Is $\text{FE}_{\text{NO50}}$ 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 ($\text{FE}_{\text{NO50}}$) remains a controversial diagnostic tool. Aim: To assess the ability of $\text{FE}_{\text{NO50}}$ to identify bronchial hyperresponsiveness (BHR) to methacholine (provocative concentration of methacholine causing a 20% fall in FEV$_1$; PC20M $\leq$ 16 mg/ml) and to establish whether or not symptoms relate to $\text{FE}_{\text{NO50}}$ and PC20M in patients with no demonstrated reversibility to $\beta_2$-agonist. Methods: We conducted a prospective study on 174 steroid naive patients with respiratory symptoms, forced expiratory volume in 1 s (FEV$_1$) $\geq$ 70% predicted and no demonstrated reversibility to $\beta_2$-agonist. Patients answered to the standardised symptom questionnaire and underwent $\text{FE}_{\text{NO50}}$ and methacholine challenge. Receiver-operating characteristic (ROC) curve and logistic regression analysis assessed the relationship between PC20M and $\text{FE}_{\text{NO50}}$, taking into account covariates (smoking, atopy, age, gender and FEV$_1$). Results: A total of 82 patients had a PC20M $\leq$ 16 mg/ml and had significantly higher $\text{FE}_{\text{NO50}}$ (19 ppb vs. 15 ppb; $p<0.05$). By constructing ROC curve, we found that $\text{FE}_{\text{NO50}}$ 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, $\text{FE}_{\text{NO50}}$ ($p = 0.0011)$ 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 $\text{FE}_{\text{NO50}}$ ($p < 0.001$ and $p < 0.05$, respectively). Conclusion: The value of $\text{FE}_{\text{NO50}} > 34$ 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, $\text{FE}_{\text{NO50}} \leq 34$ ppb does not rule out BHR and should prompt the clinician to ask for a methacholine challenge.

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 ($\text{FE}_{\text{NO50}}$) (3), the latter being much more convenient to apply in routine as it yields immediate results. Both sputum eosinophil count and $\text{FE}_{\text{NO50}}$ 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 $\beta_2$-agonist or to peak expiratory flow (PEF) variability criteria (4,5). $\text{FE}_{\text{NO50}}$ 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 ($\text{FE}_{\text{NO200}}$) cut-off of 16 ppb may help to identify patients with bronchial hyperresponsiveness to histamine or reversibility to $\beta_2$-agonist among those presenting with chronic respiratory problems.
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, FENO50 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 (FENO) measurement is easy to perform and provides immediate results. The purpose of our study was to see how FENO 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 FEV1 (PC20M) in patients referred by chest physicians for asthma diagnosis to a routine laboratory function. This study focused on patients in whom the bronchodilatation 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 (FEV1) value (> 80% predicted and FEV1/FVC > 70%). We also sought to establish how different types of respiratory symptoms relate to FENO50 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 baseline spirometric values were nor-

| Data are presented as mean ± SD (FEV1, FVC, FEV1/FVC, age) or as median (range; FENO50). PC20M is expressed as geometric mean (range). PC20M, provocative concentration of methacholine causing a 20% fall in FEV1; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FENO50, fractional exhaled nitric oxide. |
| 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 |
| FEV1, % predicted | 97 ± 13 |
| FVC, % predicted | 100 ± 14 |
| FEV1/FVC, % | 83 ± 7 |
| FENO50, ppb | 17 (4–271) |

| Data are presented as mean ± SD (FEV1, FVC, FEV1/FVC, age) or as median (range; FENO50). PC20M is expressed as geometric mean (range). PC20M, provocative concentration of methacholine causing a 20% fall in FEV1; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FENO50, fractional exhaled nitric oxide. |
| Table 1 | 159 |

Exhaled NO measurement

Exhaled nitric oxide, FENO was measured by chemiluminescence 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 NO 50 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 NO 50 (log transformed), age, gender, FEV1, 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).

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 FEV1 (95% predicted vs. 102% predicted, p < 0.001) and lower FEV1/FVC (p < 0.05) even if the average value clearly remained within the normal range. FE NO 50 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 NO 50 (p = 0.0011) and FEV1 (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 FEV1 values and higher

| Parameter | Coefficient ± SE | p-value |
|-----------|------------------|---------|
| Intercept | 3.82 ± 1.47      | 0.0091  |
| LnFENO    | 0.82 ± 0.25      | 0.0011  |
| Smoking   | −0.22 ± 0.37     | 0.56    |
| Age       | −0.02 ± 0.01     | 0.12    |
| FEV1      | −0.06 ± 0.01     | < 0.0001|
| LnFENO*atopy | 0.05 ± 0.11  | 0.65    |
| Sex       | −0.19 ± 0.33     | 0.56    |

FE NO 50, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second. Multiple logistic regression analysis. The binary outcome was bronchial hyperresponsiveness 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).

### Table 2

Demographic, functional and inflammatory characteristics for patients with and without asthma

|                      | PC20M ≤ 16 mg/ml | PC20M > 16 mg/ml |
|----------------------|------------------|------------------|
| N                    | 82               | 92               |
| Sex (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 FEV1 (%/μmol)    | 0.0033 (0.0006–0.4337)*** | 0.0004 (0.0001–0.0007)  |
| FEV1, % predicted    | 95 ± 14**        | 102 ± 12         |
| FVC, % predicted     | 99 ± 14          | 102 ± 13         |
| FEV1/FVC, %          | 82 ± 7*          | 84 ± 6           |
| FE NO 50, (ppb)      | 19 (4–271)*      | 15 (4–120)       |

Data are presented as mean ± SD (FEV1, FVC, FEV1/FVC, age) or as median (range; FE NO 50). 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 FEV1; DRS, dose-response slope; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; FE NO 50, fractional exhaled nitric oxide.
FENO values were associated with PC20M ≤ 16 mg/ml.

We constructed a ROC curve to establish the ability of FENO50 to identify bronchial hyperresponsiveness assessed by methacholine challenge (Figure 1). We found that FENO50 significantly predicted PC20 ≤ 16 mg/ml with a cut-off value of 34 ppb. However, this FENO50 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 FENO50 was 35 ppb. When referring to FENO50 normal values as defined by Travers et al. (16), we found that 22 patients (13%) had FENO50 values upper to the 95% confidence interval. The ability of FENO50 to identify airway hyperresponsiveness was high in those patients. Indeed 20 of the 22 patients with FENO50 values out of range according to Travers had bronchial hyperresponsiveness whereas this was only the case in 62 of the 152 patients in whom FENO50 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 FEV1 cut-off was best related to the prediction of the presence of a bronchial hyperresponsiveness (Figure 2). We found that FEV1 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 FENO50 and FEV1 values to predict the presence of a bronchial hyperresponsiveness to methacholine, we found that the presence of both FENO50 > 34 ppb and FEV1 ≤ 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 FENO and methacholine responsiveness**

On the whole population the dose-response slope (DRS) for methacholine weakly correlated with FENO50 (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 FENO50 (r = −0.06, p = 0.6, Figure 3).

**Relationship between respiratory symptoms and FENO50 or bronchial responsiveness**

Table 5 shows FENO50 according to the presence of respiratory symptoms in our population. Diurnal and nocturnal wheezing were associated with raised levels of FENO50 (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.
Discussion

Our results show that FENO50 > 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 bronchodilation to inhaled β2-agonist was weak. However, the sensitivity of 34 ppb cut-off is poor and FENO50 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 FENO50.

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 β2-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). FENO50 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 FENO50 value in our series is

| FENO50 | FEV1 | PC20M ≤ 16 (n = 82) | PC20M > 16 (n = 92) | Frequency 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                 |

FENO50, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; PC20M, provocative concentration of methacholine causing a 20% fall in FEV1. When FENO50 value > 34 ppb is associated with FEV1 ≤ 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 FENO50 value is ≤ 34 ppb and FEV1 > 101%, 53.3% of the patients didn’t have bronchial hyperresponsiveness to methacholine and were thus true negatives while 14.6% were false negative. The combination of FENO50 and FEV1 gave a high specificity (98.9%) but a poor sensitivity (24.4%) for identifying patients with a positive bronchial hyperresponsiveness to methacholine. The table also shows that the presence of a FENO50 > 34 ppb is more frequently associated to FEV1 ≤ 101% in patients with bronchial hyperresponsiveness than in patients without asthma (ratio = 22.4). This ratio decreases if either FENO50 or FEV1 cut-off is not reached. A FENO50 value ≤ 34 ppb associated with FEV1 > 101% is however more frequently encountered in patients with negative methacholine challenge (ratio = 1/0.3 = 3.3).
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 FENO50 cut-off > 34 ppb yielding 95% specificity and 88% positive predictive value. Our data show that 20% with confirmed asthma had FENO50 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 FENO50 and PC20M values are largely independent variables.

Indeed the correlation between DRS (dose-response slope) for methacholine and FENO50 is weak for the whole population and we did not find any significant relationship between FENO50 and PC20M in those patients diagnosed as asthmatics. This contrasts with what we have recently found concerning the relationship between FENO and sputum eosinophils in a large heterogeneous series of asthmatics encountered in daily practice (7).

The relationship between FENO 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 FENO50 and bronchial hyperresponsiveness included a significantly higher proportion of atopic patients. We found, however, by a multiple regression analysis that atopy, in contrast to FENO50, 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. In this context, it is not surprising that airway inflammation is better related to indirect than to direct bronchial hyperresponsiveness (24).

Although FENO50 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).

### Table 5 FENO50 according to the presence of respiratory symptoms in a population of patients referred for asthma diagnosis

| Symptoms       | FENO50 (ppb) | Presence of symptom | Absence of symptom |
|----------------|--------------|---------------------|--------------------|
| Diurnal cough  | N = 122      | 16 (4–213)          | 19 (4–213)         |
| Nocturnal cough| N = 62       | 14 (4–213)          | 19 (4–213)         |
| Diurnal wheezing| N = 82      | 20 (7–217)**        | 14 (4–213)         |
| Nocturnal wheezing| N = 65    | 20 (5–213)*         | 15 (4–217)         |
| Dyspnoea       | N = 111      | 17 (5–213)          | 19 (4–217)         |
| Chest tightness| N = 115      | 16 (5–217)          | 18 (4–142)         |
| Chest pain     | N = 46       | 14 (4–80)           | 18 (4–217)         |
| Exercice trigger| N = 99     | 15 (5–213)          | 19 (4–217)         |
| Humidity trigger| N = 64      | 15 (4–213)          | 18 (4–217)         |
| Fumes trigger  | N = 87       | 17 (4–150)          | 18 (4–217)         |
| Dust trigger   | N = 78       | 19 (5–142)          | 16 (4–217)         |
| Pollen trigger | N = 50       | 19 (5–118)          | 16 (4–217)         |
| Emotional trigger| N = 77     | 15 (5–213)          | 18 (4–217)         |
| Rhinitis       | N = 95       | 17 (4–142)          | 17 (4–217)         |
| Urticaria      | N = 19       | 19 (5–213)          | 16 (4–217)         |
| Pyrosis        | N = 15       | 15 (4–142)          | 19 (5–217)         |

* p < 0.05, ** p < 0.001. FENO50, 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, %) | 39 (60) 46       | 54 (28 66) 68 (24 74) |
| Nocturnal cough (Y/N, %)| 45 (45) 40       | 30 (52 37) 32 (60 35) |
| Diurnal wheezing (Y/N, %)| 45 (35 57)*     | 47 (35 57) 46 (57 38) |
| Nocturnal wheezing (Y/N, %)| 45 (35 57)     | 46 (36 56)** 19 (73 21) |
| Dyspnoea (Y/N, %)      | 45 (35 57) 46 (57 38) | 60 (22 73)** 41 (51 45) |
| Chest tightness (Y/N, %)| 45 (35 57) 46 (57 38) | 60 (22 73)** 37 (55 40) |
| Chest pain (Y/N, %)    | 45 (35 57) 46 (57 38) | 20 (62 24) 26 (66 28) |
| Exercice trigger (Y/N, %)| 45 (35 57) 46 (57 38) | 53 (29 65) 46 (46 50) |
| Humidity trigger (Y/N, %)| 45 (35 57) 46 (57 38) | 33 (49 40) 31 (61 34) |
| Fumes trigger (Y/N, %) | 45 (35 57) 46 (57 38) | 40 (42 49) 47 (45 51) |
| Dust trigger (Y/N, %)  | 45 (35 57) 46 (57 38) | 42 (40 51) 36 (56 39) |
| Pollen trigger (Y/N, %)| 45 (35 57) 46 (57 38) | 28 (54 34) 22 (70 24) |
| Emotional trigger (Y/N, %)| 45 (35 57) 46 (57 38) | 37 (45 45) 40 (52 43) |
| Rhinitis (Y/N, %)      | 45 (35 57) 46 (57 38) | 49 (33 60) 46 (46 50) |
| Urticaria (Y/N, %)     | 45 (35 57) 46 (57 38) | 25 (57 30) 23 (69 25) |
| Pyrosis (Y/N, %)       | 45 (35 57) 46 (57 38) | 39 (43 48) 52 (40 57) |

*p < 0.05, **p < 0.0001. PC20M, provocative concentration of methacholine causing a 20% fall in FEV1.
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\textsubscript{NO\textsubscript{50}} 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\textsubscript{NO\textsubscript{50}} 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 FE\textsubscript{V\textsubscript{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\textsubscript{NO\textsubscript{50}} 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\textsubscript{NO\textsubscript{50}}. 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\textsubscript{NO\textsubscript{50}}. As compared with FE\textsubscript{NO\textsubscript{50}} 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\textsubscript{NO\textsubscript{50}} 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\textsubscript{NO\textsubscript{50}} 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\textsubscript{NO\textsubscript{50}} < 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 pul-

![Proposed algorithm](image-url)

**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 performed in a routine lung function laboratory.
monary function laboratory. This is not to be neglected as methacholine challenge is time consuming and uncomfortable to the patients.

**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 Asande, 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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