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REVISITING THE ROLE OF EXHALED NITRIC OXIDE IN ASTHMA

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ABSTRACT

Purpose of review: The review focused on the most recent studies investigating fractional nitric oxide in exhaled air (FeNO) as useful biomarker for identifying specific phenotypes in asthma and as a tool for asthma diagnosis, monitoring and clinical decision making.

Recent findings: On the basis of the current literature it has been highlighted that FeNO is a clinically relevant marker in various clinical aspects of asthma: 1) FeNO is a predictor for developing asthma in persistent rhinitis or in infants with respiratory symptoms; 2) FeNO contributes to identify asthma phenotypes in both children and adults also in relation to severity; 3) FeNO is useful in monitoring the effectiveness of inhaled corticosteroids (including compliance) and biologic treatments like omalizumab; 4) FeNO, in conjunction with symptom registration and lung function measurements, contributes to asthma diagnosis and optimises asthma management.

Summary: FeNO provides further information in distinguishing different phenotypes in asthma allowing a much more appropriate control of the disease, especially in patients with difficult/severe asthma. In the future it would be interesting to shed light on the hidden biological mechanisms responsible of low or normal FeNO values in symptomatic asthmatics.

Keywords: Asthma, Wheeze, Phenotypes, FeNO, Management

INTRODUCTION

Nitric oxide (NO), a small gaseous free radical, was previously considered only a toxic environmental pollutant. The idea on NO changed when in the 1980s several studies showed that NO is a biological messenger in mammals [1]. In the last decades the important role of NO became evident in the lung: at low concentrations as smooth muscle relaxing agent, bronchoprotective factor, neurotransmitter of the inhibitory non-adrenergic non-cholinergic nervous (iNANC) system and at high concentrations as inflammatory mediator [1]. For this reason measurement devices for NO in exhaled air were developed and NO was detected for the first time in exhaled breath of mammals in 1991 [2], while elevated levels of exhaled NO were firstly described in asthmatics in 1993 [3].

Ever since, a huge amount of publications showed the role of exhaled NO as biomarker in the assessment of inflammatory activity in asthma and this review will focus on the potential clinical application of the measurement of fractional NO concentration in exhaled breath (FeNO) in asthmatics.

NITRIC OXIDE BIOSYNTHESIS AND REGULATION

In the respiratory tract, NO is produced by a wide variety of cell types, including epithelial cells, airway nerves, inflammatory cells and vascular endothelial cells [4]. NO is generated through the oxidation of the aminoacid L-arginine by NO synthase (NOS). NOS exists in three distinct isoforms (all expressed in the airways [1, 4]): 1) constitutive neural NOS (nNOS), 2) inducible NOS (iNOS) and 3) constitutive endothelial NOS (eNOS). In the lung, constitutive NOS-derived NO mediates a variety of physiological responses including lung development, airway smooth muscle relaxation, bronchoprotection against bronchoconstrictor stimuli and ciliary motility [1, 5]. High concentrations of iNOS-derived NO have been implicated in nonspecific host defense mechanisms against pathogens, as well as in chronic inflammatory diseases. Most of these effects proceed via formation

of peroxynitrite ($ONOO^-$), a highly reactive oxidant synthesized by the reaction of NO with superoxide anion (O_2^-) generated in the inflamed airways [5] (Figure 1).

Alternatively to NOS, L-arginine can be metabolised by arginase (arginase I and II) which catalyses the synthesis of polyamines and L-proline via the conversion of L-arginine to L-ornithine [6]. Finally, asymmetric dimethyl arginine (ADMA) is an important endogenous inhibitor of NO formation by arginine/NOS pathway reducing intracellular L-arginine availability. ADMA competitively inhibits NOS by displacing L-arginine from it, so that elevated ADMA results in uncoupling of NOS, reduced NO synthesis and increased production of superoxide and peroxynitrite [7].

In asthma, iNOS expression has been shown in bronchial epithelial cells [8] and during exacerbations an overexpression of epithelial iNOS [9] and increased arginase activity have been noted [10] (Figure 1).

In conclusion, NO production is modulated by a complex scenario involving L-arginine/NO pathway in conjunction with the balance (due to the different expression and activity) of all the factors metabolically involved [7].

FeNO AS INFLAMMATORY MARKER OF ASTHMA

Initially, FeNO was shown to be elevated in asthmatics compared to healthy controls [3, 11] and, in particular, in atopic *versus* non-atopic asthmatics [12]. During the last two decades several studies have been performed to assess the relationship between levels of FeNO and markers of airway inflammation in asthma. Among all the inflammatory phenotypes in asthma, there are several evidences showing the association between FeNO and the eosinophilic phenotype [13]. The relationship between eosinophilic airway inflammation and increased FeNO levels in childhood and adult asthma with different severity, before and after steroid treatment, was demonstrated in studies

on sputum [14, 15], bronchoalveolar lavage fluid [16, 17] and bronchial biopsies [18, 19]. In addition, a positive correlation between FeNO levels and mucosal eosinophil numbers in bronchial biopsies from atopic asthmatics after allergen challenge was also reported [20*] showing that increased FeNO reflects enhanced eosinophilic infiltration in asthmatics' bronchial mucosa during allergic exacerbation.

The most important risk factor for asthma is rhinitis *per se* irrespective of atopic status [21]. A recent study demonstrated that in adults with allergic rhinitis (AR) in presence or absence of asthma FeNO was higher compared to subjects with non-allergic rhinitis (NAR)-only and that among AR with asthma perennial sensitization caused higher FeNO levels than seasonal allergens [22]. Furthermore, in a population of persistent allergic rhinitis a strong correlation between FeNO levels and airway hyperresponsiveness (AHR) to methacholine (MCh) was found and a cut-off of 37 ppb was defined in order to discriminate rhinitics with AHR (PC_{20} MCh $\leq$16 mg/ml) indicating that FeNO is a useful tool in identifying subjects with rhinitis at risk of developing asthma [23*].

The mechanisms involved in asthma onset are still not well defined and most recent studies pointed out a role for FeNO as a strong risk factor of new-onset asthma in children. In infants with recurrent lower respiratory tract symptoms elevated FeNO levels were associated with maternal history of asthma and increased AHR to MCh revealing that FeNO measurements may have implications in clinical practice when therapy is considered for these infants [24]. Similarly, in a prospective study in preschool children cohort FeNO levels were elevated in those children with later asthma (at school age) compared to children not developing asthma indicating that elevated amounts of FeNO in infancy are associated with increased risk for school-age asthma [25*].

FeNO AND ASTHMA PHENOTYPES

Wheezing phenotypes are matter of discussion concerning childhood asthma and recent studies [26**, 27] explored the role of FeNO in identifying specific phenotypes related to high FeNO values. In a Dutch study, using longitudinal latent class analysis, 5 wheezing phenotypes have been characterized by different ages of onset in relation to FeNO measured at 4 and 8 years: the authors found that FeNO at 4 years was higher in intermediate onset and persistent wheeze compared to never and transient wheeze, and FeNO at 8 years was higher in persistent phenotypes of wheeze compared to never and transient wheeze, but only among children with allergic sensitization at 8 years [26**]. This study suggests that the pathophysiology of wheezing phenotypes differs between atopic and non-atopic children.

Cluster analyses identified obesity as part of a phenotype with less atopy and adult-onset asthma [28] and asthmatics with increased body mass index (BMI) showed reduced level of FeNO levels and sputum eosinophils [29]. In late-onset asthmatics, obesity is associated with a lower L-arginine/ADMA ratio explaining why FeNO is negatively related to BMI in obese late-onset asthmatics. Furthermore, reduced L-arginine/ADMA ratio is associated with increased respiratory symptoms, less allergic inflammation and lower lung volume [30**] suggesting that obesity is a factor of asthma worsening in relation to defective FeNO levels.

Moreover, FeNO has not been reported to associate directly with asthma severity [31], but in severe refractory asthma FeNO levels were higher in subjects with an eosinophilic phenotype compared to subjects with a non-eosinophilic phenotype [32]. A recent study showed that in a distinct severe adult-onset asthma phenotype (66% non-atopic) high FeNO levels and sputum eosinophils are factors associated with asthma severity suggesting that adult-onset severe asthma is a specific phenotype characterised mainly by a non-atopic status and a persistent eosinophilic airway inflammation related to high FeNO levels [33**]. Another study by Wenzel's group showed that severe asthma was associated with high expression of iNOS in bronchial epithelial cells compared to mild/moderate asthmatics and controls [34**]. The authors also found that epithelial iNOS

expression strongly predicted the magnitude of FeNO levels in severe asthmatics, and that both FeNO levels and epithelial iNOS correlated with sputum eosinophils in severe asthmatics suggesting that FeNO and eosinophilic inflammation are strongly associated with iNOS expression in severe asthma.

Because of the heterogeneity of response to standard inhaled corticosteroid (ICS) therapy in asthma, an identification of markers that predict treatment effects of specific medications will improve the likelihood of successful treatment in asthma. The EXTRA study explored the potential of three biomarkers of Th2-driven inflammation (FeNO, blood eosinophils and serum periostin) to serve as predictors of treatment effects to omalizumab in severe allergic asthmatics divided into low- and high-biomarker subgroups in order to distinguish patients with a Th2 high profile inflammatory phenotype [35**]. After 48 weeks of omalizumab there was a clinically relevant reduction in number of exacerbations in all the three higher biomarker compared with the lower biomarker subgroups indicating that these patients may achieve greater benefit from omalizumab [35**].

FeNO IN ASTHMA DIAGNOSIS AND CONTROL

Several studies focused on reference values in both adults and children taking into account the common factors affecting FeNO. Constitutive factors including age, height and gender have been reported as key determinants of FeNO values in population-based studies in non-asthmatic subjects [36, 37]. Reference equations that take constitutive factors into account have been published [38, 39]. FeNO levels in non-asthmatic children and adults have been evaluated from NHANES 2007-2010 and the predictive equations did not adequately explain the variation observed in FeNO in the general population [40**]. In line with ATS guidelines, the latter study supports the use of clinical cut-off points rather than predictive equations when interpreting FeNO values. Other data suggest that normalized values are at least as good as absolute values when assessing FeNO in relation to

diagnosed asthma [41]. Another way to identify the normal range for a patient is to reach the "personal best" FeNO level by a course of oral corticosteroids [42].

Current guidelines have highlighted the potential diagnostic role of FeNO in asthma [43]. FeNO can be very useful in differentiating asthma from other diseases, with high sensitivity and specificity at pre-specified cut-off levels [44]. Smith et al. showed that FeNO measurements in patients with undiagnosed chronic respiratory symptoms are strongly predictive of asthma diagnosis [44]. The limitations to the diagnostic role of FeNO arise principally because airway inflammation in asthma is not always associated with increased FeNO (e.g. neutrophilic airway inflammation). The recent clinical practice guidelines on FeNO indicate that 26 ppb is the optimum cut-off point for significant (3%) sputum eosinophilia [43]. The ATS guidelines suggest that a FeNO <25 ppb in adults (<20 ppb in children) is a strong indicator of an unlikely ICS responsiveness, while a FeNO >50 ppb in adults (>35 ppb in children) is a strong indicator of a likely ICS responsiveness [43].

Several studies showed that FeNO levels are increased in asthma, increasing further when asthma control deteriorates or when exacerbations occur [45]. FeNO changes during ICS therapy precede the improvement in symptoms, FEV1 and sputum eosinophilia in asthma [46]. Thus, FeNO is a sensitive predictor of loss of asthma control [47]. A recent study aimed to provide clinically relevant cut-offs by using normalised FeNO values in non-smoking, atopic asthmatics treated with ICS. It was concluded that FeNO value >300% of predicted (normal values of FeNO on the basis of age and height) identifies individuals at risk of excessive use of rescue medication and requiring oral corticosteroids within 1 year [48].

Finally, a recent report showed that asthma (diagnosed on the basis of reference standards) could be confirmed at FeNO >31 ppb (positive predictive value [PPV] 82%) and ruled out at FeNO ≤12 ppb (negative predictive value [NPV] 81%) in pulmonologists' routine work-up and that FeNO accuracy increased when patients with neutrophilic inflammation were omitted from analysis indicating that

the diagnostic accuracy of FeNO was satisfying in the whole study population and remarkably increased when inflammatory phenotypes were taken into account [49**].

FeNO IN ASTHMA MANAGEMENT

Accurate assessment and monitoring of airway inflammation in asthma is important for optimal management of patients with asthma, as symptoms of asthma may not necessarily reflect the underlying inflammatory process. [45]. FeNO predicts the likelihood of ICS responsiveness more consistently than spirometry, bronchodilator response or AHR to MCh [43, 50*]. The optimum cut-off point in the study by Smith and coworkers was 47 ppb with a negative predictive value of 89% for the change in FEV1 with ICS [51]. Even when (non-allergic) asthmatic patients do not show sputum eosinophilia, FeNO is highly predictive of ICS response at a cut-off point of 33 ppb [52].

Randomized trials designed to assess whether asthma outcomes are improved using regular FeNO measurements as basis for adjusting the ICS dose have failed to show clinically relevant benefits [53-56], although in one study ICS dose reduction was facilitated without compromising asthma control [57]. A recent meta-analysis on the efficacy of tailoring asthma interventions based on FeNO values showed that the benefits of utilising a FeNO strategy was not statistically different for asthma exacerbations, but the FeNO strategy enabled a reduction in the final daily ICS dose in adults [58*].

In childhood difficult to treat asthma poor ICS response is common and these patients are often characterized by low FeNO values. The authors evaluated whether baseline FeNO was a significant predictor of ICS response. This was a positive study and at cut-off point of >30 ppb demonstrated a sensitivity of 87% and a specificity of 91% for the identification of patients who achieved control suggesting the clinical utility of FeNO measurement in difficult to treat asthma [59].

A recent work by Gibson's group revealed that FeNO can be used used as management tool in a selected rather than in a general asthmatic population. Among 203 pregnant asthmatic women those with asthma symptoms and low FeNO (<16 ppb) received LABA and the low dose ICS whilst others with symptoms and high FeNO (>29 ppb) received higher ICS doses by step-wise titration. During pregnancy, total asthma exacerbations were significantly reduced from 0,62/pregnant patient to 0,29 in the FeNO group by a management algorithm based on FeNO and symptoms. The number of patients using ICS was greater in the FeNO group (77%) than in the control group (46%) but the mean ICS dose was lower in the FeNO group [60]. Thus, FeNO should be selectively used in a specific asthmatic population.

A 12 months study in 14 mild-to-moderate asthmatics showed a reduction of medium ICS dose, according to a step-wise algorithm, and a progressive improvement of symptoms scores associated with a decrease of FeNO levels and sputum eosinophils. These data suggest that titrating anti-inflammatory therapy, according to both FeNO and sputum eosinophils, in adults with stable asthma produces a fine long-term clinical control [61*].

Calhoun et al. compared 3 different approaches: physician-based (n=114), biomarker-based (using FeNO; n=115) and symptoms-based (n=113) management in 342 adult asthmatics [62]. There was no difference in time to treatment failure among the 3 groups. Because the patients had well controlled asthma it is not surprising that a biomarker such as FeNO, which identifies inflammation that might be discordant from symptoms, was not of benefit.

Despite these and various other encouraging study results, FeNO is not generally recommended for asthma management but a systematic assessment of published randomized trials of asthma therapy guided by FeNO concluded that the mixed results of these studies (ASTRAL studies) were due to specific design and methodological issues that may have led to incorrect conclusions [63]. Thus, new studies recruiting specific populations of asthmatics are warranted.

CONCLUSIONS

Asthma is a heterogeneous disease with several clinical, pathophysiological and inflammatory phenotypes which rule out the use of a single factor as unique marker for disease monitoring and determination of responses to treatments. Nevertheless, FeNO is currently the sole easily applicable non-invasive biomarker able to provide information about corticosteroid-sensitive airway inflammation in asthma. The role of FeNO in different aspects of asthma has been pointed out including prediction of new onset of asthma, recognition of different asthma phenotypes (especially in difficult/severe asthmatics), support of asthma diagnosis, improvement of asthma management and monitoring of ICS and biologic therapy efficacy in selected asthmatics. The author imagines a future with an appropriate use of FeNO by physicians facing clinically troublesome issues in specific asthma phenotypes.

KEY POINTS

1) FeNO is a biomarker of airway inflammatory activity and of specific phenotypes in asthma.

2) FeNO supports asthma diagnosis in conjunction with symptoms collection and pulmonary function.

3) FeNO predicts corticosteroid sensitivity in asthma and could be helpful in the management of selected asthmatic populations.

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FIGURE LEGEND

Figure 1. Schematic model of nitric oxide (NO) synthesis and regulation in the airways. L-Arginine is transported into the cell via the cationic amino acid transport (CAT) system and can be metabolised by both nitric oxide synthases [constitutive NOS (cNOS) and inducible NOS (iNOS)] and arginases (I and II). In a physiological environment, receptors activation by agonists leads to an increase in the intracellular concentration of $Ca2^{+}$ and activation of cNOS, which catalyses the synthesis of NO (at low concentrations) that can bind either thiol groups leading to S-nitrosothiols (R-SNO) or the iron of soluble guanylyl cyclase (sGC) stimulating the conversion of GTP to cGMP, all having a crucial regulatory role in airway physiology. Pro-inflammatory cytokines [interleukin-4 (IL-4), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α)] activate transcription factors and thus induce iNOS expression, which leads to the prolonged release of high amounts of NO with beneficial and detrimental effects. In addition, iNOS-derived NO might react with a broad spectrum of molecules such as superoxide (O_2) radicals and transition metals, which can lead to nitration (addition of $-NO_2$) of most classes of biomolecules ("nitrative stress"). In asthma, Th2 cytokines in conjunction with gene polymorphisms might lead to overexpression of arginase I and II, leading to the increased generation of proline and polyamines. Finally, asymmetric dimethyl arginine (ADMA) is an L-arginine analog that can competitively inhibit NOS isoforms. By competing with L-arginine, ADMA uncouples NOS, which in uncoupling conditions generates O2- and, as consequence, "nitrative stress".

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