



Association between exhaled nitric oxide and nasal polyposis in severe asthma

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ABSTRACT

Background: Although several contributions exist on the role of fractional exhaled NO (FeNO) in diagnosis and management of asthma, no studies have analysed the association between FeNO in patients with severe asthma and nasal polyposis.

Aim: We investigated the potential association between FeNO and the presence of nasal polyps in patients affected by severe asthma.

Methods: Study population included 93 severe asthmatic adult patients consecutively enrolled from four Italian specialist clinic centres from 2015 to 2018. In these patients lung function, asthma control, FeNO, blood eosinophils and CT scan of paranasal sinuses were evaluated.

Results: Nasal polyposis was observed in 28 patients (30%). Among univariate predictors (lower BMI, higher FeNO, eosinophil and neutrophil count), recursive partitioning analysis identified as best predictors of nasal polyposis high values of eosinophil count ($\geq 6.5\%$ or > 420 cells/mm³) and FeNO (≥ 39 ppb). The 40 patients with low eosinophil count and FeNO had a significant lower occurrence of nasal polyposis than those with higher values (8% vs 58%; $p < 0.001$). The stratification algorithm had a good performance in discriminate patients with and without nasal polyposis (area under the receiver operating characteristic curve of 0.768).

Conclusions: Our results show that FeNO might improve to detect nasal polyposis in patients with severe asthma and a low level of blood eosinophils counts, identifying individuals with high susceptibility to this condition.

1. Introduction

Severe asthma is considered a challenge for clinicians because it is frequently accompanied by a difficult and often unachievable control that requires a critical management. Cluster analysis of European and US cohorts of severe asthmatic patients showed several clinical, functional, and inflammatory differences among patients that let us to extend the heterogeneity of asthma also to the severe forms. Therefore, it has become imperative to sub-phenotype severe asthmatic patients looking at multiple features such as age of onset, comorbidities, atopy, and biomarkers, in view of a different management and therapeutic approach of these patients [1].

An emerging sub-phenotype is the late onset severe asthma associated with nasal polyposis [2], which the Italian severe/uncontrolled asthma registry reported with a prevalence of 30% [3]. Nasal polyposis is a frequent comorbidity of severe that significantly impact lung function and symptom control [16]. This sub-phenotype is frequently a “T2 high” eosinophilic endotype [4] characterized by the activation of type – 2 innate lymphoid cells (ICL-2) releasing interleukin (IL)-5 and IL-13, and relatively little IL-4. The same pattern of cytokine release has been demonstrated in nasal polyp tissue linked to the activation of ILC-2 [5,6]. IL-5 is the major driver of the eosinophilic blood and tissue inflammation. Among the pleiotropic effects of IL-13 on airway inflammation and remodeling, there is the inducing activity on bronchial

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epithelium of the inducible isoform of nitric oxide synthase (i-NOS), the main source of the fractional exhaled nitric oxide (FeNO).

The use of biomarkers to identify “T2 high” endotypes has today gaining increasing interest in view to sub-phenotype these patients [7] and to better drive tailored therapy [8].

Several biomarkers have been proposed, but only the eosinophils in blood and the FeNO seem to have reached a place in the clinical practice [9]. The interest in measuring FeNO in severe asthma has increased in the past years for non-invasiveness of this indirect marker of eosinophilic airway inflammation and for its proved usefulness in the diagnosis of asthma and in the management of ICS therapy [10–12]. FeNO is mainly considered a marker of the endotype type 2 high and a predictor of efficacy (in terms of the identification of responders) of some biologics as omalizumab and tralokinumab [13,14]. A significant reduction of FeNO levels has also been described after a successful treatment with lebrikizumab, dupilumab, tezepelumab [15–17].

Although several contributions exist on the role of FeNO measurement in asthma management, to our knowledge, no studies have analysed the association between FeNO and nasal polyposis in patients with severe asthma. Therefore, the aim of the study was to investigate the potential association between FeNO and the presence of nasal polyps in patients affected by this disease.

2. Patients and methods

2.1. Patients

A total of ninety-three severe asthmatic adult patients were consecutively enrolled from four Italian specialist clinic centres from 2015 to 2018. All of them had a diagnosis of severe asthma according to ATS/ERS definition of severe asthma [18] and regularly took ICS (daily use of ICS: beclomethasone HFA ≥ 1000 mcg or equivalent).

All asthmatics were assessed during a period of stability of their disease and at least four weeks after an upper respiratory tract infection. Written informed consent was obtained from all subjects, and the institutional ethics committee of each Center approved the study.

At the first visit, a complete baseline questionnaire requesting information on medical history was administered to all subjects who were then given physical examination, atopy assessment, and spirometry with bronchial obstruction reversibility test and all patients exhibited a recent (< 3 months) paranasal CT scan and/or an ENT evaluation. During the second visit, subjects underwent blood eosinophils count and FeNO measurement.

All patients enrolled were not included in randomized controlled trial or treated with biologic treatments.

2.2. Atopic status

A panel of inhalant allergens (parietaria, grass, dermatophagoides, olea, cat fur, dog hair, and molds; Allergopharma, Milan, Italy) was used. The positivity was defined as a skin-prick test of at least 3 mm weal.

2.3. Lung function

Pulmonary function tests were performed following the recommendation for standardized procedures for lung function tests [19]. Forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured using a spirometer (Sensor-medics, USA). The best value of three maneuvers was expressed as a percentage of the predicted normal value. After baseline evaluation, spirometry was repeated 15 min after the subjects had inhaled 400 µg of salbutamol, as previously reported. Reversibility of airways obstruction was expressed in terms of an increase $\geq 12\%$ and 200 ml from baseline FEV₁.

2.4. Measurement of FeNO

FeNO was measured using an electrochemical analyzer (HypairFeNO Medisoftware Exp'air, 2010) according to ATS-ERS recommendations for online measurement of FeNO in adults [20]. Briefly, subjects were studied in sitting position. FeNO was measured during slow exhalation from total lung capacity against a positive pressure to generate an exhalation flow rate of 50 mL/s. The exhalations flow rate was kept as constant as possible using a biofeedback visual display. Two measurements for each patient were performed and their mean was used in the analysis.

2.5. Statistical analysis

Data are shown as mean values $\pm$ standard deviation and categorical variables are given as frequency or percentages. Continuous variables were compared by using Student's t-test or by analysis of variance. Non parametric tests were used for variable with a non-normal distribution. Associations between categorical data were evaluated by chi-square or Fisher's exact test, as appropriate. A logistic model was performed to obtain Odds Ratios (ORs) for nasal polyposis and to perform the adjustment for other covariates. To identify patients with nasal polyposis, we used a recursive partitioning analysis based on progressive binary splits [21]. Among univariate predictors of nasal polyposis, the optimal split was selected on the basis of highest association detected among categorical variables and different cut-off values of continuous data. The process was stopped after two iterations to produce a simple decision tree and to generate the risk stratification model. Discrimination was evaluated by means of area under the receiver operating characteristic (ROC) curve that was re-evaluated in 1000 bootstrap replicate samples of the same size of the study population. A p value of < 0.05 was considered statistically significant. The analyses were made using STATA software, version 14 (StataCorp, College Station, Texas).

3. Results

Table 1 shows patients' characteristics of study population. Nasal polyposis, observed in 28 (30%) out 93 cases, was associated to lower BMI, leukotriene receptor antagonists use, higher FeNO, eosinophil and neutrophil percentage. No difference was detected in terms of demographic characteristics, age of onset, number of exacerbations, atopic status, smoking habit, drugs, FEV₁ and FVC both at baseline and after salbutamol inhalation. All associations were confirmed adjusting for gender, age, atopic status and systemic oral corticosteroids (Table 1).

At recursive partitioning analysis, eosinophil count was the best predictor of nasal polyposis (Fig. 1). Of the 37 patients with eosinophil count $\geq 6.5\%$, 20 (54%) had nasal polyposis. In the remaining 56 patients with eosinophil count < 6.5% the occurrence of nasal polyposis was observed in 8 patients (14%). Discriminating based on the value of > 420 cells/mm³ led to the same results.

A further binary split was derived in this subgroup by FeNO measurement: patients with values ≥ 39 ppb were at significant greater risk of nasal polyposis (5 out 16 patients, 31%) than those with values < 39 ppb. The 40 patients with both low eosinophil count and low FeNO had a significant lower occurrence of nasal polyposis than the 53 with higher values (8% vs 58%; $p < 0.001$). In comparison to patients with the lowest occurrence of nasal polyposis according to recursive partitioning analysis, a significant greater association was observed in subgroups identified by the presence of high eosinophil count or FeNO ≥ 39 ppb (Fig. 1). The model had good performance in discriminate patients with and without nasal polyposis (area under ROC curve 0.768); the 1000 bootstrap replicate samples confirmed this result (area under ROC curve: mean 0.759 ± 0.06 , median 0.764 with interquartile range from 0.733 to 0.798, 5–95% percentiles 0.659–0.836).

Table 1
Patients' characteristics by the presence of nasal polyposis.

	Nasal polyposis			p	Adjusted Odds Ratio for nasal polyposis	
	All	No	Yes		(by increase units)	p
	n = 93	n = 65	n = 28			
Female gender	65%	68%	57%	0.329	0.65	0.366
Age (years)	58 ± 11	58 ± 12	58 ± 10	0.566	0.97 (x10)	0.863
BMI (Kg/m ²)	27.5 ± 4.9	28.5 ± 4.8	25.3 ± 4.2	0.002	0.82 (x1)	0.005
Asthma control test	16 ± 5	16 ± 5	17 ± 6	0.341	1.01 (x1)	0.796
Age of onset (years)	38 ± 16	39 ± 17	36 ± 13	0.412	0.79 (x10)	0.247
Number of exacerbations	3.4 ± 2.6	3.3 ± 2.6	3.5 ± 2.8	0.720	1.06 (x1)	0.570
Nasal polyposis	30%	–	–	–	–	–
Rhino-sinusitis	51%	29%	100%	–	–	–
Atopic status	60%	60%	61%	0.949	0.97	0.956
Ex-smoker	26%	26%	25%	0.907	0.85	0.767
Smoker	12%	9%	18%	0.297	2.42	0.197
Immunoglobulin E (IU/ml)	371 ± 484	387 ± 565	341 ± 279	0.433	0.99 (x50)	0.832
Leukotriene Receptor Antagonists	59%	52%	75%	0.041	3.22	0.027
Systemic oral corticosteroids	34%	37%	29%	0.437	0.72	0.517
FEV1 pre-BD (% predicted)	67 ± 21	67 ± 20	68 ± 25	0.782	0.99 (x10)	0.929
FVC pre-BD (% predicted)	88 ± 22	87 ± 21	90 ± 26	0.556	1.05 (x10)	0.645
FEV1 post-BD (% predicted)	74 ± 22	75 ± 21	73 ± 27	0.784	0.93 (x10)	0.551
FVC post-BD (% predicted)	93 ± 22	93 ± 21	93 ± 25	0.953	0.99 (x10)	0.943
Reversibility FEV1 (%)	12 ± 10	13 ± 10	12 ± 10	0.445	0.99 (x1)	0.667
Exhaled Nitric Oxide (ppb)	38 ± 31	32 ± 24	50 ± 41	0.005	1.11 (x5)	0.030
Eosinophil count (cell/mm ³)	408 ± 300	334 ± 247	578 ± 345	0.001	1.16 (x50)	0.001
Eosinophil count (%)	5.7 ± 4.2	4.6 ± 3.5	8.3 ± 4.7	< 0.001	1.27 (x1)	< 0.001
Neutrophil count (cell/mm ³)	5110 ± 2267	5186 ± 2354	4927 ± 2073	0.665	0.99 (x500)	0.807
Neutrophil count (%)	59 ± 11	61 ± 11	55 ± 10	0.048	0.73 (x5)	0.040

Mean ± Standard Deviation or percentage. Odds Ratio for nasal polyposis by logistic analysis adjusting for gender, age, atopic status and systemic oral corticosteroids. Odds Ratio for continuous variables reported by the number of units increase. BMI = body mass index; FEV₁ = forced expiratory volume at 1 s; FVC = forced vital capacity; Pre-BD = pre bronchodilation; ppb = parts per billion.

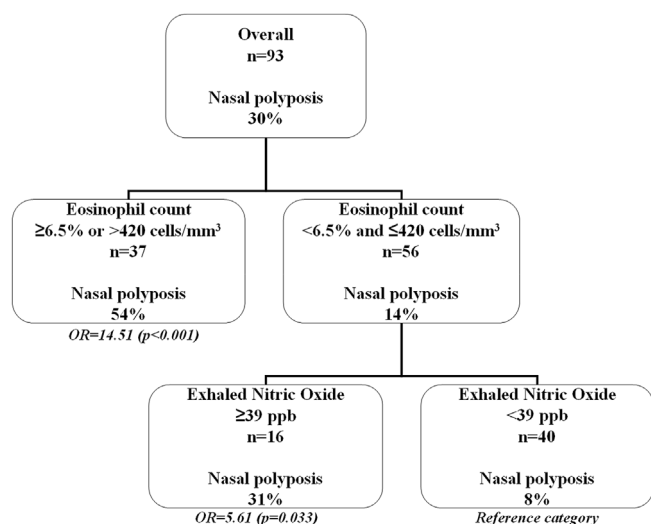


Fig. 1. Recursive partitioning analysis based on progressive binary splits to identify patients with nasal polyposis by eosinophil count and FeNO. Eosinophil count was the best predictor of nasal polyposis. In the subgroup with low eosinophil count, patients with high FeNO had a significant higher occurrence of nasal polyposis.

Table 2 shows patients' characteristics by terminal nodes in classification hierarchical tree for nasal polyposis. The group at the highest risk for nasal polyposis had a greater number of exacerbations, reversibility FEV₁ and lower neutrophil count.

4. Discussion

Our results showed that, in patients with severe asthma, the exhaled NO is significantly associated with the presence of nasal polyps. This

association was particularly relevant in patients with low eosinophils counts that, as expected, remained the strongest significant predictor of nasal polyposis.

To the best of our knowledge, this is the first study that reported as FeNO levels significantly contributed in the identification of nasal polyposis, stratifying a cohort of severe asthmatic patients based on their blood eosinophil count. We used a recursive partitioning technique to select predictors of nasal polyposis and to create a simple algorithm based for an easy patients' stratification. In comparison to parametric regression models that evaluate the impact of single independent variables, recursive partitioning is useful in identifying non-linear patterns of associations demonstrating the interplay of factors relating to outcomes. We observed several univariate associations for the presence of nasal polyps. However, based on hierarchical binary splits, only eosinophils counts and FeNO were able to classify patients with different degree of nasal polyposis occurrence. Out 28 patients with nasal polyps, three had low eosinophil count and FeNO (< 39 ppb) while 25 had a high value in at least one of two parameters. FeNO was able to improve stratification for nasal polyposis at the second stage of recursive analysis in patients with low eosinophil count.

The results from our study suggest investigating nasal polyposis comorbidity in all severe asthmatic patients with elevated FeNO levels, including the subgroups with normal or low blood eosinophil count.

The phenotype of late onset severe asthma associated with nasal polyposis has been recently recognized [2,22]. There is good evidence that the inflammatory response involved in the pathogenesis of nasal polyps is driven by T2 cytokines, such as IL-5 and IL-13, released by ILC-2 in response to several cytokines released by bronchial epithelial cell upon stimulation with different stimuli as bacteria, virus, air pollutants, in an allergen-independent manner [6]. While IL-5 is the key driver of eosinophilia, IL-13 induced inflammation is strongly associated with FeNO by i-NOS. Both these cytokines are involved in the development of nasal polyposis [6]. Thus a combination of surrogates for IL-5 (blood eosinophilia) and IL-13 (FeNO) activity would perform

Table 2
Patients characteristics by risk for nasal polyposis derived from the hierarchical tree analysis.

	High	Moderate	Low	
	n = 37	n = 16	n = 40	p
Female gender	57%	56%	75%	0.185
Age (years)	56 ± 12	62 ± 9	59 ± 12	0.780
BMI (Kg/m ²)	26.0 ± 3.8	27.4 ± 3.8	28.9 ± 5.7	0.420
Asthma control test	15 ± 6	17 ± 5	16 ± 5	0.454
Age of onset (years)	36 ± 14	42 ± 17	36 ± 16	0.415
Number of exacerbations	3.7 ± 2.5	2.8 ± 1.3	3.3 ± 3.2	0.023
Nasal polyposis	54%	31%	8%	–
Rhinosinusitis	70%	50%	33%	–
Atopic status	49%	75%	65%	0.142
Ex-smoker	30%	25%	23%	0.819
Smoker	14%	13%	10%	0.914
Immunoglobulin E (IU/ml)	485 ± 645	282 ± 248	281 ± 304	0.719
Leukotriene Receptor Antagonists	78%	31%	53%	0.003
Systemic oral corticosteroids	38%	19%	38%	0.350
FEV ₁ pre-BD (% predicted)	63 ± 22	75 ± 22	68 ± 20	0.186
FVC pre-BD (% predicted)	84 ± 23	95 ± 23	89 ± 21	0.217
FEV ₁ post-BD (% predicted)	68 ± 22	82 ± 22	76 ± 21	0.117
FVC post-BD (% predicted)	88 ± 23	97 ± 25	96 ± 21	0.290
Reversibility FEV ₁ (%)	14 ± 11	7 ± 6	13 ± 10	0.004
Exhaled Nitric Oxide (ppb)	44 ± 25	70 ± 45	20 ± 10	–
Eosinophil count (cell/mm ³)	680 ± 260	268 ± 143	212 ± 156	–
Eosinophil count (%)	10.0 ± 2.9	3.3 ± 1.7	2.6 ± 1.8	–
Neutrophil count (cell/mm ³)	4392 ± 1691	5503 ± 2242	5599 ± 2590	0.010
Neutrophil count (%)	53 ± 8	60 ± 12	64 ± 10	< 0.001

Mean ± Standard Deviation or percentage. BMI = body mass index; FEV₁ = forced expiratory volume at 1 s; FVC = forced vital capacity; Pre-BD = pre bronchodilation; ppb = parts per billion.

Daily use of ICS: beclomethasone HFA ≥ 1000 mcg or equivalent).

better than either alone in predicting the presence of polyposis, as we have shown in the present study.

Considering that FeNO is a good surrogate of sputum eosinophilia, in the sub-group of patients with high FeNO levels and low blood eosinophil count, it can be speculated that the discrepancy between systemic and local airway inflammation could be due to massive local attraction of eosinophils due to the release of chemotactic agents without heavy stimulation of bone marrow. Similarly to our results, Schleich et al. [23] found a significantly higher percentage of nasal polyposis comorbidity as well as FeNO in those with higher sputum eosinophilia in comparison with patients with lower sputum eosinophilia among patients with low blood eosinophil count.

A previous study reported in asthma patients a positive correlation between nasal polyposis and FeNO, but this evidence was found independently by the level of asthma severity [24]. Kobayashi et al. [25] showed that FeNO levels were even elevated in well controlled asthmatic patients when they were affected by eosinophilic chronic rhinosinusitis with nasal polyps, although the authors did not find a relationship with asthma severity but only with sinus CT score.

In a recent prospective 5-year follow up study, Coumou et al. [26] showed in 200 adults with newly diagnosed asthma, that the presence of nasal polyps, higher levels of FeNO, but also lower body mass index and higher numbers of blood eosinophils were significantly associated with accelerated decline in FEV₁.

However, numerous studies have shown that the prevalence of nasal polyposis is higher in severe asthmatics with values reaching 44.6% in comparison with 4.8% and 9.6% observed in mild and moderate asthmatic patients, respectively [27]. Furthermore, asthmatic patients affected by eosinophilic nasal polyposis have a higher rate of nasal polyps recurrence, higher levels of FeNO and more severe asthma in comparison with those affected by non eosinophilic nasal polyposis [28].

In the Belgian severe asthma registry, blood eosinophil count (≥ 220/mm³) with FeNO levels (> 27 ppb) were used to identify type 2 – high severe asthmatic patients. In comparison with low type – 2 asthmatics, they showed a higher prevalence of nasal polyposis reaching 24% in the late onset asthma group and 18% in the early

asthma group [29]. In addition, in patients affected by chronic rhinosinusitis, besides asthma, the presence of nasal polyposis is the major determinant of increased FeNO levels, with a significant interaction between nasal polyps and asthma [30]. Continual awareness of the coexistent eosinophilic chronic rhinosinusitis with nasal polyposis is recommended in all asthmatics with high FeNO levels [25].

Chronic rhinosinusitis and nasal polyposis represent independent risk factors for frequent severe exacerbations in severe asthma [31]. Although medical treatment of chronic rhinosinusitis with inhaled corticosteroids have been associated with improvements in asthma symptom control, FeNO, and FEV₁, paranasal sinuses may not be easily accessible for nasal corticosteroids. The ongoing inflammation of sinus mucosa may cause ostio-meatal obstruction and becomes a focus for asthma exacerbations.

Novel systemic treatments targeting type 2 cytokines may become the best options to prevent frequent severe asthma exacerbations in patients with late-onset eosinophilic asthma and nasal polyposis comorbidity. A recent systematic review and meta-analysis of *anti*-IL5 therapy demonstrated that mepolizumab and reslizumab determined a statistically significant reduction in nasal polyposis score [32]. Similarly, the IL-4/IL-13 pathway seems important for chronic rhinosinusitis with nasal polyps: the addition of subcutaneous dupilumab to nasal mometasone in comparison with the only nasal steroid reduced endoscopic nasal polyp burden after 16 weeks [33]. Although FeNO levels are strongly associated with type-2 inflammation, Modena et al. showed, in a subset of severe asthma patients with high levels of FeNO, the elevation of IFN-gamma related genes [34].

Some limitations apply to our study. Due to the kind of patients, the study population had a relatively small sample size able to detect only associations of larger magnitude. We used a bootstrap method to re-evaluate internally the performance of model for nasal polyposis. An external population should be studied to validate our findings as well as a larger cohort may be useful to evaluate other predictors with a lower level of association with nasal polyposis. Second, we enrolled also subjects who were currently smoking, a factor affecting largely FeNO and probably even blood eosinophils. However, only 11 patients were

currently smokers and we evaluated the possible interaction between smoker/non-smoker status and absolute values of FeNO or blood eosinophils without evidence of a modification in the degree of association with nasal polyposis. Moreover, model discriminative ability of the proposed cut-offs for FeNO and blood eosinophils counts in the overall study group was similar to that observed in the subgroup of non-smoker subjects (area under ROC curve 0.768 vs 0.750). Our results were mostly representative of a non-smoker population and the evaluation of different cut-offs by smoker status should be performed in a larger sample including a higher proportion of smoker patients. Finally, all the patients were under inhaled steroids treatment, which may have influenced the FeNO.

In conclusion, this study demonstrates the utility of exhaled NO in recognizing patients with severe asthma in conjunction with nasal polyposis. In particular, FeNO might improve to detect nasal polyposis in patients with a low level of blood eosinophils counts (which has the strongest ability to divide subjects into those with either high or low probability of polyposis), identifying individuals with high susceptibility to this condition.

Competing Interest

None.

Conflict of interest

All the Authors disclose any commercial interest that they may have in the subject of study and the source of any financial or material support.

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