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Association between asthma control and bronchial hyperresponsiveness and airways inflammation: a cross-sectional study in daily practice

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Summary

Background The primary end-point in the management of asthma is to obtain optimal control. The aim of this study was to assess the relationships between the markers of airway inflammation (sputum eosinophilia and exhaled nitric oxide), bronchial hyperresponsiveness (BHR) and asthma control.

Methods One hundred and thirty-four patients were recruited from our asthma clinic between January 2004 and September 2005 [mean age: 42 years, mean forced expiratory volume in 1 s (FEV_1): 86% predicted]. Eighty-six of them were treated by inhaled corticosteroids, 99 were atopic and 23 were current smokers. They all underwent detailed investigations including fractional-exhaled nitric oxide (FE_{N0}) measurement, sputum induction and methacholine challenge when FEV_1 was > 70% predicted, and filled in a validated asthma control questionnaire (ACQ6 Juniper).

Results When dividing patients into the three groups according to their level of asthma control determined by ACQ [well-controlled asthma (ACQ score ≤ 0.75), borderline ($0.75 <$ ACQ score < 1.5) and uncontrolled asthma (ACQ score ≥ 1.5)], it appeared that uncontrolled asthmatics had a greater BHR to methacholine and sputum eosinophilia than controlled asthma ($P < 0.05$, $P < 0.001$, respectively). By contrast, we failed to show significant differences in the FE_{N0} levels between the groups. With receiver-operating characteristic curves for differentiating uncontrolled (ACQ ≥ 1.5) from controlled and borderline (ACQ < 1.5) asthma, sputum eosinophilia and methacholine responsiveness were found to be more accurate than FE_{N0} (area under the curve: 0.72, 0.72 and 0.59, respectively).

Conclusion In a broad spectrum of asthmatics encountered in clinical practice, sputum eosinophilia and methacholine bronchial hyperresponsiveness, but not FE_{N0}, are associated with uncontrolled asthma.

Keywords : asthma control ; exhaled NO ; methacholine responsiveness ; sputum eosinophils

Introduction

A recent update of the GINA has placed emphasis on the concept of asthma control as being the key target of the treatment. There are several validated questionnaires to measure asthma control. Among these, asthma control questionnaire (ACQ) Juniper is certainly one of the best validated. In its original form, it takes into account not only day and night symptoms and a (β_2-agonist as needed but also the baseline airway calibre measured by forced expiratory volume in 1 s (FEV_1 [1]. Furthermore, shortened versions deleting either the FEV_1 item and/or the (β_2-agonist consumption item were shown to provide similar information on the level of asthma control achieved by treatment [2].

Recent studies have indicated that uncontrolled asthma may be associated with increased airways inflammation as reflected by increased exhaled NO levels [3] or high sputum eosinophil counts [4, 5]. Besides airway inflammation, asthma control is likely to be influenced by the magnitude of bronchial hyperresponsiveness, the key functional abnormality in asthma. The extent of bronchial hyperresponsiveness (BHR) towards direct agents in asthma was shown to relate to the severity of the disease as defined by the medication needed to obtain disease control [6], and targeting hyperresponsiveness to adjust the maintenance dose of inhaled corticoids resulted in a reduction of mild asthma exacerbations [7]. Despite being somewhat interrelated [8, 9] BHR to methacholine and airways inflammation reflect different dimensions in asthma [10]. Very few studies, however, have compared the strength of the association between the lack of asthma control and the magnitude of BHR and

airway inflammation. Besides airway inflammation and hyperresponsiveness, it is increasingly recognized that additional factors like chronic rhinosinusopathy, anxiety and depression may contribute to poor asthma control [11]. These factors might actually dilute the real influence of airway inflammation and BHR on clinical disease expression.

The purpose of our study was to assess, in a large and unselected population of asthmatics, the relationships between asthma control on the one hand and methacholine BHR and exhaled NO and sputum eosinophils on the other. The study was performed on asthmatics encountered in daily practice recruited from our asthma clinic as they come without excluding those who were already receiving inhaled corticosteroid treatment, current smokers or those experiencing an asthma exacerbation at the time of the investigations. The population was a mix of newly diagnosed asthma, together with patients with a well-established diagnosis whose disease duration was variable.

Methods

Patients

For this study, 134 consecutive asthmatics seen between January 2004 and September 2005 and whose functional and demographic characteristics are given in Table 1 were recruited from our asthma clinic. The majority of patients (86/134 - 64%) were already receiving current treatment with inhaled corticoids for more than 4 weeks before entering the study. Asthma was defined by the presence of recurrent symptoms of breathlessness, cough or wheezing associated with BHR to methacholine (when FEV_1 > 70% predicted) or/and a significant reversibility to inhaled salbutamol (when FEV_1 < 80% predicted) at a moment of the medical history. The demonstration of BHR was based on a positive methacholine challenge defined as a provocative concentration of methacholine causing a decline in FEV_1 of 20% from baseline (PC20M) ≤ 16 mg/mL. A significant reversibility of FEV_1 was defined as a broncho dilation > 12% after 400 µg inhaled salbutamol pMDI. Atopy was defined as a positive skin prick test reaction (weal ≥ 3 mm compared with control) to common aeroallergens of our area (house dust mites, cat and dog dander, grass, tree and weed pollens and moulds).

Table 1. *Demographic and functional patient characteristics*

	Well-controlled ACQ < 0.75 *n* = 31	Borderline ACQ 0.75-1.49 *n* = 32	Uncontrolled ACQ ≥ 1.5 *n* = 71
Age (years)	40±13	47±12	42±12
Sex (M/F)	16/15	16/16	35/36
Tobacco (mean pack-years)	1 CS and 10 exS (10.2)	8 CS and 0 exS (12.4)	14 CS and 14 exS (13.6)
Skin prick test[+]	28 (90%)	22 (69%)	49 (69%)
Steroid treatment	20/31 (64%)	20/32 (62%)	46/71 (65%)
Inhaled steroid dose (µg/day)	635 (0-2000)	529 (0-2000)	813 (0-4000)
Oral steroid dose (mg/day)	0	0	24 (6 patients)
FEV_1 (°/o predicted)	101±11	88±13*	81±27*
FEV_1/FVC (°/o)	80.6±4.5	76.5±6.5	79±21
PC20M (mg/mL)	6.3 (0.17-16)	3.9 (0.05-16)*	1.6 (0.06-16)*
Dose-response slope (%/µmol)	0.00113 (0.00011-0.1089)	0.00212 (0.0001-0.08289)	0.00411* (0.00015-0.1089)
FE_{N0} (p.p.b.)	47.9 (11.4-130)	30.6 (2.8-222)	50.2 (4.1-244)
Sputum eosinophils (%)	0.4 (0-31.2)	1.4 (0-26)	5.6 (0-93.4)***

FEV_1, FEV_1/FVC are expressed as mean±SD while DRS, FE_{N0} and sputum eosinophil count are expressed as median (range); PC20M is expressed as the geometric mean (range).
**P<0.001 as compared with well controlled.*
**P<0.05 as compared with well controlled.*
***P<0.05 as compared with borderline.*
FE_{N0}, fractional exhaled nitric oxide; ACQ, asthma control questionnaire; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; SD, standard deviation; DRS, dose-response slope; PC20M, decline in FEV_1 of 20% from baseline; CS, current smokers; exS, ex-smokers.

Study design

The patients attended our asthma clinic on 1 day, during which they filled in an ACQ and underwent, in this order, an exhaled NO measurement, a methacholine challenge (when FEV_1 was > 70% predicted after stopping a short-acting (β_2-agonist for 8 h and a long-acting (β_2-agonist for 24 h) and a sputum induction. The study was approved by our local ethics committee and subjects gave their informed consent.

Asthma control

The degree of asthma control was evaluated by the ACQ score from the six-item Juniper ACQ questionnaire deleting the FEV_1 from the original questionnaire [2] (0 = totally controlled and 6 = severely uncontrolled). ACQ6 was chosen in order to avoid the mathematical influence of baseline airway calibre on the level of BHR. Patients were considered 'well controlled' when the ACQ score was lower than 0.75 and 'uncontrolled' when the ACQ score was > 1.5. Between these two values, the control was assumed to be 'borderline'. These values were chosen in accordance with the analysis derived from the GOAL study as published by Juniper [12].

Exhaled nitric oxide

Exhaled nitric oxide was measured by a chemolumines-cence analyser (NIOX, Aerocrine, Stockholm, Sweden) at a flow rate of 50 mL/s, in accordance with the recommendations of the ATS/ERS task force [13]. The measurement was performed before spirometry and methacholine challenge.

Methacholine challenge

Spirometry was performed using an electronic spirometer connected in real time to a computer (Spirobank, MIR, Rome, Italy). All manoeuvres were repeated three times and the best FEV_1 value was selected by the software program (Winspiro, MIR). Methacholine challenge was performed according to a slightly modified Cockroft's method. Patients successively inhaled by tidal breathing for 2min fourfold increasing concentrations of methacholine chloride from 0.06 to 16mg/mL as described previously [8]. The aerosol was generated by a jet nebulizer (Hudson, Temecula, CA, USA), whose characteristics were described previously [14]. The provocative concentration of methacholine causing PC20M was calculated by linear interpolation from the dose-response curve. Furthermore, in order to express responsiveness as a continuous variable without censoring subjects, methacholine responsiveness was also expressed as a dose-response slope (DRS) and expressed as $\%FEV_1$ fall/cumulative μmol methacholine. After the provocative challenge, each patient inhaled 400μg salbutamol given by a metered dose inhaler through a spacer, and the sputum induction was started 30 min later.

Sputum induction and processing

After premedication of the subjects with 400 μg inhaled salbutamol (pMDI+spacer), sputum was induced by inhalation of a hypertonic saline (NaCl 4.5%) combined with additional salbutamol [15] delivered by an ultrasonic nebulizer (Ultra-Neb 2000, De Vilbiss, Somerset, PA, USA) with an output set at 0.9mL/min. When post-bronchodilation FEV_1 was lower than 65% of the predicted value, induction was performed with physiologic fluid (NaCl 0.9%) combined with salbutamol. Each subject inhaled the aerosol for three consecutive periods of 5min for a total time of 15 min. For safety, $FEV_1$was monitored every 5 min and the induction was stopped when FEV_1 delined by > 20% from the post-bronchodilation value.

The whole sputum was collected in a plastic container, weighed and homogenized by adding three volumes of PBS, vortexed for 30 s and centrifuged at 800 g for 10 min at 4 °C. The supernatant was separated from a cell pellet, which was resuspended in a solution containing 5 mM DTT without Ca^{2+} and Mg^{2+}, filtered and used to perform squamous and total cell count using a manual haemocytometer. Cell viability was checked by trypan blue exclusion. The differential performed on cytospins stained with Diff-Quick after counting 500 cells.

Statistical analysis

Comparisons between the three groups were performed using the Kruskall-Wallis test, followed, when significance emerged, by Dunn's test for pairwise comparisons. Correlations were tested using the Spearman coefficient of correlation. Receiver-operating characteristic (ROC) curves were constructed to define the accuracy of FEV_1, FEV_1/forced vital capacity (FVC), fractional-exhaled nitric oxide (FE_{N0}), sputum eosinophilia and methacholine BHR to differentiate controlled and borderline from uncontrolled asthma. Cut-point was defined as the value allowing to correctly classify most of the events. *P*-values < 0.05 were considered as

statistically significant.

Results

Overall exhaled NO and sputum eosinophil percentage were correlated ($r = 0.54$, $P < 0.0001$), and both sputum eosinophilia and exhaled NO were inversely correlated with PC20 M ($r = -0.41$ and $P < 0.001$ and $r = -0.34$ and $P < 0.01$, respectively).

When dividing patients in to three groups according to their asthma control as determined by the ACQ [well-controlled asthma (ACQ score ≤ 0.75), borderline (0.75 < ACQ score < 1.5) and uncontrolled asthma (ACQ score ≥ 1.5)], we found that uncontrolled asthmatics displayed a greater bronchial responsiveness to methacholine than those who were controlled ($P < 0.05$) (Fig. 1a, Table 1). We failed to show significant differences in FE_{N0} between the three groups (Fig. 2a, Table 1) while sputum eosinophilia was significantly higher in the uncontrolled group than in the controlled and the borderline group ($P < 0.001$ and $P < 0.05$, respectively) (Fig. 3a, Table 1). How treatment with inhaled corticoids may influence the relationships between these variables and asthma control is shown in Figs 1b, 2b and 3b. Among patients regularly receiving inhaled corticoids, uncontrolled asthmatics still exhibited greater methacholine BHR than their controlled or borderline counterparts (Fig. 1b). FE_{N0} values were not significantly higher in the uncontrolled corticosteroid-naïve group as compared with the other groups not treated with corticoids. However, it clearly appeared that patients receiving inhaled corticoids had lower FE_{N0} than those without corticoids irrespective of the level of asthma control (Fig. 2b). As for sputum eosinophils, Fig. 3b clearly shows that treatment with inhaled corticoids failed to normalize sputum cell counts when asthma remained uncontrolled.

Fig. 1. *(a) Methacholine bronchial hyperresponsiveness expressed as the dose-response slope according to asthma control assessed by the mean Juniper asthma control questionnaire (ACQ)6 score. Bars represent the median, (b) Represents corticosteroid-treated and corticosteroid-naïve patients separately.*

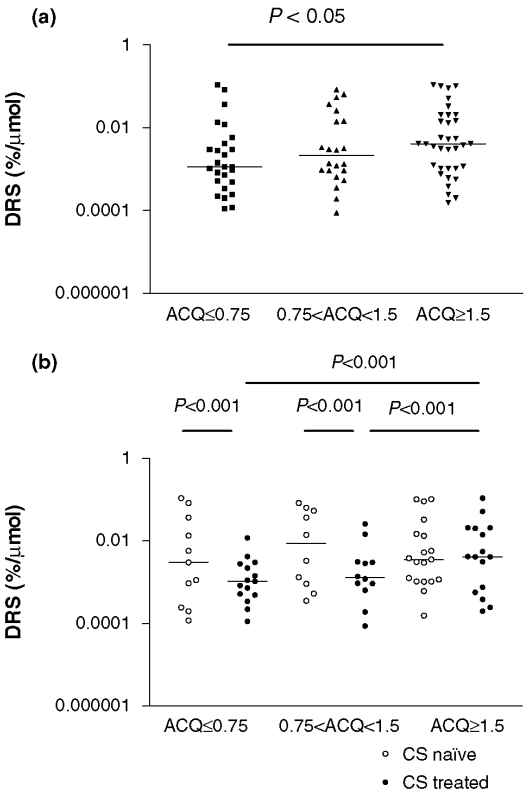


Fig. 2. *(a) Exhaled nitric oxide values according to the level of asthma control assessed by the mean Juniper asthma control questionnaire (ACQ)6 score. Bars represent the median, (b) Represents corticosteroid-treated and corticosteroid-naive patients separately.*

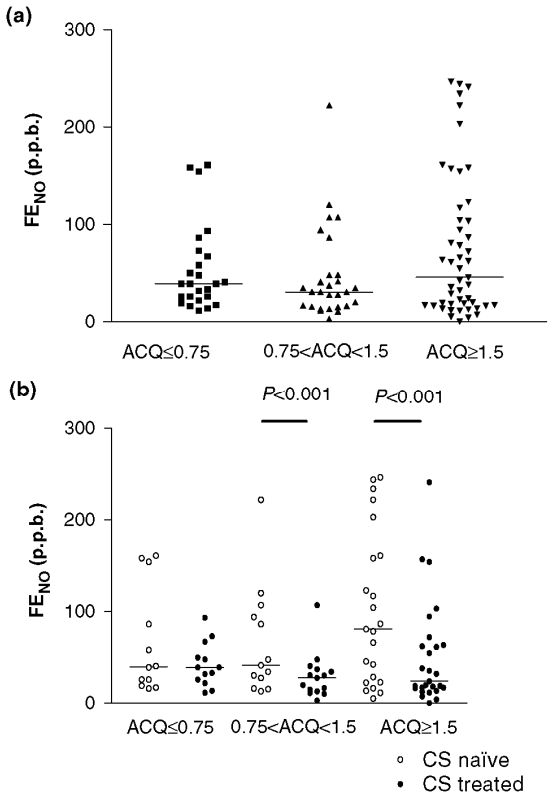


Fig. 3. *(a) Sputum eosinophil count according to asthma control assessed by the mean Juniper asthma control questionnaire (ACQ)6 score. Bars represent the median, (b) Represents corticosteroid-treated and corticosteroid-naïve patients separately.*

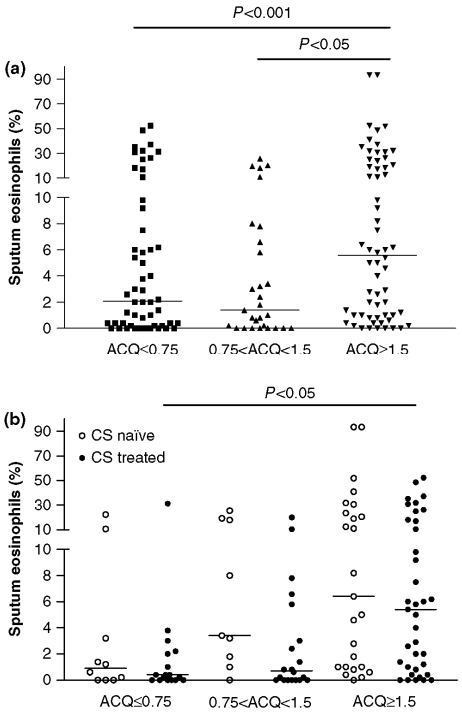


Fig. 4. *Receiver-operating characteristic curves of dose-response slope (DRS) (upper panel), fractional exhaled nitric oxide (FE_{NO}) (middle panel) and sputum eosinophil count (lower panel) in differentiating uncontrolled [asthma control questionnaire (ACQ) ≥ 1.5] from controlled and borderline asthma (ACQ < 1.5).*

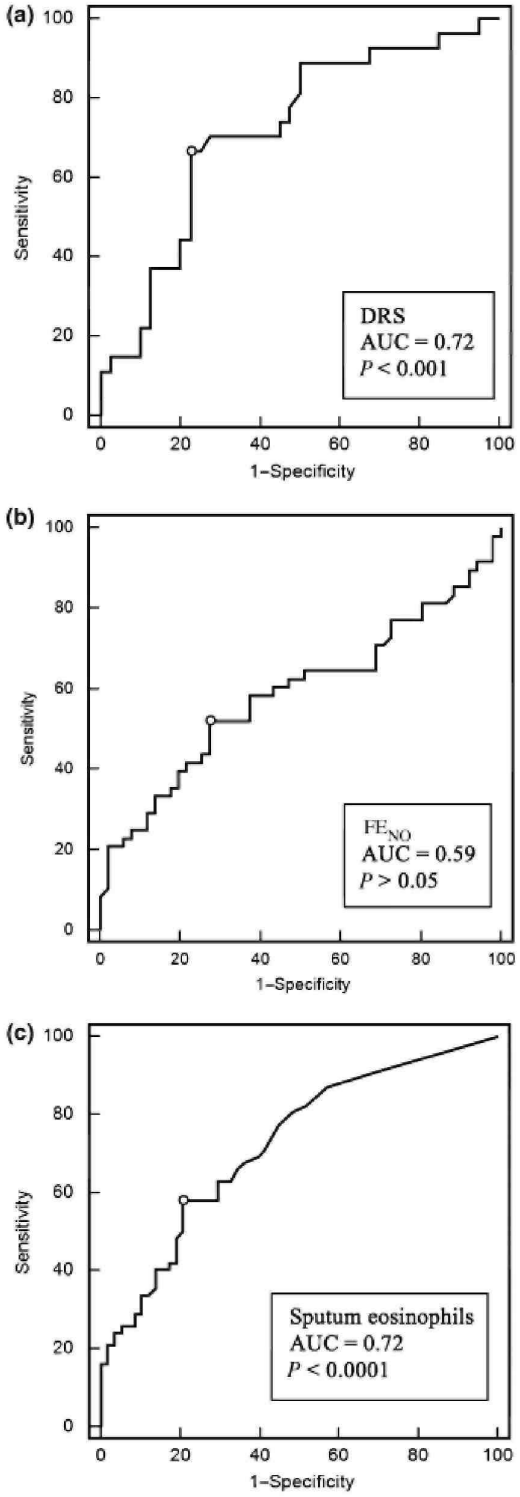
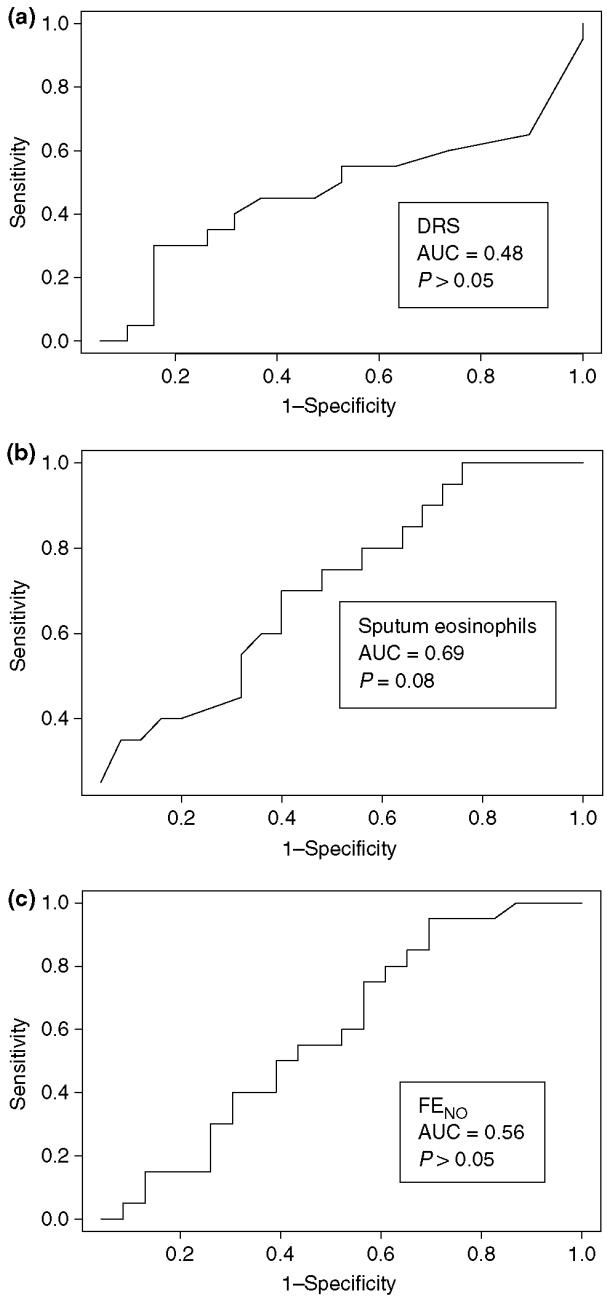


Fig. 5. *Receiver-operating characteristic curves of dose-response slope (DRS) (upper panel), fractional exhaled nitric oxide (FE_{N0}) (middle panel) and sputum eosinophil (lower panel) count in differentiating uncontrolled (asthma control questionnaire ACQ > 1.5) from controlled and borderline asthma (ACQ< 1.5) inpatients not receiving inhaled corticosteroids.*



ROC curve analysis shows that the sputum eosinophil count and methacholine responsiveness were better than FE_{N0} for identifying uncontrolled asthmatics [area under the curve (AUC) = 0.72 vs. 0.72 vs. 0.59, respectively) (Fig. 4). ROC curves for baseline lung calibre as reflected by FEV_1 and the ratio FEV_1/FVC yielded AUC reaching 0.62 and 0.63, respectively (Table 2).

ROC curves in asthmatics not receiving inhaled corticoids indicate that the sputum eosinophil count remained the best variable to predict uncontrolled asthma while methacholine responsiveness and FE_{N0} were poor in this case (Fig. 5).

The 'best' cut-off value for differentiating uncontrolled from controlled and borderline asthma was 3.8% for sputum eosinophilia with a sensitivity and a specificity of 58% and 79%, respectively (Table 2). Likewise, the

cut-off value for PC20M was 3.4 mg/mL with a sensitivity and a specificity of 70%. As for $FEV_1$and FEV/FWC, the best cut-off values were 69% predicted and 84%, respectively (Table 2).

A bronchodilation test was performed in only 24 patients; 21 out of them were found in the uncontrolled group. Among these patients, the extent of reversibility was correlated to the ACQ values (r = 0.36, P = 0.08).

Six patients were considered as having severe exacerbation by the time of the study based on the need to prescribe oral corticoids. Mean±SEM ACQ was 4.2 (3.4-4.9) and the median (range) sputum eosinophil count was 22% (1.2-48). FE_{N0} was measurable in only one of those patients (241 p.p.b.).

Table 2. *Diagnostic performance of baseline lung function, methacholine hyperresponsiveness and airway inflammatory parameters to identify uncontrolled asthma (ACQ ≥ 1.5)*

	AUC	*P*-value	Cut-off value	Sensitivity (%)	Specificity (%)
FEV_1 (°/o predicted)	0.62	<0.05	69	31	94
FEV_1/FVC (°/o)	0.63	<0.01	84	82	43
PC20M (mg/mL)	0.69	< 0.005	3.4	70	70
DRS (o/o/nmol)	0.72	< 0.001	0.0035	67	77
FE_{N0} (p.p.b.)	0.59	>0.05	54.7	52	73
Sputum eosinophils (%)	0.72	<0.0001	3.8	58	79

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; ACQ, asthma control questionnaire; DRS, dose-response slope; AUC, area under the curve; PC20M, decline in FEV_1 of 20% from baseline.

Discussion

This cross-sectional study, conducted on a large and heterogeneous population of asthmatics, shows that uncontrolled asthma is associated with the degree of sputum eosinophilia and methacholine BHR but not with the levels of exhaled NO.

The relationship between sputum eosinophilia and poor asthma control comes as a confirmation of previous data reported by Romagnoli et al. [5] on a limited series of patients and is keeping with the role attributed to airway eosinophils in asthma exacerbation occurring in moderate to severe asthma [16]. Our finding points to a threshold of4% sputum eosinophilia as the best level to predict uncontrolled asthma. This threshold is only slightly higher than the threshold recognized to identity an abnormally high sputum eosinophil percentage, which lies between 2% and 3% according to a study performed on healthy subjects [4, 8, 17-19]. This indicates that the appearance of a modest eosinophilic airway inflammation in asthma may rapidly be associated with symptom expression of the disease. It is of interest to note that the association of sputum eosinophils with uncontrolled asthma clearly persisted when considering patients regularly treated with inhaled corticoids. Although inhaled corticoids are often extremely effective in reducing eosinophilic inflammation in mild asthmatics tightly selected for controlled drug trials [20, 21], our data indicate that the picture may not be the same in patients encountered in real life. The reasons for this may be numerous and may include factors independent of the drug efficacy itself such as poor adherence or awkward device utilization but also factors that cause true resistance to the drug such as active smoking [22]. As demonstrated by the overlap in the sputum eosinophil count between our different groups, the link between eosinophilic inflammation and asthma symptoms is of course not absolute. A recent cross-sectional study using a cluster analysis on a large series of asthmatics seen in secondary care has clearly pointed to a subgroup of patients with few symptoms but an intense sputum eosinophilia while another subgroup was characterized by a high symptom score without sputum eosinophilia [23].

In contrast to sputum eosinophilia, it appears that exhaled NO was poorly related to the level of asthma control. Although it may seem to be surprising in view of previous data [24, 25], it should be kept in mind that our study was cross-sectional and conducted in a real-life setting including all types of asthmatics as they come to the asthma clinic. It includes a majority of patients already treated with inhaled corticoids, with some of them being current smokers, factors that are recognized to decrease the level of exhaled NO [26, 27] and therefore potentially altering the relationship between exhaled NO and asthma control. As pointed out by Bates et al. FE_{N0} reacts particularly quickly and well before symptoms to a treatment with inhaled corticoids [28]. However, neither the percentage of patients treated with inhaled corticoids nor that of current smokers was significantly

different between the uncontrolled asthmatics vs. the other two groups. Our data clearly showed that exhaled NO levels were more influenced by inhaled corticoids than by the level of asthma control. The persistence of a significant association between eosinophils, but not between FE_{N0} and asthma control, might be linked to the different sensitivities of the two inflammatory markers to the doses of inhaled corticoids. In contrast to most asthma studies, here we did not systematically exclude those patients who had symptoms of viral infection during the 4 weeks preceding the visit. These infections are a well-established cause of asthma exacerbation [29]. Nevertheless, as viral infections are thought to increase exhaled NO together with asthma symptoms [30], they are not supposed to break the relationship between exhaled NO and asthma control. On the other hand, several well-conducted longitudinal studies found exhaled NO to be less predictive of asthma control deterioration than sputum eosinophils, when stepping down inhaled corticoids [31-33]. Taken together, these data lead us to consider the sputum eosinophil count, although being a less convenient technique, as a more robust inflammatory marker than exhaled NO in asthmatics encountered in daily practice. Our data support the recommendation of using sputum eosinophil counts to assess global asthma control [34].

The degree of methacholine BHR was found to be proportional to the level of asthma control, with lower PC20 and greater DRS values in uncontrolled asthmatics. The extent of responsiveness to methacholine is thought to mainly reflect abnormal behaviour of smooth muscle and/or airway remodelling rather than airway inflammation, which is better assessed by responsiveness to indirect mediators like adenosine [35]. It is noteworthy that approximately one-third of our controlled asthmatics had normalized their BHR with PC20 > 16mg/mL thanks to a regular treatment with inhaled corticoids. This is in keeping with the effect of a long-term treatment with inhaled corticoids on bronchial responsiveness towards direct constricting agents, which, on average, improves the PC20 by 1-2 doubling dilution [36, 37]. The greater BHR found in uncontrolled asthmatics suggests that airway instability is a factor of poor asthma control. This concept is supported by the demonstration that taking into account the level of BHR when adjusting the dose of inhaled corticoids results in a reduction of mild exacerbations in asthmatics [7]. Interestingly, the association between hyperresponsiveness and uncontrolled asthma is essentially observed in those patients already receiving inhaled corticoids. This would suggest that a component of methacholine responsiveness that is poorly responsive to corticoids (remodelling part) contributes to asthma symptoms. Thermoplasty, a new technique for asthma treatment that targets airway smooth muscle by radio-frequency, may well improve asthma control through an attenuation of BHR [38].

Although providing significant ROC curves in differentiating uncontrolled from controlled asthma, FEV_1 and the ratio FEV_1/FVC proved to be less accurate than methacholine responsiveness and sputum eosinophils to predict the lack of control of asthma symptoms. This emphasizes the need to go beyond spirometry when dealing with uncontrolled asthmatics.

In conclusion, our cross-sectional study shows that, in a large heterogeneous population of mild to moderate asthmatics encountered in daily practice, uncontrolled asthma is associated with increased BHR to methacholine and an increased sputum eosinophil count, but not with increased exhaled NO. This should prompt the clinician to consider measuring these parameters when asthma remains uncontrolled despite treatment with inhaled corticoids.

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