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Is FE_{NO50} useful diagnostic tool in suspected asthma?

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SUMMARY

Background: Asthma diagnosis is based on the presence of symptoms and the demonstration of airflow variability. Airway inflammation measured by fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s (FE_{NO50}) remains a controversial diagnostic tool. **Aim:** To assess the ability of FE_{NO50} to identify bronchial hyperresponsiveness (BHR) to methacholine (provocative concentration of methacholine causing a 20% fall in FEV_1; PC20M ≤ 16 mg/ml) and to establish whether or not symptoms relate to FE_{NO50} and PC20M in patients with no demonstrated reversibility to β_2-agonist. **Methods:** We conducted a prospective study on 174 steroid naive patients with respiratory symptoms, forced expiratory volume in 1 s (FEV_1) ≥ 70% predicted and no demonstrated reversibility to β_2-agonist. Patients answered to a standardised symptom questionnaire and underwent FE_{NO50} and methacholine challenge. Receiver-operating characteristic (ROC) curve and logistic regression analysis assessed the relationship between PC20M and FE_{NO50}. taking into account covariates (smoking, atopy, age, gender and FEV_1). **Results:** A total of 82 patients had a PC20M ≤ 16 mg/ml and had significantly higher FE_{NO50} (19 ppb vs. 15 ppb; $p < 0.05$). By constructing ROC curve, we found that FE_{NO50} cut-off value of 34 ppb was able to identify not only BHR with high specificity (95%) and positive predictive value (88%) but low sensitivity (35%) and negative predictive value (62%). When combining all variables into the logistic model, FE_{NO50} ($p = 0.0011$) and FEV_1 ($p < 0.0001$) were independent predictors of BHR whereas age, gender, smoking and atopy had no influence. The presence of diurnal and nocturnal wheezing was associated with raised FE_{NO50} ($p < 0.001$ and $p < 0.05$, respectively). **Conclusion:** The value of $FE_{NO50} > 34$ ppb has high predictive value of PC20M < 16 in patients with suspected asthma in whom bronchodilating test failed to demonstrate reversibility or was not indicated. However, $FE_{NO50} \leq 34$ ppb does not rule out BHR and should prompt the clinician to ask for a methacholine challenge.

What's known

Fractional exhaled nitric oxide (FE_{NO}) is a noninvasive marker of eosinophilic airway inflammation. Some studies have suggested it may be helpful in diagnosing asthma but it remains debated.

What's new

We conducted a field study assessing the potential of fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s (FE_{NO50}) in steroid-naive patients with suspected asthma sent by respiratory physicians to a routine function laboratory to perform a methacholine challenge. Our results show that $FE_{NO50} > 34$ ppb had a high positive predictive value to identify patients with significant bronchial hyperresponsiveness. However, $FE_{NO50} \leq 34$ ppb does not rule out BHR and should prompt the clinician to ask for a methacholine challenge. We calculated that FE_{NO} measurement could spare methacholine challenge in 20% of patients.

Introduction

Asthma diagnosis is usually based on symptoms such as cough, breathlessness, dyspnoea and wheezing together with the demonstration of airflow variability. The airway inflammatory component of the disease is an important feature which is an integral part of the asthma definition (1). Airway inflammation can be non-invasively assessed by measuring sputum eosinophil count (2) and fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s (FE_{NO50}) (3), the latter being much more convenient to apply in routine as it yields immediate results. Both sputum eosinophil count and FE_{NO50} have been proposed as a useful diagnostic tool in mild to moderate asthma. In this group of patients airway inflammatory markers proved to be superior to classic FEV_1 reversibility

to β_2-agonist or to peak expiratory flow (PEF) variability criteria (4,5). FE_{NO50} was shown to reflect airway eosinophilic inflammation in asthma patients seen in clinical practice (6,7).

Asthma diagnosis remains a challenge in clinical practice (8) and either reversibility test or bronchial provocation challenge is required to confirm the diagnosis. There is a need for a simple, quick and reliable test in those patients with suggestive symptoms of asthma. Early studies have suggested that fractional exhaled nitric oxide, measured at a flow rate of 200 ml/s (FE_{NO200}) cut-off of 16 ppb may help to identify patients with bronchial hyperresponsiveness to histamine or reversibility to β_2-agonist among those presenting with chronic respiratory symptoms and normal baseline lung function (9). Factor analysis has, however, revealed that airway inflammation and bronchial hyperresponsiveness towards methacholine load in different clusters in patients with long disease duration (10,11). On the other hand, FE_{NO20} has been shown to correlate with new onset wheeze in longitudinal population study (12).

Airway hyperresponsiveness assessed by methacholine challenge is time consuming and unpleasant to the patient whereas fractional exhaled nitric oxide (FE_{NO}) measurement is easy to perform and provides immediate results. The purpose of our study was to see how FE_{NO} measured at a flow rate of 50 ml/s may actually reflect the presence of methacholine bronchial hyperresponsiveness assessed by the provocative concentration that causes a 20% fall in FEV_1 (PC20M) in patients referred by chest physicians for asthma diagnosis to a routine laboratory function. This study focused on patients in whom the bronchodilation test did not allow to ascertain asthma diagnosis either because of being negative or not done given a high baseline forced expiratory volume in 1 s (FEV_1) value (> 80% predicted and FEV_1/FVC > 70%). We also sought to establish how different types of respiratory symptoms relate to FE_{NO50} and PC20M.

Methods

Subject characteristics and study design

We conducted a prospective study on a series of 237 patients recruited from the University Hospital of Liege between March 13, 2009 and December 30, 2009. These patients were addressed by their respiratory physician for a methacholine challenge to detect asthma. Subjects referred to methacholine challenge were those in whom the bronchodilating test failed to demonstrate reversible airways obstruction or those in whom baseline spirometric values were normal giving a low probability for a bronchodilating test to be significant. The patients studied here had either baseline $FEV_1 \geq 80\%$ predicted and FEV_1/FVC ratio $\geq 70\%$ or bronchodilation < 12% from baseline and 200 ml after 400 µg inhaled salbutamol in case of baseline FEV_1 was < 80% predicted or FEV_1/FVC ratio < 70%. Patients already receiving inhaled corticosteroids were excluded from the study. The demographic and functional characteristics of the 174 corticosteroid naïve patients are summarised in Table 1.

Table 1 *Demographic, functional and inflammatory characteristics for 174 steroid naive patients*

No.	174
Sexe (M/F)	72/102
Age, years	41 ± 16
Atopy (Y/N)	84/90
Current smoking (Y/N)	59/115 (34%)
PC20 < 16 mg/ml (Y/N)	82/92
FEV_1, % predicted	97 ± 13
FVC, % predicted	100 ± 14
FEV_1/VC, %	83 ± 7
FE_{NO50}, ppb	17 (4-271)

Data are presented as mean ± SD (FEV_1, FVC, FEV_1/VC, age) or as median (range; FE_{NO50}). PC20M is expressed as geometric mean (range). PC20M, provocative concentration of methacholine causing a 20% fall in FEV_1; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FE_{NO50}, fractional exhaled nitric oxide.

After receiving and signing inform consent, patients were asked to complete a questionnaire concerning their symptoms and FE_{NO} was measured at a flow rate of 50 ml/s. The subjects underwent methacholine challenge after refraining from using bronchodilators for the appropriate time (8 h for short-acting bronchodilators and 24 h for long-acting bronchodilators) as long as the baseline FEV_1 value was not less than 70% predicted. Asthma

was diagnosed based on airway hyperresponsiveness demonstrated by inhaled concentration of methacholine provoking a 20% fall in FEV_1 of less than 16 mg/ml (13,14). Methacholine challenges were performed according to a slightly adapted Cockroff's tidal-breathing method as previously described (15). Subjects were characterised as atopic if they had at least one positive skin prick test (wheal > 3 mm as compared with negative control) or specific IgE (> 0.35 KU/l; Phadia) for at least one common aero-allergen (cat, dog, house dust mites, grass pollen, tree pollen and a mixture of moulds). This study was conducted with the approval of the Ethics Committee of CHU Liege.

Questionnaires

Symptoms were assessed using a standardised questionnaire which covered symptoms and smoking habits. Symptoms listed were diurnal and nocturnal cough, diurnal and nocturnal wheezing, dyspnoea, chest tightness, chest pain, exercise trigger, humidity trigger, fumes trigger, dust trigger, pollen trigger, emotional trigger, rhinitis, urticaria and pyrosis.

Exhaled NO measurement

Exhaled nitric oxide, FE_{NO} was measured by chemi-luminescence using a nitric oxide monitor set at a flow rate of 50 ml/s (NIOX, Aerocrine, Sweden). The analyser was calibrated daily with a known NO concentration.

Statistical analyses

Results were expressed as mean ± standard deviations (SD) for continuous variables. The median and range were preferred for skewed distributions. For categorical variables, the number of observations and percentages were given in each category. Comparisons between different subgroups were performed by using a Kruskal-Wallis test. The Receiver-operating characteristic (ROC) curve was constructed to determine the value of FE_{NO50} which best identified a bronchial hyperresponsiveness in the whole population. Logistic regression analysis was used to assess the relationship between the binary outcome (PC20M ≤ 16 mg/ml) and a set of covariates, individually or in combination. Covariates included FE_{NO50} (log transformed), age, gender, FEV_1, smoking and atopy. The results were considered to be significant at the 5% critical level ($p < 0.05$). Calculations were done using SAS Version 9.1 (SAS Institute, Cary, North Carolina, USA).

Table 2 *Demographic, functional and inflammatory characteristics for patients with and without asthma*

	PC20M ≤ 16 mg/ml	PC20M > 16 mg/ml
N.	82	92
Sexe (M/F)	33/49	39/53
Age, years	38 ± 18*	44±15
Atopy (Y/N)	43/39 (52%)	41/51 (45%)
Smoking (Y/N, %)	25/57 (30%)	34/58 (37%)
PC20, mg/ml	2.44 (0.02-16)	
DRS FEV_1 (%/µmol)	0.0033 (0.0006-0.4337)***	0.0004 (0.0001-0.0007)
FEV_1, % predicted	95 ± 14**	102 ± 12
FVC, % predicted	99 ± 14	102 ± 13
FEV_1/VC, %	82 ± 7*	84 ± 6
FE_{NO50}, (PPb)	19 (4-271)*	15 (4-120)

Data are presented as mean ± SD (FEV_1, FVC, FEV_1/VC, age) or as median (range; FE_{NO50})· PC20M is expressed as geometric mean (range), *p < 0.05, **p < 0.001, ***p < 0.0001. PC20M, provocative concentration of methacholine causing a 20% fall in FEV_1; DRS, dose-response slope; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FE_{NO50}, fractional exhaled nitric oxide.

Results

Asthma diagnosis

Among the 174 patients referred for a methacholine challenge, 82 had a PC20M ≤ 16 mg/ml and were thus considered as being asthmatics. The demographic and functional characteristics of patients according to their level of bronchial responsiveness towards methacholine are given in Table 2. Patients with positive methacholine challenge had lower baseline FEV_1 (95% predicted vs. 102% predicted, $p < 0.001$) and lower FEV_1/FVC ($p < 0.05$) even if the average value clearly remained within the normal range. FE_{NO50} was significantly higher in

patients with positive methacholine challenge than in their negative counterparts (19 ppb vs. 15 ppb, $p < 0.05$).

When combining all variables into the logistic model, FE_{NO50} ($p = 0.0011$) and FEV_1 ($p < 0.0001$) were independent predictors of bronchial hyperresponsiveness to methacholine whereas age ($p = 0.12$), gender ($p = 0.56$), smoking status ($p = 0.56$) and atopy ($p = 0.65$) were not significant (Table 3). Lower baseline FEV_1 values and higher FE_{NO} values were associated with PC20M ≤ 16 mg/ml.

We constructed a ROC curve to establish the ability of FE_{NO50} to identify bronchial hyperresponsiveness assessed by methacholine challenge (Figure 1). We found that FE_{NO50} significantly predicted PC20 ≤ 16 mg/ml with a cut-off value of 34 ppb. However, this FE_{NO50} cut point offers much greater specificity (95%) and positive predictive value (PPV) (88%) than sensitivity (35%) and negative predictive value (NPV) (62%). In patients with a negative methacholine challenge the upper limit of the 95% CI of FE_{NO50} was 35 ppb. When referring to FE_{NO50} normal values as defined by Travers et al. (16), we found that 22 patients (13%) had FE_{NO50} values upper to the 95% confidence interval. The ability of FE_{NO50} to identify airway hyperresponsiveness was high in those patients. Indeed 20 of the 22 patients with FE_{NO50} values out of range according to Travers had bronchial hyperresponsiveness whereas this was only the case in 62 of the 152 patients in whom FE_{NO50} was within the normal range according to Travers et al. (Odds ratio 14.5, $p < 0.0001$).

We constructed a ROC curve to identify which FEV_1 cut-off was best related to the prediction of the presence of a bronchial hyperresponsiveness (Figure 2). We found that FEV_1 significantly predicted PC20M ≤ 16 mg/ml with a cut-off value of 101%. The sensitivity and specificity of this threshold was 71% and 57%, respectively ($p = 0.0001$, AUC = 0.67).

When combining FE_{NO50} and FEV_1 values to predict the presence of a bronchial hyperresponsiveness to methacholine, we found that the presence of both $FE_{NO50} > 34$ ppb and $FEV_1 \leq 101\%$ predicted gave a high specificity (98.9%) but a poor sensitivity (24.4%) for identifying patients with positive methacholine challenge (Table 4).

Relationship between FE_{NO} and methacholine responsiveness

On the whole population the dose-response slope (DRS) for methacholine weakly correlated with FE_{NO50} ($r = 0.18$; $p = 0.03$). Among those patients positive to methacholine there was, however, no relationship between the magnitude of bronchial hyperresponsiveness (PC20M) and the level of FE_{NO50} ($r = -0.06$, $p = 0.6$, Figure 3).

Relationship between respiratory symptoms and FE_{NO50} or bronchial responsiveness

Table 5 shows FE_{NO50} according to the presence of respiratory symptoms in our population. Diurnal and nocturnal wheezing were associated with raised levels of FE_{NO50} ($p < 0.001$ and $p < 0.05$, respectively). Table 6 shows the proportion of symptoms according to the results of methacholine challenge. Patients reporting dyspnoea, diurnal and nocturnal wheezing and chest tightness were more likely to have positive methacholine challenge.

Table 3 *Relationship between bronchial hyperresponsiveness to methacholine and a set of covariates including FE_{NO50}, smoking status, age, FEV_1, atopy and sex*

Analysis of Maximum	**Likelihood Estimates**	
Parameter	**Coefficient ± SE**	**p-value**
Intercept	3.82 ± 1.47	0.0091
$LnFE_{NO}$	0.82 ± 0.25	0.0011
Smoking	-0.22 ± 0.37	0.56
Age	-0.02 ± 0.01	0.12
FEV_1	-0.06 ± 0.01	< 0.0001
$LnFE_{NO}$*atopy	0.05 ± 0.11	0.65
Sex	-0.19 ± 0.33	0.56

FE_{NO50}, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second. Multiple logistic regression analysis. The binary outcome was bronchial hyperresposiveness to methacholine (PC20M ≤ ou > 16 mg/ml). Covariates included FENO (log-transform), smoking status, age, FEV1, atopy and gender. When combining all variables into the logistic model, we found that only FENO and FEV1 were significant predictors of the presence of a bronchial hyperresponsiveness to methacholine. Akaike's Criterion (AIC) reached a minimum for this model (AIC = 206.2).

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Table 4 *Combination of FE_{NO50} and FEV_1 to predict the presence of a bronchial hyperresponsiveness to methacholine challenge according to FE_{NO50} and FEV_1 cut-off value defined by the ROC curve*

		PC20M ≤ 16 ($_n$ = 82)		PC20M > 16 (n = 92)		Frequence
FE_{NO50}	FEV_1	*n*	% (A)	*n*	% (B)	ratio A/B
> 34	≤ 101	20	24.4	1	1.1	22.4
> 34	> 101	11	13.4	2	2.2	6.2
≤ 34	≤ 101	39	47.6	40	43.5	1.1
≤ 34	> 101	12	14.6	49	53.3	0.3

FE_{NO50}, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; PC20M, provocative concentration of methacholine causing a 20% fall in FEV_1. When FE_{NO50} value > 34 ppb is associated with FEV_1 ≤ 101% predicted, 24.4% of patients have bronchial hyperresponsiveness to methacholine and are thus true positives while there are only 1.1% of false positive. When FE_{NO50} value is ≤ 34 ppb and FEV_1 > 101%, 53.3% of the patients didn't have bronchial hyperresponsiveness to methacholine and were thus true negatives while 14.6% were false negative, fhe combination of FE_{NO50} and FEV_1 gave a high specificity (98.9%) but a poor sensitivity (24.4%) for identifying patients with a positive bronchial hyperresponsiveness to methacholine. fhe table also shows that the presence of a FE_{NO50} > 34 ppb is more frequently associated to FEV_1 ≤ 101 % in patients with bronchial hyperresponsiveness than in patients without asthma (ratio = 22.4). This ratio decreases if either FE_{NO50} or FEV_1 cut-off is not reached. A FE_{NO50} value ≤ 34 ppb associated with FEV_1 > 101% is however more frequently encountered in patients with negative methacholine challenge (ratio = 1/0.3 = 3.3).

Figure 1 *Receiver-operating characteristic curve (ROC) for the whole group determining exhaled nitric oxide value which best identified the provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 s, FEV_1 ≤ 16 mg/ml. Cut-off point: 34 ppb (Specificity: 95.4%, Sensitivity: 35.4%, positive predictive value: 88%, negative predictive value: 62%, p = 0.0033. AUC = 0.62)*

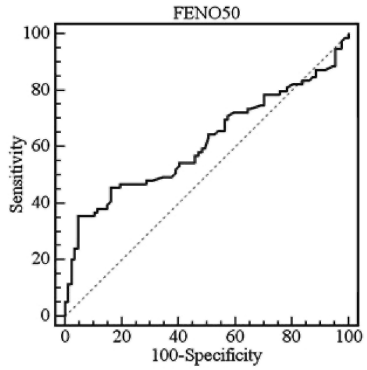


Figure 2 *Receiver-operating characteristic curve (ROC) for the whole group determining % predicted forced expiratory volume in 1 s (FEV_1) value which best identified the provocative concentration of methacholine causing a 20% fall in FEV_1 ≤ 16 mg/ml. Cut-off point: 101% (Specificity: 57%, Sensitivity: 71%, positive predictive value: 59%, negative predictive value: 68%, p = 0.0001. AUC = 0.67)*

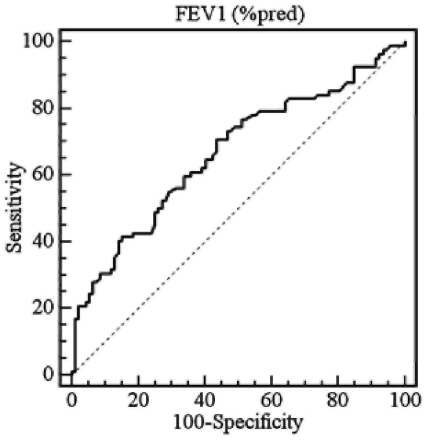


Figure 3 *Spearman test for patients exhibiting a 20% fall in forced expiratory volume in one second (FEV_1) for a provocative concentration of methacholine ≤ 16 mg/ml We didn't find any correlation between fractional exhaled nitric oxide, measured at a flow rate of 50 ml/s (FE_{NO50}) and provocative concentration of methacholine causing a 20% fall in FEV_1 (PC20M) in those patients considered as asthmatics (r = -0.06, p = 0.59)*

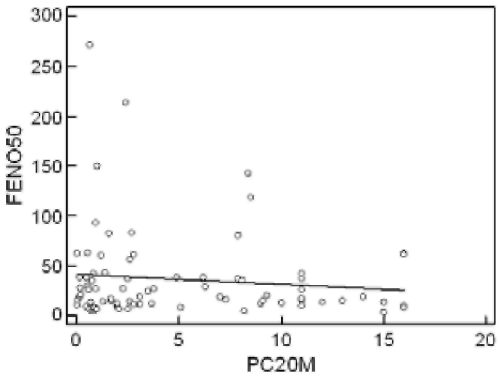


Table 5 *FE_{NO50} according to the presence of respiratory symptoms in a population of patients referred for asthma diagnosis*

Symptoms	FE_{NO50} (PPb)	
	Presence of symptom	**Absence of symptom**
Diurnal cough	16 (4-271)	19 (4-213)
	N = 122	*N* = 52
Nocturnal cough	14 (4-213)	19 (4-271)
	N = 62	*N*= 112
Diurnal wheezing	20 (7-271)**	14 (4-213)
	***N* = 82**	*N* = **92**
Nocturnal wheezing	20 (5-213)*	15 (4-271)
	***N* = 65**	*N* = **119**
Dyspnoea	17 (5-213)	15 (4-271)
	N = 111	*N* = 63
Chest tightness	16 (5-271)	18 (4-142)
	N = 115	*N* = 59
Chest pain	14 (4-80)	18 (4-271)
	N = 46	*N* = 128
Exercice trigger	15 (5-213)	19 (4-271)
	N = 99	*N* = 75
Humidity trigger	15 (4-213)	18 (4-271)
	N = 64	*N* = 110
Fumes trigger	17 (4-150)	18 (4-271)
	N = 87	*N* = 87
Dust trigger	19 (5-142)	16 (4-271)
	N = 78	*N* = 96
Pollen trigger	19 (5-118)	16 (4-271)
	N = 50	*N* = 124
Emotional trigger	15 (5-213)	18 (4-271)
	N =77	*N* = 97
Rhinitis	17 (4-142)	17 (4-271)
	N = 95	*N* = 79
Urticaria	19 (5-213)	16 (4-271)
	N = 48	*N* = 126
Pyrosis	15 (4-142)	19 (5-271)
	N = 91	*N* = 83

*p < 0.05, **p < 0.001. FE_{NO50}, fractional exhaled nitric oxide.

Table 6 *Proportion of symptoms according to the results of the methacholine challenge in a population referred for asthma diagnosis*

Symptoms	PC20M < 16 mg/ml	PC20M > 16 mg/ml
Diurnal cough (Y/N, %)	54/28 (66)	68/24 (74)
Nocturnal cough (Y/N, %)	30/52 (37)	32/60 (35)
Diurnal wheezing (Y/N, %)	47/35 (57)*	35/57 (38)
Nocturnal wheezing (Y/N, %)	46/36 (56)**	19/73 (21)
Dyspnoea (Y/N, %)	60/22 (73)**	41/51 (45)
Chest tightness (Y/N, %)	60/22 (73)**	37/55 (40)
Chest pain (Y/N, %)	20/62 (24)	26/66 (28)
Exercice trigger (Y/N, %)	53/29 (65)	46/46 (50)
Humidity trigger (Y/N, %)	33/49 (40)	31/61 (34)
Fumes trigger (Y/N, %)	40/42 (49)	47/45 (51)
Dust trigger (Y/N, %)	42/40 (51)	36/56 (39)
Pollen trigger (Y/N, %)	28/54 (34)	22/70 (24)
Emotional trigger (Y/N, %)	37/45 (45)	40/52 (43)
Rhinitis (Y/N, %)	49/33 (60)	46/46 (50)
Urticaria (Y/N, %)	25/57 (30)	23/69 (25)
Pyrosis (Y/N, %)	39/43 (48)	52/40 (57)

*$p < 0.05$, **$p < 0.0001$. PC20M, provocative concentration of methacholine causing a 20% fall in FEV_1.

Discussion

Our results shows that FE_{NO50} > 34 ppb has a high positive predictive value to identify bronchial hyperresponsiveness to methacholine in patients who had respiratory symptoms suggestive for asthma and in whom the respiratory physician had no argument for airway flow variability either because baseline calibre was considered to be normal or because bronchodila-tion to inhaled β_2-agonist was weak. However, the sensitivity of 34 ppb cut-off is poor and FE_{NO50} values below this threshold clearly do not rule out bronchial hyperresponsiveness. Furthermore, we found that, among a list of respiratory symptoms, wheezing was the symptom that was the most convincingly associated with raised FE_{NO50}.

Airway hyperresponsiveness and airway inflammation are acknowledged to be key but largely independent features of asthma (10,11). In routine many asthmatics are diagnosed based on the association between chronic respiratory symptoms and the demonstration of airway variability. Reversibility to inhaled β_2-agonist and methacholine/histamine bronchial challenge are the most common ways used to confirm suspected asthma. In those patients with normal baseline lung function it was shown that bronchodilation test and peak expiratory flow rate variability perform rather weakly to ascertain the diagnosis (4,5). FE_{NO50} has been advocated as a useful tool to make asthma diagnosis in steroid naive patients with respiratory symptoms (5,9). The population selected in our study is somewhat slightly different from those described in previous studies in that only patients in whom asthma diagnosis remains uncertain after reversibility testing and/or baseline spirometry were sent to our routine function laboratory for a methacholine challenge. Furthermore, it is of interest to note that the proportion of atopic patients was rather low (50%) and the proportion of active smokers rather high (35%) for a population of mild to moderate steroid naive asthmatics. Dupont (9) and Smith (5) excluded smokers and the series of Smith et al. (5) included 76% of atopic subjects whereas their proportion was not mentioned in the study of Dupont et al. The relatively weak proportion of atopy and the presence of smokers certainly explain why the average FE_{NO50} value in our series is clearly lower than that reported in patients attending an asthma clinic (7,17).

Our results show that bronchial NO may predict methacholine hyperresponsiveness reflected by PC20M ≤ 16 mg/ml with FE_{NO50} cut-off > 34 ppb yielding 95% specificity and 88% positive predictive value. Our data show that 20% with confirmed asthma had FE_{NO50} value > 34 ppb. In contrast to the specificity, sensitivity of 34 ppb threshold is poor and a value below this threshold clearly does not exclude the presence of bronchial hyperresponsiveness. It is important to realise that FE_{NO50} and PC20M values are largely independent variables.

Indeed the correlation between DRS (dose-response slope) for methacholine and FE_{NO50} is weak for the whole population and we did not find any significant relationship between FE_{NO50} and PC20M in those patients diagnosed as asthmatics. This contrasts with what we have recently found concerning the relationship between

FE_{NO50} and sputum eosinophils in a large heterogeneous series of asthmatics encountered in daily practice (7).

The relationship between FE_{NO} and airway hyperresponsiveness is controversial and conflicting results have been published (5,18-23). Compared with our patients, the studies showing a more convincing relationship between FE_{NO} and bronchial hyperresponsiveness included a significantly higher proportion of atopic patients. We found, however, by a multiple regression analysis that atopy, in contrast to FE_{NO50}, was not an independent predictor of bronchial hyperresponsiveness. On the other hand, Smith et al. (5) used hypertonic saline, an indirect stimulus, to measure bronchial responsiveness. It is recognised that airway inflammation is better related to indirect than to direct bronchial hyperresponsiveness (24).

Although FE_{NO50} and PC20M reflect different dimensions in asthma, it does not exclude functional relationship between the two variables. It is admitted that part of the bronchial hyperresponsiveness in asthma is linked to an airway eosinophilic inflammation that can be attenuated by corticosteroids (25).

Furthermore, nitric oxide may itself contribute to bronchial hyperresponsiveness by increasing airway oedema as it is a potent vasodilator responsible for plasma exudation from bronchial vessels (26) and the transformation of NO in peroxynitrite was shown to induce airway hyperresponsiveness in guinea pigs (27). This may explain the good specificity of FENO to detect methacholine responsiveness even if it is not perfect as increased FE_{NO50} may be observed in other pathological conditions such as eosinophilic bronchitis (28) where bronchial hyperresponsiveness is absent. It is also interesting to notice that FE_{NO50} values outside the normal range as defined by Travers et al. (16), whilst being rather rare in our series (13%), carries a high odds ratio (14.5) in favour of bronchial hyperresponsiveness. This observation highlights the fact that consistent airway inflammation may be a determinant factor of bronchial hyperresponsiveness.

The multiple logistic regression analysis confirmed the effect of baseline airway calibre as a strong independent predictor of the presence of bronchial hyperresponsiveness to methacholine. Some studies have shown a correlation between FEV_1 and bronchial hyperresponsiveness (22,29,30). This suggests that airway geometric factors are involved in the mechanisms of bronchial hyperresponsiveness in asthma. Beyond geometry there is also solid argument to support the role of bronchial smooth muscle dysfunction in determining hyperresponsiveness to direct constricting agent (31). Although atopy was shown to correlate with bronchial hyperresponsiveness in epidemiological and clinical studies (32), our data suggest that its influence may be mediated by an increase in airway inflammation as atopic patients clearly exhibited higher FE_{NO50} than non-atopic (19 ppb vs. 15 ppb, $p < 0.01$). We therefore believe that it is not atopy *per se* that matters in determining bronchial hyperresponsiveness but rather the fact that atopy may favour airway inflammation in case sensitised patients are exposed to a relevant allergen. It is important to emphasise that smoking status did not impact on bronchial hyperresponsiveness to methacholine in our study. Smoking has been shown to induce airway hyperresponsiveness in the general population (33). Our data show that smoking may be less critical when considering selected patients based on the presence of chronic respiratory symptoms.

There are only limited data on the precise relationship between the type of symptoms and airway inflammation. In our study diurnal and nocturnal wheezing were associated with proximal airway inflammation as reflected by raised levels of FE_{NO50}. Leuppi et al. reported the same observation in a population of children (34). Although it is admitted that asthma may sometimes be revealed by isolated cough (35), our data show that cough is generally poorly related to methacholine hyperresponsiveness and to FE_{NO50}. As compared with FE_{NO} bronchial hyperresponsiveness to methacholine is associated with a broader spectrum of symptoms including not only wheezing but also dyspnoea and chest tightness which are likely to better reflect airflow limitation than wheezing alone.

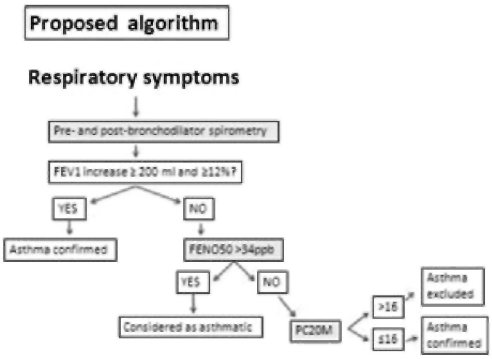
Our study had not the purpose to study asthma phenotypes but rather to validate an inflammometer as a diagnostic tool for the currently accepted definition of asthma according to GINA. A key issue is to know whether or not those patients with chronic respiratory symptoms and high FE_{NO50} are better responsive to inhaled corticoids irrespective of their level of bronchial hyperresponsiveness and their 'asthma' label. This has been suggested by pilot monocentric study (36) but has to be confirmed in a study conducted on a larger scale.

Conclusion

We conclude that FENO measurement may be useful to the clinician in diagnosing asthma in patients with chronic respiratory symptoms in whom bronchodilating test failed to demonstrate reversibility or was not indicated. However, the poor sensitivity of FE_{NO50} to detect bronchial hyperresponsiveness should prompt the clinician to ask for a methacholine challenge when asthma is suspected based on clinical history in case of

FE_{NO50} < 34 ppb (Figure 4). According to our data, application of the algorithm proposed in Figure 3 could save 20% of methacholine challenges performed in a routine pulmonary function laboratory. This is not to be neglected as methacholine challenge is time consuming and uncomfortable to the patients.

Figure 4 *Proposed algorithm for asthma diagnosis. Values < 34 ppb should prompt the clinician to ask for a methacholine challenge when asthma is suspected based on clinical history. The application of the proposed algorithm could save 20% of the methacholine challenge perfomed in a routine lung function laboratory*



Author contributions

Dr F. Schleich: read and met the International Committee of Medical Journal Editors criteria for authorship, designed this study, extracted data, performed the analysis, wrote the first draft of the manuscript and read and approved the final manuscript. Dr Asandei, Dr Manise, Ms Sele: read and met the International Committee of Medical Journal Editors criteria for authorship, read and approved the final manuscript. Ms Seidel: read and met the International Committee of Medical Journal Editors criteria for authorship, performed the analysis, read and approved the final manuscript, Prof. Dr Louis: read and met the International Committee of Medical Journal Editors criteria for authorship, designed this study, critically revised the manuscript and read and approved the final manuscript.

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Disclosures

The authors have reported that no potential conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

References

1 Global Initiative for Asthma. *Global Strategy for Asthma Management and Prevention (GINA).* National Institutes of Health; National Heart, Lung, and Blood Institute, 2011. Available from: http:// www.ginasthma.org/ (accessed 9 November 2011).

2 Brightling CE. Clinical applications of induced sputum. *Chest* 2006; **129:** 1344-8.

3 Taylor DR, Pijnenburg MW, Smith AD et al. Exhaled nitric oxide measurements: clinical application and interpretation. *Thorax* 2006; **61:** 817-27.

4 Hunter CJ, Brightling CE, Woltmann G et al. A comparison of the validity of different diagnostic tests in adults with asthma. *Chest* 2002; **121:** 1051-7.

5 Smith AD, Cowan JO, Filsell S et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. *Am J Respir Cut Care Med* 2004; **169:** 473-8.

6 Berry MA, Shaw DE, Green RH et al. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005; **35:** 1175-9.

7 Schleich FN, Seidel L, Sele J et al. Exhaled nitric oxide thresholds associated with a sputum eosinophil count >/=3% in a cohort of unselected patients with asthma. *Thorax* 2010; **65:** 1039-44.

8 Luks VP, Vandemheen KL, Aaron SD. Confirmation of asthma in an era of overdiagnosis. *Eur Respir J* 2010; **36:** 255-60.

9 Dupont LJ, Demedts MG, Verleden GM. Prospective evaluation of the validity of exhaled nitric oxide for the diagnosis of asthma. *Chest* 2003; **123:** 751-6.

10 Gronke L, Kanniess F, Holz O et al. The relationship between airway hyper-responsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. *Clin Exp Allergy* 2002; **32:** 57-63.

11 Rosi E, Ronchi MC, Grazzini M et al. Sputum analysis, bronchial hyperresponsiveness, and airway function in asthma: results of a factor analysis. *J Allergy Clin Immunol* 1999; **1:** 232-7.

12 Olin AC, Rosengren A, Thelle DS et al. Increased fraction of exhaled nitric oxide predicts new-onset wheeze in a general population. *Am J Respir Crit Care Med* 2010; **181:** 324-7.

13 Boushey HA, Sorkness CA, King TS et al. Daily versus as-needed corticosteroids for mild persistent asthma. *N Engl J Med* 2005; **352:** 1519-28.

14 Crapo RO, Casaburi R, Coates AL et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; **161:** 309-29.

15 Louis R, Sele J, Henket M et al. Sputum eosinophil count in a large population of patients with mild to moderate steroid-naive asthma: distribution and relationship with methacholine bronchial hyperresponsiveness. *Allergy* 2002; **57:** 907-12.

16 Travers J, Marsh S, Aldington S et al. Reference ranges for exhaled nitric oxide derived from a random community survey of adults. *Am J Respir Crit Care Med* 2007; **176:** 238-42.

17 Michils A, Baldassarre S, Van Muylem A. Exhaled nitric oxide and asthma control: a longitudinal study in unselected patients. *Eur Respir J* 2008; **31:** 539-46.

18 Dupont LJ, Rochette F, Demedts MG et al. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naive patients with mild asthma. *Am J Respir Crit Care Med* 1998; **1:** 894-8.

19 Henriksen AH, Lingaas-Holmen T, Sue-Chu M et al. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000; **15:** 849-55.

20 Jatakanon A, Lim S, Kharitonov SA et al. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 1998; **53:** 91-5.

21 Silvestri M, Spallarossa D, Battistini E et al. Dissociation between exhaled nitric oxide and hyperresponsiveness in children with mild intermittent asthma. *Thorax* 2000; **55:** 484-48.

22 Ichinose M, Takahashi T, Sugiura H et al. Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre. *Eur Respir J* 2000; **15:** 248-53.

23 de Gouw HW, Grunberg K, Schot R et al. Relationship between exhaled nitric oxide and airway hyperresponsiveness following experimental rhinovirus infection in asthmatic subjects. *Eur Respir J* 1998; **11:** 126-32.

24 Joos GF, O'Connor B, Anderson SD et al. Indirect airway challenges. *Eur Respir J* 2003; **21:** 1050-68.

25 Cockcroft DW, Davis BE. Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006; **118:** 551-9.

26 Rapoport RM, Murad F. Endothelium-dependent and nitrovasodilator-induced relaxation of vascular smooth muscle: role of cyclic GMP. *J Cyclic Nucleotide Protein Phosphor Res* 1983; 5: 281-96.

27 Sadeghi-Hashjin G, Folkerts G, Henricks PA et al. Peroxynitrite induces airway hyperresponsiveness in guinea pigs *in vitro* and *in vivo*. *Am J Respir Crit Care Med* 1996; **153:** 1697-701.

28 Brightling CE, Symon FA, Birring SS et al. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax* 2003; **58:** 528-32.

29 Sparrow D, O'Connor GT, Rosner B et al. The influence of age and level of pulmonary function on nonspecific airway responsiveness. The Normative Aging Study. *Am Rev Respir Dis* 1991; **1:** 978-82.

30 Hardaker KM, Downie SR, Kermode JA et al. Predictors of airway hyperresponsiveness differ between old and young patients with asthma. *Chest* 2011; **139:** 1395-401.

31 Solway J, Fredberg JJ. Perhaps airway smooth muscle dysfunction contributes to asthmatic bronchial hyperresponsiveness after all. *Am J Respir Cell Mol Biol* 1997; **17:** 144-6.

32 Britton J, Pavord I, Richards K et al. Factors influencing the occurrence of airway hyperreactivity in the general population: the importance of atopy and airway calibre. *Eur Respir J* 1994; **7:** 881-7.

33 Plaschke PP, Janson C, Norrman E et al. Onset and remission of allergic rhinitis and asthma and the relationship with atopic sensitization and smoking. *Am J Respir Crit Care Med* 2000; **1:** 920-4.

34 Leuppi JD, Downs SH, Downie SR et al. Exhaled nitric oxide levels in atopic children: relation to specific allergic sensitisation, AHR, and respiratory symptoms. *Thorax* 2002; **57:** 518-23.

35 Corrao WM, Braman SS, Irwin RS. Chronic cough as the sole presenting manifestation of bronchial asthma. *N Engl J Med* 1979; **300:** 633-7.

36 Smith AD, Cowan JO, Brassett KP et al. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005; **352:** 2163-73.