnea, a major symptom of asthma, is often used as a marker of disease severity and monitoring of dyspnea is being recommended in self-management plans for asthma (1). Nonetheless, a wide variation in dyspnea perception associated with bronchoconstriction (PB) has been described in asthmatic patients, and a significant percentage of asthmatics has been demonstrated to have a low PB (2,3), these patients being subject to delay the assumption for rescue treatment. The influence of clinical, functional, psychological, and pathological factors has been advocated to explain the wide variation in PB among asthmatic subjects (4–6). The number of factors involved is PB also explain the discrepancies in terms of the relationship between changes in dyspnea and function data reported in treating severe asthma attacks (7–9). Rodrigo and Rodrigo (7) and Janson et al. (8) reported on independence of symptoms, treatment regimen and airway function. Noseda et al. (9) maintained that the reduction in dyspnea could not be ascribed to an improvement of forced expiratory volume in 1 s (FEV1) after a spontaneous acute asthma attack.

Nonetheless, in evaluating the factors involved in breathlessness during acute asthma exacerbation, the following should be considered: (i) the reduced expiratory flow is only one of the many mechanisms that occur during an asthma attacks (10,11), (ii) dynamic lung hyperinflation (DH) contributes importantly to acute breathlessness in asthma for a given level of bronchoconstriction (10).

Our hypothesis is that changes in DH along with changes in airway inflammation and narrowing contribute to explain a part of the variability of PB during remission of spontaneous asthma attack. To validate this hypothesis, we carried out the present study in patients with exacerbated asthma before and after a short course of corticosteroid treatment.
PATIENTS AND METHODS

We studied 12 consecutive patients, aged 27–62, with exacerbation of asthma. The diagnosis of chronic bronchial asthma had been previously established according to NHLBI (1) on the basis of history of episodes of dyspnea with wheezing and bronchial hyperresponsiveness to histamine (provocative concentration of histamine causing 20% fall in FEV$_1$—PC20 FEV$_1$ < 8 mg/ml). A previous diagnosis of atopy was established on the basis of skin-prick allergy tests with a battery of common aeroallergen extracts. In 7 of the 12 patients, skin-prick tests were positive. An exacerbation of asthma was defined by the presence of dyspnea at rest with wheezing or nocturnal symptoms disturbing sleep.

Lung function

Baseline pulmonary function testing was performed by measuring static and dynamic lung volumes with a water-sealed spirometer (Pulmonet Godart), as previously reported (12). The normal values for lung volumes are those proposed by the European Community for Coal and Steel (13). Peak expiratory flow (PEF) was measured using mini-Wright peak flow meters (Clement Clarke International Ltd., Harlow, U.K.). Patients were instructed on the correct use of the meter and the recording form. After instruction, they performed the test every morning upon rising (morning PEF) and every night (evening PEF) before assuming bronchodilator and recorded the highest value of three measurements on the recording form (14).

Dyspnea measurement

Dyspnea was evaluated by the Visual Analogue Scale (VAS) (15). Subjects were asked to rate their sensation of dyspnea every morning (morning VAS) and every night (evening VAS) before administering bronchodilator, by placing a vertical mark on a horizontal 10 cm line labelled “no breathlessness at all” at the left end and “the most breathlessness ever experienced” at the right end. The dyspnea score was expressed as the distance of the mark to the left end of the VAS in mm (15).

Induction and analysis of sputum

Induction of sputum was performed according to the method of Pin et al. (16). Briefly, 10 min after fenoterol inhalation (200 µg), hypertonic saline was nebulized with an ultrasonic nebulizer (Fison; Fisons Corp., Rochester, NY, U.S.A.) and was inhaled for 5-min periods for up to 20 min. The concentration of saline was increased at intervals of 10 min from 3 to 4%. FEV$_1$ was measured every 5 min during inhalation of hypertonic saline solution. The sputum induction procedure did not cause troublesome symptoms and the FEV$_1$ did not decrease by more than 20% in any subject. Every 5 min subjects were asked to try to cough sputum into a Petri dish and to collect saliva in a separate container. Cytological analysis and eosinophilic cationic protein (ECP) measurement were performed according to Ronchi et al. (17): two or three plugs free of salivary contamination were suspended in dithiothreitol (DTT) solution (0.1%) and incubated for 30 min at 37°C for slide making. Cells were centrifuged at 1500 rpm for 10 min and then re-suspended in saline. Three sputum slides were then prepared for cytological examination by cytocentrifugation. Cells were air-dried and stained with May–Grumwald–Giemsa stain. Cell differentials were determined by counting 200 non-squamous cells on each sputum slide. The volume of the remaining portion of sputum samples was determined and an equal volume of DTT (0.1%) was added. The samples were mixed by vortex and incubated at 37°C for 20 min. The samples were then centrifuged at 1000 g for 10 min. The supernatants were aspirated and frozen at −70°C for later ECP analysis. ECP was assessed by a fluoroenzymoimmunoassay (CAP ECP FEIA Kabi Pharmacia, Pharmacia Diagnostics AB, Uppsala, Sweden). Anti-ECP, covalently coupled to immuno-CAP, reacted with the ECP in the specimens. After washing, enzyme-labelled antibodies against ECP were added to form a complex. After incubation, unbound enzyme anti-ECP was washed away and the bound complex was then incubated with a developing agent. After stopping the reaction, the fluorescence of the eluate was measured in Fluoro-Count 96 (Kabi Pharmacia). The sensitivity of this technique is <0.5 mg/l. ECP was determined in duplicate.

Protocol

All patients were seen before any asthma treatment in hospital (day I). Ten patients were admitted to hospital for 3–5 days. Spirometry and sputum were performed on the first day of observation (day I) and repeated 15 days later (day II). Patients were asked to measure PEF and dyspnea sensation by using the VAS in the morning and evening. Values obtained on days I and II were used for the statistical analysis.

After obtaining respiratory function data and collecting sputum, patients were treated with i.v. corticosteroids (methylprednisolone 0.5–1 mg/kg/day) progressively tapered over 15 days and inhaled β$_2$-agonists as appropriate. From day I to day II, the patients also received inhaled steroids (fluticasone or beclomethasone by MDI (metered dose inhaler)).

Data analysis

PEF and VAS variability were assessed as within-day variation (amplitude%mean) (18,19). Amplitude%mean
(amp%mean) was defined as: (highest PEF [or VAS]—lowest PEF [or VAS])/(mean value of either) 100.

**Statistical analysis**

Mean values and standard errors of the mean (SE) were calculated for all variables. Data obtained on days I and II were compared using the Wilcoxon test for paired samples. Regression analysis was performed using Spearman’s correlation coefficient. \( P < 0.05 \) was considered statistically significant. All statistical analyses were carried out using the Statgraphics for Windows 5.0 package (Manugistics, Rockville, MD, U.S.A.).

**RESULTS**

**Dyspnea**

On days I and II, morning VAS were significantly higher than evening VAS \( (P=0.005 \text{ and } 0.03, \text{ respectively}) \) and on day II, morning VAS was significantly lower than on day I \( (P=0.003) \) (Fig. 1). VAS variability in terms of amp%mean was significantly higher on day I than on day II \( (P=0.02) \).

**Pulmonary volumes and flow**

On day I, FEV1 was 61\% (± 3.5) of the predicted value and inspiratory capacity (IC) was 2.7 l (± 0.18). The FEV1 % increase from baseline after \( \beta_2 \)-agonist inhalation was 25 (± 3.15). On day II, FEV1 was 88\% (± 3.8) of the predicted value and IC was 3.2 l (± 0.26), both changes being significant \( (P < 0.01) \) (Fig. 2). Both on days I and II, morning PEF was significantly lower than evening PEF \( (P=0.003 \text{ and } 0.03, \text{ respectively}) \). On day II, both morning and evening PEF were significantly higher than on day I \( (P=0.003 \text{ and } 0.005, \text{ respectively}) \) (right panel of Fig. I). PEF variability in terms of amp%mean was significantly higher on day I than on day II \( (P=0.02) \).

**Cell biology**

On day I, sputum was obtained spontaneously while on day II, it was obtained after induction with hypertonic saline. Sputum eosinophils were above the normal range \( (0–2.2\%) \) in all patients \( (33\% ± 7.5) \) before treatment and significantly \( (P=0.002) \) reduced \( (0.8 ± 0.2) \) after treatment. Sputum neutrophils were increased in eight patients but unaffected by treatment: from 27\% ± 6.9 to 36\% ± 7.5 \( (P=ns) \). Compared to the normal range in our laboratory \( (0–70\mu g/l) \) sputum ECP was markedly higher \( (2812\mu g/l ± 1301) \) before treatment and significantly decreased \( (351\mu g/l ± 166) \) \( (P=0.001) \) after treatment even if in eight patients it remained above the normal range (Fig. 3).

**Correlations**

The reduction in variability of dyspnea sensation measured as amp%VAS \( (\text{amp%VAS measured on day I}−\text{amp%VAS measured on day II}) \) significantly correlated with increase in FEV1 %predicted value \( (%pv) \) \( (P=0.02; \rho=0.74) \) and improvement of dyspnea expressed as decrease in VAS \( \text{(DVAS: VAS measured in the morning on day I}−\text{VAS measured in the morning on day II}) \) significantly correlated with increase in FEV1 \( (P=0.03; \rho=0.72) \) (Fig. 4). No significant correlation was found between IC

![Image](image1.png)

**Fig 1.** Mean morning (circles) and evening (squares) VAS and PEF on days I and II. Lines represent standard errors.

![Image](image2.png)

**Fig 2.** Forced expiratory volume in one second (FEV1) and inspiratory capacity (IC) on days I and II. Mean values with standard error (filled circles and bars) and individual data points (empty symbols) are shown; pv: predicted value.
DISCUSSION

In this study, we found that in patients with asthma exacerbation FEV1, IC, PEF and VAS significantly improved with treatment while sputum eosinophils and sputum ECP significantly decreased. Unlike FEV1, neither airway inflammation outcomes, nor PEF nor IC related to dyspnea.

Dyspnea, one of the main symptoms of asthma, is often used to assess the severity of the disease. Self-management plans for asthmatic patients recommend the monitoring of dyspnea (I). In order to measure dyspnea sensation during and after an exacerbation of asthma, we chose to administer VAS to our patients at different times of the day. VAS has been adopted in asthma for the subjective measurement of dyspnea and provides a reliable and sensitive measure (20,21).

Diurnal variability of VAS (amp%mean) has been used to measure variability in dyspnea sensation (I5). Our study, which shows a significant reduction in absolute value and variability of VAS, supports this application of VAS.

Several lines of evidence indicate that eosinophilic airway inflammation may have either a positive or a detrimental effect on perception of bronchoconstriction depending on whether patients are ICS naive or ICS treated (4−6). It is well known that inflammation in asthma is mostly characterized by the presence and activation of eosinophils (22,23) and that the release of eosinophil cationic proteins is responsible for the airway damage. To assess the inflammatory response of the bronchial mu-

cosa, we performed cytological and biochemical (ECP) analyses of sputum which have been recently proposed as a non-invasive method to investigate airway inflammation in stable asthma (I6,17,24). More recently, the examination of sputum has also been proposed as a valid guide for monitoring asthma treatment after a severe exacerbation (25). In our study, sputum eosinophils and ECP, but not neutrophils, were markedly reduced at the end of observation. These results are in line with the findings of Pizzichini et al. (25) who found a reduction in both sputum eosinophils and sputum ECP in acute asthma treated with prednisone. However, we were not able to observe any significant correlation among airway inflammation outcomes, and dyspnea measurements and their respective improvements. This may be explained with the demonstration that in acute asthma clinical and functional measurement do not accurately reflect the airway inflammatory response to anti-inflammatory therapy (25,26).

Our results showing that short-term change in FEV1 predicted short-term changes in breathlessness are not in line with the results by Janson et al. (8). Different experimental models and severity of airway obstruction may explain the different results. Nonetheless, it has also been shown that hyperinflation partly accounts for inter-subject variability in breathlessness for a given level of FEV1 decrease in methacholine-induced asthma and it is the strongest predictor of symptom recovery (10). Although not precisely defined in its mechanisms, DH is associated with important negative consequences: (i) increase in mechanical load for the respiratory muscles already burdened with substantial resistive work (27,28); (ii) attendant inspiratory threshold load at the beginning of inspiration in some patients (27,28); (iii) decreased ability of inspiratory muscles to pressure generation (29) despite the increased respiratory drive (30). All these factors play an important role in breathlessness (31).

Despite the significant increase in IC paralleled the significant decrease in VAS, the two variables did not relate
to each other, neither did IC predict any amount of the variability in VAS. What is the explanation for this? It has long been shown that TLC does not change in airway obstruction of asthma (32) so that IC represents the mirror image of end expiratory lung volume (EELV): the lower the former, the higher the latter during an asthma attack. Assuming similar reciprocal change in our study, we should conclude that change in EELV was not an important contributor to breathlessness. One reason for this may be that decrease in IC was minimal (see Fig. 2) such as not to influence pressure production and thereby inspiratory muscle effort. The discrepancy between our study and that of Lougheed et al. (10) showing that FRC (functional residual capacity) contributed to dyspnea after adjustment for FEV₁ decrease with methacholine, likely depends on the following: (i) a smaller increase in EELV in our study and (ii) the different experimental model and the different experiences suffered during an asthma attack vs induced bronchoconstriction.

Increased spontaneous variation in airway caliber has often been described as a characteristic hallmark of asthma (33,34) and the measurement of PEF has been universally accepted as a simple and valuable clinical tool for assessing the degree of airway obstruction (35). PEF measurements are now recommended in the guidelines for diagnosis and management of asthma and diurnal PEF variability is considered one of the indicators of asthma severity (I). Nonetheless, in the present study, improvement in PEF values and a reduction in its variability after corticosteroid therapy did not provide any information about the symptom of breathlessness.

In conclusion, we found that in patients with acute asthma, nor dynamic lung hyperinflation nor markers of inflammation such as sputum cellular and biochemical profile are related to dyspnea sensation. Thus, in this condition, PEF, dyspnea measurements, lung function and sputum outcomes may all provide independent information on the state of the disease.

REFERENCES


