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Fractional Exhaled Nitric Oxide (FE_{NO}) in the management of asthma: a position paper of the Italian Respiratory Society (SIP/IRS) and Italian Society of Allergy, Asthma and Clinical Immunology (SIAAIC)

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ABSTRACT

Asthma prevalence in Italy is on the rise and is estimated to be over 6% of the general population. The diagnosis of asthma can be challenging and elusive, especially in children and the last two decades has brought evidences that asthma is not a single disease but consists of various phenotypes. Symptoms can be underestimated by the patient or underreported to the clinician and physical signs can be scanty. Usual objective measures, like spirometry, are necessary but sometimes not significant. Despite proper treatment, asthma can be a very severe condition (even leading to death), however new drugs have recently become available which can be very effective in its control. Since asthma is currently thought to be caused by inflammation, a direct measure of the latter can be of paramount importance. For this purpose, the measurement of Fractional Exhaled Nitric Oxide (FE_{NO}) has been used since the early years of the current century as a non-invasive, easy-to-assess tool useful for diagnosing and managing asthma. This SIP-IRS/SIAAIC Position Paper is a narrative review which summarizes the evidence behind the usefulness of FE_{NO} in the diagnosis, management and phenotypization of asthma.

Key words: Asthma diagnosis; asthma management; Fractional Exhaled Nitric Oxide (FE_{NO}).

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Introduction and aim of the Position Paper

Asthma affects more than 300 million individuals worldwide. It is characterized by chronic airway inflammation leading to respiratory symptoms (dyspnea, wheezing, chest tightness and cough). Symptoms can vary over time and intensity and are coupled with variable expiratory airflow limitation [1].

The prevalence of asthma in Italy is constantly increasing over time and it is estimated to be over 6% of the general population [2,3]. The diagnosis of asthma can be challenging. The clinical presentation can be not very specific for the disease and it requires the demonstration of variable airflow limitation by means of lung function tests (*i.e.*, spirometry, bronchodilation test, bronchial challenges, PEF measurements) and the diagnostic process can be even more challenging in children. Moreover, subjects with asthma can be unaware of their sickness or anyway underestimate it, due to infrequent symptoms or exacerbations [1]. Symptoms can be classically triggered by exposure to allergens, respiratory tract infections, exercise, exposure to cold air, tobacco smoke or pollution, and tend to be more frequent at night.

Chronic airway inflammation, enhanced by the above mentioned triggers, contributes to induce airflow limitation that can be assessed by lung function tests. This airflow limitation is classically reversible after administration of inhaled bronchodilators (an increase in Forced Expiratory Volume in the first second FEV_1 of at least 200 mL and higher than 12% compared with basal value achieved after inhalation of salbutamol 400 mcg) or after prolonged (*i.e.*, after at least 4 weeks) treatment with inhaled corticosteroids (ICS). Airway hyperresponsiveness can be assessed by bronchial challenge with bronchoconstrictor drugs such as methacholine [1]. Histamine or mannitol can also be used. Diagnosis in children is even more challenging, since lung function tests cannot be carried out before 5 years of age.

Once clinical and functional diagnosis of asthma has been established, further evaluations, including a complete allergological workup, should be done in order to identify possible specific triggers or predisposing conditions that may have an impact on asthma management. In this context, Fractional Exhaled Nitric Oxide (FE_{NO}) assessment could be taken into consideration to stratify patients according to the airway inflammatory involvement [1].

Treatment of asthma is based on controller drugs with anti-inflammatory activity [mainly ICS, but also other add-on drugs such as leukotriene-receptor antagonists (LTRA)], possibly associated with long-acting bronchodilator drugs [generally long-acting beta2-agonists (LABA), or long-acting muscarinic agents (LAMA) for more severe patients] depending on the severity of the disease [1]. The aim of the treatment is to achieve the complete asthma control, defined as the absence of symptoms, normal lung-function and no future risk of adverse events [1]. This should be obtained using the least level of treatment as possible, optimizing the adherence and the ability to correctly use inhaler's devices as otherwise patients could not benefit from the given therapy [4]. This emphasizes the essential role of education just after diagnosis and in follow up [1]. If symptoms are inadequately controlled despite treatment, adherence and the ability to use inhalers' devices should be revised, common comorbidities should be properly treated and any other known risk factors should be removed. Persistence of poor asthma control and/or frequent exacerbations and/or impaired lung function despite high dose of ICS plus another controller and/or maintenance treatment with oral corticosteroids (OCS) defines "severe asthma" [5]. A referral to a severe asthma clinic should be taken into consideration also for treatment with novel biological agents [6]. For instance, the Severe Asthma Network in Italy (SANI) represents a network of Italian severe asthma centers, by the Italian Respiratory Society (SIP-IRS) and the Italian Society of Allergy, Asthma and Immunology (SIAAIC) [6,7].

Nowadays, asthma is thought to be a complex, multi-factorial disease characterized by variable clinical presentations in different subset of patients (the so-called "phenotypes"), resulting from heterogeneous expression of inflammatory pathways, involving both innate and adaptive immune systems (the so-called "endotypes") [8]. Accordingly, asthma can be classified at least in two different subgroups: one characterized by high expression of type-2 cytokines and associated inflammatory cells ("type-2 high" endotypes), classically presenting with eosinophilic phenotypes; the other with low expression of type-2 cytokines and characterized by predominant neutrophilic airway inflammation [9]. A 'mixed' endotype has been also described coming from an overlap of these syndromes [10].

The identification of phenotypes passes through a careful evaluation of any single clinical aspect, lung function patterns, sputum and systemic inflammatory involvement. The identification of endotypes passes through reliable and possibly non-invasive biomarkers of inflammation. This is crucial for establishing a precision medicine-based management [11], including the possibility to treat patients with novel biologic agents that are showing impressive results in controlling more severe asthmatics [12]. Despite the central role of airway inflammation in the pathogenesis of asthma, the most commonly used diagnostic algorithms, such the one proposed by the Global Initiative for Asthma (GINA) [1], do not include any assessment of airway inflammation. FE_{NO} is a non-invasive, easy-to-assess tool to measure airway inflammation, both in adults and children [13,14], and it could be useful for both asthma diagnosis and management. This SIP-IRS/SIAAIC position paper is a narrative review which aims to summarize the evidence behind the usefulness of FE_{NO} in the diagnosis, management and phenotypization of asthma.

Nitric oxide

Nitric oxide (NO) is a colorless gas that is only slightly soluble in water (<2 mmol under standard temperature and pressure). NO is a paramagnetic molecule with an odd number of electrons, which implies that it is a radical with an unpaired electron that implies an extreme reactivity responsible for many of its biological effects [15]. The kinetics of NO autoxidation in aqueous solution depends on its concentration [16]. Accordingly, the half-life is not a constant value and is inversely proportional to the NO concentration, becoming much longer as nitric oxide dilution increases. At physiological concentrations (1 μM to 10 nM), the half-life of NO due to the reaction with oxygen (O_2) is estimated to be in the 9-to-900 min range [17]. It has been reported that when the partial oxygen pressure increases in aqueous solution from 150 to 700 mmHg, the NO half-life decreases from 6.2 to 3.8 s [18]. The short half-life and the reactive structure of NO, in the absence of efficient free NO storage, require a carefully controlled enzymatic NO synthetic activity regulated through complex mechanisms of activation and inactivation.

NO production can occur *via* enzymatic and non-enzymatic pathways [19]. The enzymatic synthesis occurs from the semi-essential amino acid L-arginine and oxygen *via* three isoforms of an enzyme named nitric oxide synthase (NOS), identified in various tissues, and it is classically classified as constitutive and inducible. In fact, NOS-1 and NOS-3 isoforms are constitutively expressed, while the third one (NOS-2) is generally expressed in activated cells, although the latter may also be constitutively expressed and be active in the paranasal sinuses [20]. NOS enzymes have different requirements for the activation [21].

Indeed, NOS-1 and NOS-3 are calcium-calmodulin dependent, and are activated in response to a calcium signal. Enzyme activation occurs rapidly and transiently. Production of NO is equally transient, providing a rapid pulse-like signal. NOS-1 and NOS-3 enzymes produce small amounts of NO. Differently from NOS-1 and -3, NOS-2 contains tightly bound calmodulin. NO synthesis does not seem to be regulated but rather controlled at the transcriptional level, and once the enzyme is expressed it will produce large amounts of NO for prolonged periods, depending on how long the enzyme is present in a given cell or tissue. NOS-2 expression is dependent on transcription factors such as nuclear factor κB (NF-κB), activated by pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1b (IL-1b) [21], interleukin 4 (IL-4) [22] and interleukin 13 (IL-13) (which upregulates NOS-2 expression and activity) [23].

Apart from enzymatic synthesis pathways, endogenous production of both NO and H2S can occur through other non-enzymatic processes that are less well understood. NO can be produced *in vivo* by a reduction of NO_3– (nitrate) to NO_2– (nitrite), and NO_2– can produce NO. In fact, at low pH, nitrite will form nitrous acid (HNO_2), which decomposes to various nitrogen oxides, including NO. Nitrite may come from either dietary intake or saliva, through a reduction of nitrate to nitrite performed by bacteria in the oral cavity.

Figure 1 summarized the enzymatic and non-enzymatic synthesis of nitric oxide.

Biological functions of nitric oxide

The biological effects of NO in humans are numerous and involve the whole organism, and only some of those effects are exerted by direct actions [15]. Indeed, certain physiological and pathophysiological effects of NO are likely to be due to derivatives of NO rather than by this molecule itself. The direct actions of NO occur at low concentrations by binding to a number of molecular targets such as metal containing proteins and DNA. These interactions lead to enzyme activation or inhibition. The most notable of such processes is the reaction of NO with the heme group of guanylyl cyclase [15]. The subsequent activation of this enzyme is responsible for the conversion of guanosine trisphosphate (GTP) into cyclic guanosine monophosphate (cGMP). cGMP in turn activates protein kinases that perform several regulatory functions, including smooth muscle relaxation, neuronal transmission, and inhibition of platelet aggregation.

On the other hand, NO can inhibit other metallo-proteins such as cytochrome P-450, cytochrome oxidase and catalase. NO may also modulate other oxidative reactions by interacting directly with high energy free radicals, for example inhibiting lipid peroxidation and reducing the generation of pro-inflammatory lipids.

In contrast to the direct actions of NO, its indirect effects are mediated by reactive nitrogen oxide species (RNOS) derived from the interactions of NO with O_2 or O_2•– superoxide anion [24]. Indeed, reactive nitrogen oxides have been suggested to be important mediators of the pathophysiological events underlying a broad spectrum of inflammatory responses. The most common reactive nitrogen oxide species produced *in vivo* are dinitrogen trioxide (N_2O_3) and peroxynitrite (ONOO–, an unstable structural isomer of nitrate, NO_3–), which can induce both nitrosative and oxidative chemical stresses often associated with pathological situations such as inflammation. An example of such phenomena can be mediated by the potent cytotoxic and oxidant peroxynitrite and its conjugate peroxynitrous acid ONOOH, which can oxidize thiols, nitrate tyrosine and guanosine, as well as cleave DNA [24]. RNOS can react with sulfhydryl containing amino acids that irreversibly inactivate enzymes and other proteins. NO targets many enzymes in this way, particularly those important for the mitochondrial respiratory chain, which is essential for ATP synthesis [24].

The role of NO in inflammation remains elusive. It is likely that an excessive amount of NO produced by NOS-2 exerts the same types of effects as does the "physiological" NO, including relaxation of smooth muscle cells and vasodilatation. Thus, the increased NO levels in inflammation may be involved in hyperemia, edema and hypotension. Furthermore, NO may reduce apoptosis of inflammatory cells such as eosinophils. On the other hand, at high concentrations NO downregulates adhesion molecules, suppresses activation of inflammatory cells, and induces their apoptosis [25].

However, a different perspective on NO homeostasis in airway inflammation has been outlined [26]. In particular, it has been suggested that an increased arginase activity, in conjunction with an abnormal cellular uptake of L-arginine, may represent a major causative factor of NOS dysfunction in asthma. L -arginine is a substrate for both arginase and NOS, and therefore these enzymes might affect each other activity through substrate competition. In an allergic inflammatory microenvironment, pro-inflammatory cytokines and "oxidative stress" might upregulate the production of NOS-2-derived NO through activation of transcription factors [27]. In this situation, the synthesis of strong oxidizing reactive nitrogen species (RNS), such as peroxynitrite, leads to cell damage in the airways of asthmatics. In addition, upregulation of arginase in an inflammatory microenvironment is able to limit L-arginine bioavailability for NOS-2, which can result in the production of both NO and O_2 as a consequence of the substrate deficiency. This effect promotes an amplification of peroxynitrite formation, leading to an enhanced cytotoxic action in the airways. It might thus be speculated that a similar pathway can be activated in the inflammatory diseases of the upper airways such as allergic rhinitis or nasal polyposis, though this hypothesis needs to be experimentally validated.

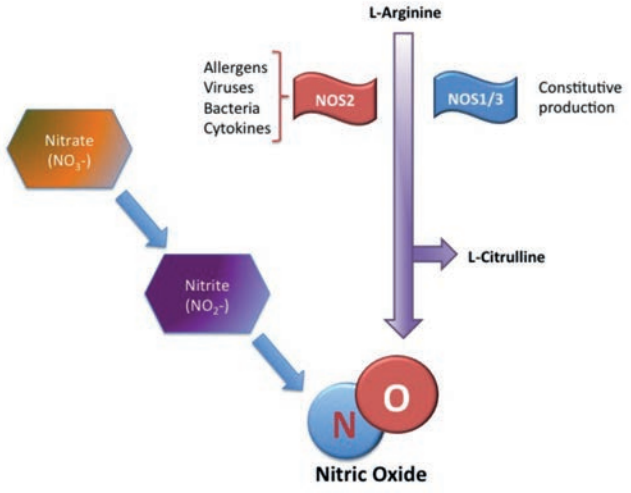

Figure 1. Non-enzymatic (left side) and enzymatic (right side) synthesis of nitric oxide. NOS, Nitric Oxide Synthase.

Assessment of FE_{NO}

Since Gustafsson *et al*. [28], who reported the first detection of NO from human expired breath, several techniques have been developed. Nowadays, the most used are chemiluminescence, electrochemical sensors and laser-based technology, all of which present advantages and disadvantages in a clinical setting. FE_{NO} could be measured both online and offline [29,30]. Online measurement may provide a better data quality but offline measurement is often more practical [31,32].

The chemiluminescence method is the gold standard for exhaled NO analysis. NO molecules contained in the gas sample are detected because of the radiation created after their reaction with ozone (O_3), generated in the instrument. The reaction between NO and O_3 generates nitrogen dioxide molecules (NO_2^*) in an electronically excited state. The subsequent reversion of these molecules to their lower energy ground state causes the emission of electromagnetic radiation (photons), with wavelengths ranging between 600-3000 nm, which can be detected and amplified by a photomultiplier tube. The resulting output signal is determined and corresponds linearly to the NO concentration in the sample, provided that O_3 is present in excess.

The chemiluminescence equipment is highly sensitive, with a detection threshold at ppb (parts-per-billion, $1:10^9$) level and a very fast response time (0.5-0.7 s). In addition, the technique allows for direct analysis of the breath *in situ*, or indirectly by sampling the breath in a balloon that can be analyzed later. However, to ensure reliability frequent instrument calibration is often required, which is achieved by using concentration of NO up to hundreds of ppb. In addition, these analyzers need a source of external NO-free air to generate ozone within the equipment, and a vacuum pump system, which rise manufacturing costs with prices ranging between 18,000 and 41,000 EUR [33]. Furthermore, chemiluminescence analyzers are quite large, weighing between 25 and 45 kg. All these limitations have restricted the use of chemiluminescence analyzers in routine clinical applications or home monitoring, and currently remain in use solely for laboratory analysis.

Current commercially available chemiluminescence FE_{NO} analyzers include NOA 280i (Sievers, GE Analytical Instruments, Boulder, CO, USA), NIOX (Circassia, Oxford, UK), Logan model LR2149 (Logan Research; Rochester, Kent, UK) and CLD 88 (Eco Medics, Duernten, Switzerland).

Electrochemical sensors can also be used to measure exhaled NO as they convert the gas concentration into an electrical signal [34]. The principle is based on the amperometric technique, which is achieved in the electrochemical instrument by a buffer system that allows retention of the last portion of the exhalation sample. Subsequently, the sample is transferred to the sensor for analysis where the target gas undergoes a chemical reaction in the presence of active catalytic sensor, and a measurable physical change is emitted within an electrical circuit. The sensor output signal, which presents a high sensitivity, is directly proportional to the partial pressure, and therefore to the concentration, of NO in the sample. The optimization of NO selectivity and sensitivity from the exhaled breath sample relies on catalyst and electrolyte composition with a complex arrangement of diffusion barrier membranes and a specific chemical filter system.

Several electrochemical or infrared sensor devices are commercially available: NIOX VERO (Circassia, Oxford, UK), NObreath (Bedfont Scientific Ltd, Kent, UK), Medisoft (Hypair, Dinant, Belgium) and Vivatmo pro (Bosch, Waiblingen, Germany).

The NIOX VERO device is hand-held and portable (less than 1 kg), and can be used for both adults and children [35]. It is precalibrated, designed to ensure a service- and calibration-free system, with a sensor that needs replacing between 100 and 300 measurements. Patients have to produce a 10-second exhalation of breath at an exhalation pressure of 10-20 cmH_2O in order to maintain a stable flow rate of 50±5 mls^{-1}. A calibrated electrochemical sensor evaluates the final 3 s of exhalation expressing the result in ppb in the 5-300 ppb range. The NObreath is a monitoring device that requires 12 seconds of exhalation of breath in adults and 10 seconds in children. It weighs approximately 400 g, including batteries, which last for up to 120 procedures. As the instrument does not have a set lifetime, it is strongly suggested that the sensor cells be replaced every 2 years [35]. The Medisoft device is semi-portable (weighing approximately 10 kg), and allows for repeatable analysis of exhaled NO using an internal sample bag for off-line measurements. It has a software package that provides a step-by-step, on-line quality control. The measurement range is 0-600 ppb. The NO cells are long lasting, typically 24 months or longer. The Vivatmo-PRO device is a portable device with an infrared sensor, which gives a rapid response and may not require storage of the sample in a chamber. In a recent study it has been showed a good correlation among some of these devices although the absolute exhaled NO measurements may differ to a clinically relevant extent [36]. Table 1 summarizes the performance characteristics of the most representative FE_{NO} analysers.

Recently, the use of optical sensors based on different laser technologies and detection methods has been developed for measuring of NO concentrations [37]. Schematically, these sensors include a laser source that produces light that interacts with gas molecules, a gas cell containing the sample to be analyzed, and, finally, the detection system. For NO detection, the light source in the optical sensors must probe at the fundamental and strongest absorption band, centered in the mid-infrared region at 5.3 μm ranging from 5.1 to 5.7 μm.

Previously, the main limitation of the laser-based NO sensors in this spectral range was the interference from several other gases, such as CO_2 and H_2O. Hence, only specific absorption NO lines could be targeted, requiring only sensors that could generate the specific light spectra to be used.

Other methods are being developed, based on new technologies, like the smart solid-state microsensors [38]. The optical sensor can be used to detect low levels of NO concentration, utilizing laser technology to measure the decrease of light intensity due to absorption by NO; several laser-based sensors have been developed to detect also 0.3 ppb of NO within 1 s [39-42].

The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have agreed on a highly standardized procedure for measurements of lower respiratory tract exhaled NO [43]. According to the guidelines for FE_{NO} measurement in adults, a single breath sample is instantly analyzed as the subject performs a breathing maneuver. The subject makes an inhalation to total lung capacity (TLC) with scrubbed air, not to contaminate the sample with possibly high NO from the environmental air, and then

Table 1. Performance characteristics for representative FE_{NO} analyzers.

Characteristics	Chemiluminescence	Electrochemical	Laser
Weight	40 kg	1 kg	
Sensitivity	< 1ppb	>5 ppb	1 ppb
Response time	<1 s	>10 s	1 s
External calibration	Yes	No	No
Price	50 kEUR	4 kEUR	>100 kEUR

exhales for 10 s at a specified 5-20 cmH_2O pressure. This pressure is necessary to ensure the closure of the soft palate, minimizing the risk of contamination of the exhaled NO from the paranasal sinuses, where NO concentrations are very high. The guidelines also recommend an exhaled flow of 50 ml/s ($FENO_{50}$), based on the hypothetical assumption that the region of interest for the NO excretion is within the lower parts of the airways. This relates to the reasoning that the airways are considered similar to a basic tubing system through which the expired air is led. If there is no NO depletion within the airway walls during the air passage, a steady state condition and thereby a stable exhaled concentration level (plateau) is reached, corresponding to the chosen exhalation flow rate. The exhalation flow rate can influence the exhaled concentration level, with low flows resulting in higher levels and *vice versa*. A normal FE_{NO} concentration in healthy adults is in the 10-20 ppb range. In inflammatory diseases such as bronchial asthma, not treated with anti-inflammatory medication, the exhaled NO values can reach more than 100 ppb.

Interpretation of FE_{NO} results

FE_{NO} values can be influenced by several non-disease-related factors, thus filling in a questionnaire for NO measurement is recommended [44]. Confounding factors could be related to the patient, like genetics, sex, weight and height, diet (*i.e.*, coffee) or taking drugs such as anti-inflammatory medications; also current smoking and atopy seem to influence FE_{NO} levels [45,46]. Allergen exposure is associated with higher levels of FE_{NO} but they could decrease during the early phase of allergic response [47]. Smoking is an important determinant of FE_{NO} levels and current smokers exhibit lower levels of FE_{NO} in comparison to ex-smokers and never smokers [48]. Both active and passive smoking have effects on lowering FE_{NO} as demonstrated in healthy adults and in both adults and children suffering from asthma, regardless their allergy status [49]. Different mechanisms are suggested for explaining the reduced FE_{NO} in smokers such as downregulation of NO synthetase by NO from cigarette smoke, increased breakdown of NO or lack of the required supply of tetrahydrobiopterin [50]

FE_{NO} could be influenced also by viral respiratory infections [43]. Age seems to be important too, especially in children [51], but correlation between age and sex has still to be defined [52-54]. Recently, also ethnicity seems to have a role in FE_{NO} results, impacting clinical management [55-57].

Moreover, there are also technical confounding factors in FE_{NO} measurement, like the NO analyzer used [58], measurement technique, exhalation flow rate or nasal NO contamination. Spirometry could also influence FE_{NO} results, thus it should not be performed first [43]. FE_{NO} could increase due to bronchodilation and it could decrease due to bronchoconstriction [47,59]. Over-distension during a profound inhalation can affect FE_{NO} levels also because the patient may not have control over exhalation flow rate [44]. Thus, it is mandatory to correctly interpret FE_{NO} results in each patient, referring to the clinical context in which the test is being done; it is also important to report the device used to make FE_{NO} measurement, how many measurements have been made and the flow rate (50 ml/s for approved FDA devices) [43]. All the measurements performed can be included but at least the mean value should be reported [43].

Reference values have been described for FE_{NO} in adults [51,52,60,61] and children [59,62-64]. In clinical practice, FE_{NO} <25 ppb in adults (<20 ppb in children) is considered the normal value. FE_{NO} levels between 25-50 ppb in adults (20-35 ppb in children) should be contextualized within the clinical context [43].

The eosinophilic asthma phenotype is characterized by sputum eosinophils ≥3% and identifies patients with a good response to corticosteroids and T2 immunomodulators. FE_{NO} values >50 ppb (>35 ppb in children) are likely connected with airway eosinophilic inflammation and this data may be used to predict a response to anti-inflammatory therapy, while low FE_{NO} <25 ppb (<20 ppb in children) correlates with less eosinophilic inflammation and responsiveness to corticosteroids [43]. Diagnostic FE_{NO} cut point in well controlled asthma is usually indicated by normal values [65]. FE_{NO} >30 ppb was associated with uncontrolled asthma [66].

According to GINA guidelines, following the diagnosis of severe asthma, FE_{NO} ≥20 ppb is considered the cut-off characterizing Type 2 inflammation severe asthma and it is used to assess this asthma phenotype, together with other markers like blood and sputum eosinophils [1]. Is has been suggested a FE_{NO} cut point of 21 ppb that best fits ≥3% sputum eosinophils in corticosteroid-naive patients [67]

Reference values are meant to be used as a general guide, mindful that they can have significant changes in different patients [1,43].

Very few data from studies analyzing clinically important change of FE_{NO} in individual patients is available [62,68-73] and different are the results depending on the considered outcome. Considering simply the within-subject coefficient of variation, in healthy subject is approximately 10% (corresponding to a raw change up to 4 ppb) [62,68] while it increases to about 20% in patients with asthma [71-73], therefore leading the ATS experts to recommend a change of at least 20% to indicate a significant rise or fall in FE_{NO} over time or following an intervention [43]. If the considered outcome is the transition from good control to poorly controlled asthma, a Minimal Clinically Important Difference (MCID) ranging from 16 ppb to 25 ppb (corresponding to an up to 60% increase from baseline) has been demonstrated [71-73]. On the other hand, considering the change in FE_{NO} during an acute event, the increase of values has been described as 50% higher in acute asthma attacks compared with when stability was restored [70], and up to 150 ppb during exposure to a relevant allergen [74,75] or acute infection.

Extended nitric oxide analysis

The measurement of exhaled NO at just one exhalation flow rate does not allow identification of NO production sites within the respiratory system. Therefore, mathematical models have been created to calculate the production within lung. George *et al*. [76] and Hogman *et al.* [77] have extensively reviewed the different models. When the exhaled NO at different flow rates is detected in breath sampler, the NO production sites in the respiratory system can be calculated. In particular, the NO flux from the airway wall to the lumen ($J_{aw}NO$) and fraction of NO in the gas-phase alveolar region (C_ANO) can be calculated when NO measurements are acquired at multiple high flow rates. Additional mathematical calculations with NO measurements obtained at both low and high flow rates can give the airway tissue concentration of NO released by the rigid conducting airway system ($C_{aw}NO$) and the transfer factor indicating the total airway compartment diffusion capacity ($D_{aw}NO$). Hence, extended NO analysis can shed light on the NO production sites of the respiratory system in patients.

The clinical application of measuring FENO at different flow rates is yet limited to some research setting, however information on the contributions of the bronchi (bronchial NO flux) and the peripheral lung (alveolar NO concentration) to exhaled NO is intriguing [78.]

Increased alveolar NO concentration has been reported in

severe, nocturnal and treated asthma [79] while NO flux from bronchial lumen (J'awNO) was associated to cough variant asthma and non-asthmatic eosinophilic bronchitis [80].

In all chemilumiscence analyzers, expiratory flow rates can be modified by resistors, allowing an extended NO analysis. On the other hand, most electrochemical sensors are not suitable for multiple flow analysis. An exception is the Medisoft that allows evaluation of exhaled NO at multiple flow rates.

FE_{NO} in asthma diagnosis

As the GINA guidelines suggest [1], FE_{NO} cannot be used as the only parameter for ruling in or ruling out a diagnosis of asthma. Its values are higher than normal in asthmatics that are characterized by Type 2 airway inflammation, and, as previously reported, several factors can affect FE_{NO} levels (smoke, bronchoconstriction, viral respiratory infections).

On the other hand, the British National Institute for Health Care Excellence (NICE) guidelines [81] recommend FE_{NO} testing in combination with other diagnostic options to help diagnose asthma in adults and children when diagnosis is unclear (*i.e.*, in case of normal lung function), and in those for whom, after initial clinical examination, an intermediate probability of having asthma is present or in those with confounding factors as obesity, anxiety, *etc*… [82]. FE_{NO} measurement is recommended by NICE also as an option to support asthma management in people who are symptomatic despite using inhaled corticosteroid (ICS) treatment.

FE_{NO} measurement could also be used in differentiating Cough-Variant Asthma (CVA) from other causes of chronic cough [83-86], to distinguish pre-school wheezing phenotypes and to assess the risk of later asthma or impaired lung growth and lung dysfunction in children [87].

According to the available literature, high FE_{NO} values increase the probability of asthma diagnosis, while a negative test does not necessarily exclude asthma [43]. Data from secondary care patients showed a sensitivity of 43–88% and specificity of 60–92% [88] for diagnosis of asthma. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 54-95% and 65-93%, respectively [43]. Thus, around 1 in 5 people with a positive FE_{NO} test will not have asthma (false positives), and 1 in 5 people with a negative FE_{NO} test will have asthma (false negatives). However, even if data on FE_{NO} specificity and sensibility are heterogeneous and could vary between studies, FE_{NO} seem to have higher specificity than sensitivity for the diagnosis of asthma, so FE_{NO} measurement is better at ruling in rather than to ruling out asthma diagnosis [89]. Sensitivity but not specificity could vary significantly among different FE_{NO} devices [89]. Specificity is optimized if higher FE_{NO} cut-off is used. ATS guidelines show that FE_{NO} >36 ppb had a sensitivity of 78% and a specificity of 72% for sputum eosinophilia, while a FE_{NO} <26 ppb has a negative predictive value of 85% [43].

FE_{NO} and asthma comorbidities

Atopy

Atopy, defined as sensitization to common inhalant allergens, in the absence of allergic diseases, such as rhinitis, has been consistently reported to be associated with increased FE_{NO} values, when compared to values observed in non-atopic control subjects, in children, but not in adults [90]. In children, exhaled NO correlates with the degree of IgE sensitization, in terms of both the number of positive skin-prick tests [91] and IgE-antibody concentrations [92]. Recently it has been shown that increased levels of exhaled NO in adolescents, 12-15 years old, precede incident self-reported allergic symptoms to cat and dog within four years [93].

Rhinitis

FE_{NO} values are generally reported to be higher in adults with allergic rhinitis (AR) when compared to healthy controls and patients with non-allergic rhinitis (NAR) [94]. As up to one third of patients with rhinitis may have asthma, it is interesting to report that AR with/without asthma had significantly higher FE_{NO} levels than patients with NAR without asthma, while subjects with NAR and asthma exhibited elevated FE_{NO} levels, similar to AR [95]. Natural pollen exposure was found to cause a significant FE_{NO} elevation in allergic individuals. Thus, IgE-mediated allergy has been reported to be responsible for elevated FE_{NO} [96]. FE_{NO} values that were lower in AR compared with asthma were shown to reach similar levels after allergen exposure [97]. In patients with rhinitis and asthma-like symptoms, the presence of asthma was associated with higher FE_{NO} values [98]. In a consecutive series of patients referred to an allergy clinic for chronic persistent rhinitis symptoms, airway inflammation, evaluated by increased values of FE_{NO}, and diagnosis of asthma were significantly more prevalent in patients with AR and chronic rhinosinusitis (CRS) compared to patients with non-allergic rhinitis [99]. One every four subjects with allergic rhinitis and very high FE_{NO} values (>50 ppb) have been shown to develop asthma at follow up according to a recent report [100].

Chronic rhinosinusitis

Chronic rhinosinusitis is classified into CRS with nasal polyps (CRSwNP), characterized by eosinophilic inflammation and CRS without nasal polyps (CRSsNP). Asthma is more frequently seen in CRSwNP patients than in CRSsNP patients. FE_{NO}, blood eosinophil counts, number of eosinophils in nasal polyps, and total IgE are generally all higher in CRSwNP patients than in CRSsNP patients. FE_{NO} values in CRSwNP patients without asthma showed significantly higher FE_{NO} values than CRSsNP patients without asthma, while no significant difference in FE_{NO} was seen between patients with CRSwNP with and without asthma [101]. The presence of nasal polyps in patients with CRS was associated with increased asthma prevalence as well as increased FE_{NO} levels. Respiratory symptoms without bronchial hyperresponsiveness were associated with eosinophilic airway inflammation and increased FE_{NO} only in patients with nasal polyps [102], suggesting eosinophilic airway inflammation even in patients without asthma.

FE_{NO} and asthma control

Its intrinsic feature as a biomarker of the underlying T2-mediated airway inflammation in asthmatics [103], its ability to predict asthma exacerbation [104,105] and the prompt reduction after anti-inflammatory treatment initiation [106,107] theoretically make FE_{NO} as a promising biomarker of poor asthma control.

Many studies investigated this aspect both in children and in adults with contradictory results reported so far: some Authors reported higher FE_{NO} levels in uncontrolled or partially controlled asthmatics both in adults [64,108,109] and children [110-112], while others failed to find such a correlation [113-119]. A recent metanalysis including many of these studies concluded that there is only a weak correlation between FE_{NO} levels and current asthma control [120].

This apparent contradiction between the promising role of FE_{NO} as a biomarker of asthma control and the reported results may be explained looking at the clinical characteristics of patients

included into the different studies: in fact, a better correlation between high FE_{NO} levels and poor asthma control was seen in patients not on regular treatment for asthma [111,112,121], while patients regularly treated with ICS seem to demonstrate weaker or no correlation between FE_{NO} and asthma control [111-113,116-119,121]. It is also possible that the presence of sino-nasal comorbidities (such as allergic rhinitis or chronic rhinosinusitis with nasal polyps), which are frequently associated with asthma, may have an influence on the correlation between FE_{NO} and asthma control, as they may independently increase FE_{NO} levels [99,102].

The performance of FE_{NO} as a biomarker of asthma control increases when its assessment is combined with lung function parameters [122-123], when it is used to predict the future risk of losing asthma control [124-128], or when its increase in a given time is considered as a marker of loss of asthma control [119].

It is therefore suggestable that combined measurement (at first clinical evaluation and during regular follow ups) of FE_{NO} and lung function, which are both easy to obtain in clinical practice, may help clinicians to predict the achievement of asthma control after treatment initiation. Moreover, considering the easiness of FE_{NO} assessment even in pre-school children, its use to determine asthma control can be reasonable particularly when lung function and self-reported control level are difficult to obtain [110].

FE_{NO} and response to asthma treatments

Glucocorticoids are known to reduce eosinophilic inflammation that characterizes most of the asthma phenotypes; therefore, FE_{NO} has the potential properties to be the perfect tool for monitoring the response to inhaled and/or systemic corticosteroid treatments in asthmatic patients.

In steroid-naïve patients, FE_{NO} has been shown to be a reliable predictor of responsiveness to ICS, with high levels associated with favorable response to the treatment [105, 129-131]. Higher baseline FE_{NO} levels were indeed able to predict ICS response in terms of improvement of lung function both in adults [132-134] and children [135], and in terms of reduction of symptoms [133,134] (at least in eosinophilic phenotypes) and improvement of asthma-related quality of life [133]. The study by Cowan *et al.* [134] showed that, despite FE_{NO} was able to predict the improvement of asthmatic symptoms only in patients with eosinophilic asthma after a short course of 4 weeks of ICS, its high baseline levels were associated to a significant reduction in bronchial hyperresponsiveness also in patients with non-eosinophilic asthma, suggesting that these subjects may need longer ICS treatment to obtain also a clinical response associated with the prompt lung function improvement. A further subanalysis of the same study confirmed the same results [136].

The ability of FE_{NO} to predict ICS response could be even more clinically relevant when evaluating patients with non-specific respiratory symptoms (such as isolated cough) and not already clearly diagnosed with asthma [133,137]. A double-blind randomized placebo-controlled trial published by Price *et al.* [133] showed that higher baseline FE_{NO} was associated with better response to ICS in patients with undiagnosed, non-specific respiratory symptoms. Interestingly, in this study FE_{NO} performance in predicting ICS response was superior than baseline lung function assessment, blood eosinophil count and clinical opinion of asthma. This study confirmed, using a more robust study design, previous similar observation in single-blind design and with limited number of patients [137].

More controversial is the effect of leukotriene-receptor antagonists (LTRA) on FE_{NO}, as some studies showed a prompt and sustained reduction of it [138-143], while others failed to find this association [144-146]. Further studies are therefore needed to clarify if this class of drugs affects FE_{NO}.

In any case, when a response to ICS and/or LTRA has been found, FE_{NO} decreases rapidly, generally more quickly than other asthmatic features, such as lung function parameters, symptoms or airway hyperreactivity [106]. This rapid response to anti-inflammatory treatments, together with similarly rapid increase before worsening of asthma control and exacerbations [104,147] led researchers to investigate the potential therapeutical strategies based on tailoring the treatment level according to FE_{NO} assessment [131]. A recent metanalysis [131] combining data from three previously published Cochrane reviews [148-150], highlighted that tailoring of asthma therapy based on FE_{NO} results in a significant reduction of exacerbations in adults and a similar tendency in children, compared to guidelines-based therapeutical strategies; interestingly, these results were obtained without an increase need in ICS dose, reinforcing the benefit that may be achieved from a FE_{NO} -based strategy for tailoring asthma therapy.

FE_{NO} and adherence to treatment

A proportion of patients with asthma remains symptomatic despite prescription of adequate treatment and they should be distinguished into two categories: patients with possible severe asthma ("difficult-to-control" asthmatics) and those with other causes of poor asthma control ("difficult-to-treat" asthmatics) [151]; among these causes, nonadherence to ICS is a major determinant of poor asthma control and treatment failure accounting about 50% of those who had been prescribed long-term treatment [152]. Distinguishing patients with difficult-to-control asthma who may respond to ICS if properly addressed from those really affected by refractory asthma is an important clinical challenge.

FE_{NO} has been largely investigated as possible tool to identify nonadherence [68,124,153-159]: elevated FE_{NO} levels were constantly associated with nonadherence, despite the heterogeneity of methods used to assess the adherence to treatment, both in children and adults; this ability to identify poorly adherent patients was constantly reported to be greater for FE_{NO} than for other parameters such as lung function or patient-reported symptoms. Fewer are the reports of poor correlation between FE_{NO} and adherence to treatment, probably due to the very small number of patients enrolled [159].

McNicholl *et al.* [130] developed the so-called "FE_{NO} suppression test", a practical objective procedure for assessing nonadherence in difficult-to-treat asthma; they enrolled asthmatic patients with persistently elevated FE_{NO} despite treatment and administered them inhaled budesonide 1600 mg for 7 consecutive days under their direct observation. FE_{NO} was daily measured for 8 days, then weekly for 4 weeks to test its suppression after directly observed inhaled corticosteroid (DOICS) treatment; if FE_{NO} persisted to be higher than 40 ppb after seven days of DOICS, intramuscular triamcinolone 80 mg was administered, to demonstrate FE_{NO} responsiveness to high-dose systemic corticosteroids. A composite measure comprising prescription records, adherence interview, blood testing, and inhaler technique, was used to assess nonadherence. Using this study design, they were able to reveal that suppression of FE_{NO} after DOICS had a sensitivity of 67%, a specificity of 95% and a positive predictive value of 92% in identifying nonadherent patients and differentiating them from patients with proper severe asthma. A subsequent study from the same group of Authors demonstrated that the FE_{NO} suppression test is applicable in a routine clinical care of reference centers for severe asthma, with the help of an integrated remote monitoring technology specifically developed [158].

FE_{NO} in severe asthma

Severe asthma is well known to be a heterogeneous disease that comprises multiple factors and that predisposes approximately 10% of asthmatic patients to suffer daily symptoms and acute exacerbations despite the intake of high-dose inhaled corticosteroids (ICS), oral corticosteroids (OCS), and other controllers [5]. Recently, identification of severe asthma mediated by Type 2 inflammation has resulted in the successful launch of several biologic therapies that target specific inflammatory phenotypes. Several biomarkers have been proposed for the Type 2 severe asthma, such FE_{NO}, eosinophils in blood or in sputum and periostin.

The last GINA guidelines confirmed the role of FE_{NO} as a useful, easy to perform and cost-effective phenotyping test for severe asthma management [1]. The possibility of refractory Type 2 inflammation should be considered if FE_{NO} ≥20 ppb is found while the patient is taking high-dose ICS, together with blood eosinophils (>150/µl), sputum eosinophils (>2%) or allergen-driven asthma; blood eosinophils and FE_{NO} should be repeated up to 3 times, on lowest possible OCS dose, before excluding Type 2 severe asthma.

FE_{NO} ≥20 ppb may also predict an increase in exacerbations. FE_{NO} demonstrated itself to be the strongest predictor of exacerbation in severe asthmatic patients treated with high dose ICS and OCS when compared to peripheral blood eosinophils and periostin [104].

A recent study by Price and collaborators showed that in a population of asthmatic in ICS treatment, the combination of high FE_{NO} and high blood eosinophil count (≥300 cell/µl) was associated to a significant increase in severe exacerbation rate, up to four times or twice if categorized in FENO≥50 ppb or FENO≥35 ppb respectively compared to patients with non-high FENO and non-high blood eosinophils [159]. Furthermore, several studies support the use of FE_{NO} as a marker to guiding OCS increase or escalation in severe as well as in mild-moderate asthmatics [160,161]. If patient has good response to Type 2 targeted therapy, internet-based monitoring of symptom control and FE_{NO} may help the clinician to decide if gradually decreasing or stopping OCS [1]. On the other hand, the international ERS/ATS guidelines suggest that clinicians do not use FE_{NO} to guide OCS therapy in adults with severe asthma [5]. Recently, FE_{NO} has also started to be proposed as a predictor of efficacy of biologic therapies. The story in this direction started with the EXTRA study [163] in which FE_{NO} showed to be able to identify responders to omalizumab. GINA guidelines [1] also confirm that FE_{NO} ≥20 ppb, associated with blood eosinophils ≥ 260/µl, allergen-driven symptoms and childhood-onset asthma, predicts a good response to anti-IgE biologic treatment.

Several later studies supported the role of FE_{NO} as a good predictor of efficacy to omalizumab [164,165]. However, if FE_{NO} seems to well identify responders to omalizumab, no efficacy has been demonstrated for alveolar concentration of nitric oxide that did not modify its concentrations after treatment with omalizumab [166]. On the other hand, contrasting data are available on FE_{NO} as a marker of good response to mepolizumab. In the DREAM study [167], no statistical differences were found in FE_{NO} after treatment with mepolizumab compared to baseline values, suggesting that FE_{NO} may not be responsive to modulation through IL-5 pathway. Accordingly, with the DREAM study, Farah *et al.* demonstrated that treatment with mepolizumab improves small airway function, but not spirometry and FE_{NO} [168].

FE_{NO} has also been evaluated in the identification of patients eligible for dupilumab, a human anti IL-4 and IL-13 antibody. Castro *et al*. [169] found that the subgroup of severe asthmatic with higher FE_{NO} treated with dupilumab experienced a more significant clinical efficacy in terms of reduction of exacerbation and functional improvement compared to asthmatic with lower FE_{NO}. Wenzel *et al.* [170] also confirmed the best role of FE_{NO}, among other Type 2 inflammation biomarkers, in predicting the best responder to dupilumab.

Lebrikizumab, a humanized anti IL-13 antibody, showed a marked reduction in FE_{NO} values correlated to improvement in asthma control compared to placebo [171], while tralokinumab, another anti IL-13 humanized antibody, showed controversial results regarding its utility in reducing asthma exacerbation rate in relation with FE_{NO} values: in the STRATOS 1 study high-FE_{NO} group (>37 ppb) showed a lower exacerbation rate *versus* placebo, while in the STRATOS 2 study this finding was not confirmed [172]. In addition, in the MESOS trial tralokinumab showed a significant reduction in FE_{NO} values in moderate-to-severe asthmatic patients [173].

Finally, treatment with tezepelumab, a humanized antibody targeting thymic stromal lymphopoietin (TSLP), was associated with a substantial and persistent decreasing in blood eosinophil counts and FE_{NO} levels [174].

FE_{NO}, as well as eosinophils, are biomarkers easy to measure in clinical practice and their combined evaluation can identify patients with frequent exacerbations and stratify the appropriate therapy for Type 2 inflammation-predominant severe asthma [175].

FE_{NO} in childhood asthma

The first reports about the high level of FE_{NO} in children with asthma date from 1997 [176,177]. FE_{NO} represents an interesting way to monitor airway inflammation, because of its non-invasive nature and the relatively easy use. In fact, measurement can be obtained in most children starting from 5-6 years old and results are available in a few minutes.

Recommendations for FE_{NO} measurements in children have been published and are used worldwide [30,178]. FE_{NO} measurement is performed with a deep inhalation through the mouth and slow exhalation, with feedback of the flow rate for the subject. Velum closure is mandatory and achieved by using a positive pressure of 5-20 cmH_2O against exhalation. An approved measure is one in which the flow rate is within 10% of the target value, *i.e.* 45-55 $ml \cdot s^{-1}$ [30,170].

FE_{NO} levels correlate with eosinophilic counts in induced sputum (5) and bronchoalveolar lavage fluid as well as with eosinophil infiltration of the airways and peripheral eosinophilia, mainly in atopic children [179,180]. Correlations were also found with serum total IgE, serum eosinophil cationic protein (ECP) and the number of positive skin prick tests [179-181]. Consequently, FE_{NO} is considered a marker of the most common asthma endotype in children, characterized by Th2-mediated airway inflammation, eosinophilia and responsiveness to inhaled steroids [182]. Moreover, some studies suggest that low FE_{NO} levels predict a non-eosinophilic asthma phenotype better than high levels can predict an eosinophilic one [183]. It has also been suggested that FE_{NO} can help us to identify early-onset asthma among preschool-age children with recurrent wheezing [179,184,185]. At last, several studies demonstrated that FE_{NO} is increased in atopic children with and without asthma, suggesting that atopy and asthma could be cofactors in determining elevated FE_{NO} levels [181,186,187]. It has been also established that baseline FE_{NO} levels are elevated in children with exercise-induced bronchoconstriction and relate with the degree of post-exercise bronchoconstriction, suggesting that FE_{NO} may be a predictor of airway hyperresponsiveness to exercise, especially in asthmatic children sensitive to indoor allergens [188].

On these bases, it has been proposed a role of FE_{NO} in asthma

diagnosis in children. Sensitivity and specificity of FE_{NO} measurements were showed to be acceptable to discriminate asthma from other non-asthmatic conditions in previous clinical studies [189], though normal FE_{NO} levels do not exclude the diagnosis of asthma, especially in non-atopic subjects. In fact, FE_{NO} levels of atopic asthmatic are higher than those of non-atopic asthmatics [190]. Furthermore, it has to be considered that there is a large range in FE_{NO} levels among children, which may reflect the natural heterogeneity in baseline epithelial nitric oxide synthase activity with the contribution of other non-eosinophilic factors.

Normal values of FE_{NO} and feasibility in children have been assessed, showing that FE_{NO} levels in healthy children are below 15 to 25 ppb [60,191]. These values range is the result of several factors: age, gender, height, ethnicity, allergic sensitization, serum total IgE, infections, a nitrate rich diet, exercise, smoking, environmental nitric oxide, time of the day, season and environmental pollution [182,192-194). A systematic review and meta-analysis on eight current diagnostic accuracy studies, including 2,933 cases of diagnostic performance of FE_{NO} in children with asthma, indicates a FE_{NO} values range from 19 ppb to 25 ppb as the best cut-point to diagnose asthma [195]. This range showed the equal highest Youden's index (sensitivity + specificity -100).

In a study performed in preschool children, FE_{NO} was higher in those with a frequent recurrent wheeze and a stringent index for the prediction of [asthma asthma predictive index (API)] than in those with a loose index or those with recurrent or chronic cough but no history of wheeze [196,197]. Furthermore, in infants with eczema but not yet wheezing, exhaled nitric oxide was shown to be capable to provide important insights into the risk of asthma later in childhood and its airway characteristics [198]. The body of these data is in favour of the idea that objective measurement of FE_{NO} in addition to the clinical characterization may improve the possibility of defining disease presentation and of predicting disease progression in preschool children.

Therefore, currently, FE_{NO} may be regarded as a potential complementary tool in asthma diagnosis pathway in children.

There is also a strong interest to use FE_{NO} as a guide for asthma treatment, considering that FE_{NO} reflects airway inflammation. In fact, in some studies FE_{NO} is validated as a useful tool both in diagnosing and managing children with atopic asthma [60]. Data suggest that using FE_{NO} to tailor the dose of ICS cannot be recommended in routine clinical practice, because of the danger of excessive doses of treatment without significant changes in clinical outcomes. In fact, two meta-analyses of paediatric studies showed that FE_{NO} monitoring lead to increased use of ICS, without significant influence on lung function outcomes (FEV_1 levels) compared to conventional management [199,200]. Actually, a guideline-based approach still remains essential [199]. It has been suggested that FE_{NO} may be more appropriate for tapering, rather than for stepping up anti-inflammatory treatment and could be used mainly as an indicator of the child's compliance with the prescribed therapy [182]. In fact, the relatively rapid shift of FE_{NO} levels after steroid treatment suggests its utility in monitoring adherence to and response to therapy [43,201].

For these reasons different authors suggested to use FE_{NO} to rationalize corticosteroid therapy in asthmatic patients, together with the traditional clinical tools (history, physical examination and lung function tests) [43]. Nevertheless, the issue to consider FE_{NO} as a clinical tool to manage asthma treatment in children is still under debate [202].

Some previous studies showed that FE_{NO} increased in uncontrolled asthmatic children, especially during exacerbations [202]. In particular, a study, in which 22 children allergic to mites underwent twice-daily fractional exhaled nitric oxide measurement before, during and after period of natural exposure to mite allergens, observed significant differences between the mite-free baseline FE_{NO} level and FE_{NO} levels measured during natural mite exposure and after natural mite exposure [203]. Moreover, six children reported asthma symptoms during the mite exposure, and an increase in FE_{NO} was observed in each case [203].

The usefulness of FE_{NO} for monitoring children with moderate-to-severe asthma is still unclear. In fact, studies aimed to evaluate FE_{NO} usefulness as a predictor of asthma exacerbations show conflicting results. Moreover, a consensus about the optimal FE_{NO} cut-point level to define high risk of exacerbation still lack. Cabral *et al.* showed no benefits in tapering ICS doses in atopic children by monitoring FE_{NO} levels, suggesting that this tool has a limited value as a predictor of asthma exacerbations [204]. Conversely, some data reported that FE_{NO} might be helpful in predicting and preventing exacerbations. In a study based on daily FE_{NO} values and symptom scores over 192 days in 41 atopic asthmatic children, Stern *et al*. have demonstrated that fluctuation in FE_{NO} values and their cross-correlation to symptom scores give information on asthma severity and control [205]. They found that the majority of subjects had the strongest positive relationship between FE_{NO} values and symptom scores on the same day. Children who had a severe or moderate exacerbation had a stronger positive cross-correlation between FE_{NO} values and symptom scores, suggesting that concordance of FE_{NO} values and symptom scores is an indicator of increased risk of exacerbation [205]. In another study, Gagliardo *et al.* found a significant correlation between FE_{NO} levels and other markers of inflammation, such as sputum eosinophilia and IL-8, and the number of severe exacerbations in asthmatic children [206]. Van der Valk *et al*. collected longitudinal daily FE_{NO} measurements in relation to exacerbations in atopic asthmatic children [207]. They have found changes in FE_{NO} prior to moderate, but not severe exacerbations. Probably, moderate exacerbations are preceded by increased eosinophilic airway inflammation and the level of cross-correlation between FE_{NO} levels and symptoms could identify children at risk for exacerbations. However, the study sample size was small and the therapeutic intervention with ICS could have modified the association between FE_{NO} and exacerbations [207]. At last, in a study based on forty-two children with confirmed asthma, Chang *et al*. has found that FE_{NO} values were associated with an increased risk for subsequent loss of asthma control 4 weeks after ICS withdrawal (40). Moreover, subjects with high FE_{NO} values had an earlier LAC respect subjects with normal FE_{NO} [208]. Their findings suggest that FE_{NO} values may be useful to predict subsequent loss of asthma control among asymptomatic children after ICS interruption. In this setting, FE_{NO} level may contribute for clinical follow up decision during childhood asthma after ICS withdrawal. Discordant data were found also about the correlations between FE_{NO} and Asthma Control Test scores, both in adults and in children [209]. A study on 200 asthmatic children (47 of them with newly diagnosed asthma and without any regular controller therapy) has pointed out that the assessment of asthma control by Children-ACT questionnaire in children is significantly related to the level of FE_{NO} in newly diagnosed patients, but not in those already under regular follow up [111].

In conclusion, due to the complex nature of the disease, asthma control in children needs more than only one item in assessment and both physician evaluation and other objective testing are necessary. FE_{NO} may provide useful information about airway inflammation, playing a complementary role in the management of asthma.

FE_{NO} in respiratory diseases other than asthma

Chronic obstructive pulmonary disease

The clinical value of FE_{NO} measurements in patients with established chronic obstructive pulmonary disease (COPD) is not presently clear. According to a recent systematic review and meta-analysis [210] patients with stable COPD had a mild elevation of FE_{NO} levels compared to healthy controls, with FE_{NO} levels much higher in ex-smokers than in current smokers. No association was found between FE_{NO} levels and exacerbated COPD. Some studies show that, at least in the short term, the response to corticosteroids is likely to be greater in patients with COPD who also have elevated FE_{NO} [211]. A raised FE_{NO} had been shown to predict FEV_1 response to ICS in COPD [212,213].

In a significant number of patients, an overlap syndrome comprising features of both asthma and COPD is found [214]. The airway inflammatory cell infiltrate may be mixed, including eosinophilic inflammation. Asthma-COPD overlap (ACO) is characterized by persistent airflow limitation and several manifestations usually associated with both asthma and COPD. A GINA/GOLD document on ACO recommended that both FE_{NO} and blood eosinophils be used as inflammatory biomarkers for differentiating ACO from COPD [215]. According to a recent study, for patients naïve to ICS, FE_{NO} level >25.0 ppb combined with a blood eosinophil count >250 cells/µl showed high specificity (96.1%) for differentiating ACO from COPD [216].

Obstructive sleep apnea

Obstructive sleep apnoea (OSA) is a sleep disorder that may lead to metabolic abnormalities and increased cardiovascular risks. Airway and systemic inflammation has been proposed to have a central role in the pathophysiology of OSA [217]. Inflammation involving the nose, the uvula, the soft palate and the pharyngolaryngeal tract promotes and aggravates oropharyngeal inspiratory muscle dysfunction, upper airway narrowing and collapsibility. A slightly increase in levels of NO were detected in the exhaled air of OSA compared to healthy subjects, generally between 20 and 25 ppb, it is more evident in nonsmoking OSA and after sleep and it seems to reflect bronchial neutrophilic inflammation [218]. Increased FENO in OSA is not consistently positively related to the severity of OSA (apnoea / hypopnoea index) thus excluding a clear role for screening OSA in adults. [219] On the contrary, nasal NO (nNO) might have a greater value that FENO in the fact that correlates to AHI and time of SpO2<90%, potentially reflecting upper airway inflammation in OAS patients. A nNO higher that 626 ppb could be recommended for confirming OSA by polysomnography [220].

Non-asthmatic eosinophilic bronchitis

Non-asthmatic eosinophilic bronchitis (NAEB) is characterized by chronic irritable dry cough, sputum eosinophilia and being responsive well to glucocorticosteroids [221]. In contrast to asthma, NAEB presents no airflow obstruction and airway hyperresponsiveness [221]. Some reports indicate that FE_{NO} levels in patients with NAEB were significantly higher than those in other causes of chronic cough [222]. According to a systematic review and meta-analysis [223] FE_{NO} test might not be precise enough to predict NAEB in non-asthmatic patients with chronic cough. Hierarchical summary receiver operating characteristic curve analyses suggested sensitivity and specificity were 72% and 83%, respectively, with optimal cutoff levels ranging from 30 to 40 ppb. Even if FE_{NO} measurement might not fully replace induced sputum analyses, the clinical utility of FE_{NO} should not be dismissed in the non-asthmatic population with cough, where FE_{NO} may help to identify corticosteroid-responsive patients among the non-asthmatic population with cough.

Acute eosinophilic pneumonia

Acute eosinophilic pneumonia (AEP) is to be suspected in patients with progressive and severe dyspnea less 1-2 weeks in duration and a chest radiograph showing diffuse parenchymal opacities. At presentation eosinophilia is not present in peripheral blood, while there is typical BAL eosinophilia (> 25%). Among 60 subjects prospectively enrolled with pulmonary infiltrates and a febrile illness and who were clinically suspected to have AEP, the pretreatment FE_{NO} levels of the patients with AEP were significantly higher than those of the patients without AEP. The cut-off value (23.5 ppb) showed that the maximal area under the receiver operating characteristic curve predicted AEP with a sensitivity of 87% and a specificity of 83% [224].

FE_{NO} measurement has been shown to be useful for differentiating AEP from other types of acute-onset interstitial lung diseases, regardless of the blood eosinophil levels [225]: forty patients with a combination of illness ≤4 weeks in duration and diffuse radiographic infiltrates were classified into groups based on the etiology; the median FE_{NO} value of patients with AEP (48.1 ppb) was significantly higher than that of the other groups (17.4 ppb in cryptogenic organizing pneumonia, 20.5 ppb in hypersensitivity pneumonia, and 12.0 ppb for sarcoidosis). The area under the receiver's operating characteristic curve (AUC) for FE_{NO} to identify AEP was 0.90 with a cut-off of 23.4 ppb [225].

Chronic eosinophilic pneumonia

Chronic eosinophilic pneumonia (CEP) is characterized by chronic respiratory symptoms, bilateral peripheral lung opacities, pulmonary eosinophilia, and peripheral eosinophilia. Symptoms and radiopacities resolve rapidly after corticosteroid treatment, but they recur frequently after tapering or discontinuing the medication.

FE_{NO} levels were measured in 18 patients with CEP at several assessment points over one year, showing positive correlation with peripheral eosinophil count [226]. The median FE_{NO} levels were significantly higher in uncontrolled compared to controlled CEP. The FE_{NO} level of 66.0 ppb showed the largest area under the curve (0.835) for predicting exacerbation of CEP (sensitivity 80%, specificity 84%). Authors concluded that FE_{NO} may be useful for monitoring eosinophilic parenchymal inflammation and determining the appropriate corticosteroid dose in CEP [226].

Nasal nitric oxide

As shown by Lundberg *et al.* in 1995 [226], nasal cavity and upper airways represent the major source of nitric oxide detected in the respiratory tract of adult healthy subjects [227]. They found a continuous nitric oxide synthesis in paranasal sinuses, yielding very high nitric oxide concentration (3000-25000 ppb) contributing to that found in nasal air.

The nasal nitric oxide, which represents more than 90% of the total [228], is produced by all three NOS isoforms that have been identified in the upper airways in epithelial cells of nasal mucosa, in parasympathetic neurons innervating nasal vessels, in endothelial cells and in ciliated epithelial cells [229]. Interestingly, the NOS found in the paranasal sinuses is essentially calcium independent [20], a characteristic usually related to NOS-2, but it is constitutively expressed and resistant to steroids, the latter being typical features of constitutive NOSs.

Nasal NO can have several physiological functions including the participation in non-specific host defense against bacterial, viral and fungal infections [230], preserving a sterile microenvironment with-

in the paranasal sinuses, regulating cilia motility [231,232] and the nasal airway resistance to airflow, and entering in the humidification and warming mechanisms of inhaled nasal air flow [233]. Nasal NO has also been hypothesized to improve the ventilation–perfusion ratio in the lungs by the auto-inhalation [231,232], and to act as an aerocrine messenger between the upper and lower airways [234]. However, none of these actions has been directly associated with the high levels of NO detected in the nose [19].

As in the lower airways, nasal NO can exert the biological effects of NO by a direct action [21], although some of its physiological and pathophysiological effects (especially its pro-inflammatory actions) are likely to be activated by NO derivatives and not by the molecule itself. As discussed above, it can form complexes of metal-containing proteins, leading to enzyme activation or inhibition, or directly interact with high energy free radicals and modulate other oxidative reactions like lipid peroxidation inhibition, and limit the generation of pro-inflammatory lipids [21]. Furthermore, the NO indirect effects are mediated by reactive nitrogen oxide species (RNOS) originating from its interactions with O_2 or O_2•– [21].

Differently from lower airways, there are several methods to measure nasal NO (nNO). Currently, two methods of nNO assessment are recommended: nasal aspiration via one nostril during velum closure, and nasal exhalation through a tight facemask with fixed flow [30,43]. In the first method, nNO is aspirated from patients by the intrinsic suction of analyzer through a line with a disposable foam olive inserted into one nostril while palate is close by exhaling through the mouth (20–40 s) into a disposable resistor (with a resistance of at least 10-cm H_2O). Alternatively, nNO is aspirated while the subject breath holds with the velum elevated. In this case, a suction pump aspirates air through a nasal olive placed in one nostril with the subject holding his/her breath after inspiration to total lung capacity. In the second method, the nasal exhalation through a tight facemask with a stable fixed flow is used. The subject starts inhaling NO-free air from the analyzer through the nose during a full inspiration to total lung capacity, and then exhales through a tightly fitting mask covering the nose connected to the analyzer. The obtained NO values can be in parts per billion (ppb) or in nl/min (multiplying nasal NO concentration (ppb) by the sampling flow rate).

Differently from exhaled nitric oxide, nNO measurements have been proposed as diagnostic tool in only a few diseases. In primary ciliary dyskinesia (PCD), nNO is by far the most effective screening tool [235], with a specificity of 88%, a sensitivity of 100%, and a positive predictive value of 89% for a correct diagnosis when using a nNO cut-off concentration of 105 ppb [236]. It has also been reported that a value of nNO less than 100 ppb or 77 nl/min would strongly suggest PCD [237] and Collins *et al.* [238] using the same cut-off found a sensitivity of 93% and specificity of 84%, with a positive predictive value of 42.6% and a negative predictive value of 99%.

Some authors suggest that nasal NO measurements could also be useful in screening for cystic fibrosis (CF) patients, as they present low levels of exhaled NO [239,240]. However, the NO metabolism in CF airways is complex and not yet completely understood, and therefore it is of limited value in the diagnosis of CF.

In analogy with other inflammatory diseases, nNO has been proposed also for the diagnosis of allergic rhinitis [94,241] and for the diagnosis, prognosis or treatment evaluation of other sino-nasal diseases [242,243]. However, as showed by Phillips *et al.* [244], data referring to the sino-nasal application of nNO measurements have produced no clear evidence of clinical relevance, except for the impact of sinus surgery. A possible explanation is that nNO measurements in sino-nasal pathologies are currently hampered by confounding factors such as the continuous gas exchange between the nasal airway and paranasal sinuses [245], which may affect the ability to detect alterations in nNO occurring in sino-nasal disorders [246]. However, a very recent study suggests to use nNO also for differentiate AR patients from healthy subjects and may be significantly correlated with nasal symptoms and nasal patency of rhinitis patients [247]. A few data are consistent with the finding of lower nNO levels in patients with CRS compared with controls, and they reported an inverse correlation between nNO level and CT changes in patients with CRS [248]. Moreover, testing for nNO was highly predictive of separating CRSwNP , who had the lowest values, from patients with CRSsNP, and nNO cutoff value of less than 442 ppb was associated with the best combination of sensitivity and specificity, with a PPV of 87% and an NPV of 91% in detecting CRSwNP [242]. A more recent report confirmed the acceptability of the receiver operating characteristic curves in differentiating patients as CRSwNP, CRSsNP, and healthy controls and the correlation with sinus computed tomography and Sinonasal Outcome Test Scores [249,250].

The evidence that measurements of nNO during humming (which is the production of a tone without opening the lips or forming words) are correlated with ostial function [246,251,252], has led to its potential use as test for osteo-meatal patency. In normal conditions, humming causes a great increase in nNO (humming responders), whilst, when there is an obstruction of osteo-meatal complex, this maneuver does not cause any increase in NO (humming non-responders). This method may represent a suitable non-invasive test to assess sinus ostium block [246], and might be useful for screening of sinus disorders and for both post-medical and post-surgery follow up in patients with bilateral nasal polyposis [253] and in patients with allergic rhinitis [254]. Therefore, it is likely that the humming test may also characterize an on–off response in the presence of advanced ostium disease [255].

Nasal NO has been also investigated in other non-respiratory diseases such as inherited retinal dystrophies [256].

In conclusion, the use of nNO in diagnosis and monitoring of respiratory disorders (*e.g.*, allergic rhinitis, sinusitis, nasal polyposis, CF) is potentially of interest, but more research is needed before we understand how clinically useful these tests are.

Cost effectiveness of using FE_{NO} in asthma diagnosis and management

As described above, FE_{NO} measurement can be used for different purposes: from diagnosis to management of asthma, including the evaluation of corticosteroid responsiveness and adherence, and phenotypization of patients with severe asthma.

FE_{NO} is also promisingly useful in properly prescribing and monitoring the treatment with novel biological agents together with other biomarkers of T2 inflammation such as serum IgE and peripheral blood eosinophils.

Naturally not all the described possible uses have the same degree of evidences. Guidelines and reviews have graded the evidence for each of the possible uses. While NICE guidelines on asthma released in 2017 include FE_{NO} assessment among the first-line evaluations for suspect asthma together with lung function tests in both children and adults [75], a Cochrane review [148] concluded that strategies based on tailoring asthma medications dose according only to FE_{NO} levels do not have enough evidence to be translated into clinical practice. Another Cochrane review stated that while the use of FE_{NO} to guide asthma therapy in children may be beneficial in a subset of children, it cannot be universally recommended for all children with asthma [149].

Due to the characteristics of the current technology, the measurement of FE_{NO} is not expensive and in 2017, in the USA, the pro-

posed reimbursement by Medicaid was around 20 $ [257], a price that may lead to a cost-saving policy, both in diagnosing and in the follow managing asthma.

A retrospective observational study conducted in USA on patients hospitalized or treated in emergency department for asthma, demonstrated that direct costs related to asthma exacerbations can be reduced and almost halved by the use of FE_{NO} for monitoring asthmatic patients [258].

In Spain it was calculated that adding FE_{NO} to standard asthma care in adults saved 62.53 € per patient/year in adults and improved QALY, with a potential net yearly saving of €129 million in the budget of primary care settings [259].

In Italy the situation is patchy; even if FE_{NO} measurement has been recognized at national level as a diagnostic test that can deserve reimbursement from public health care system, the Italian State-Region Conference has not approved a specific code and reimbursement tariff yet. The result is that each region can use different codes (relating to other tests) to classify and price FE_{NO} measurements. Only one region has a specific code for FE_{NO}. All the other ones use existing codes (not specific to FE_{NO}) to get the reimbursement. The tariff also is a haphazard one. It spans from 23.20 euro to 73.00 euro. The rough median is around 24.00 euro.

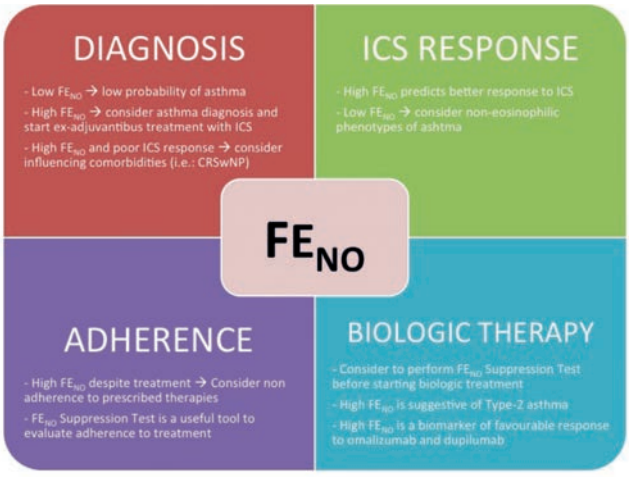

Figure 2. Fractional exhaled nitric oxide (FE_{NO}) usefulness in different aspects of the management of asthma.

Conclusions

FE_{NO} is a non-invasive, cheap and easy-to-assess method to assess airway inflammation, and it has a series of possible advantages in the management of asthma, both in adults and children (Figure 2):

- in the **diagnostic process**, in which high values of FE_{NO}, in patients with consistent symptoms, confirm the suspect of asthma and the need to do further tests to rule in the diagnosis [43]; on the other hand, low values of FE_{NO} are rarely associated with a final diagnosis of asthma, and therefore they should suggest to investigate other possible differential diagnosis [43]. These were the evidence that brought the NICE guidelines [75] to recom-

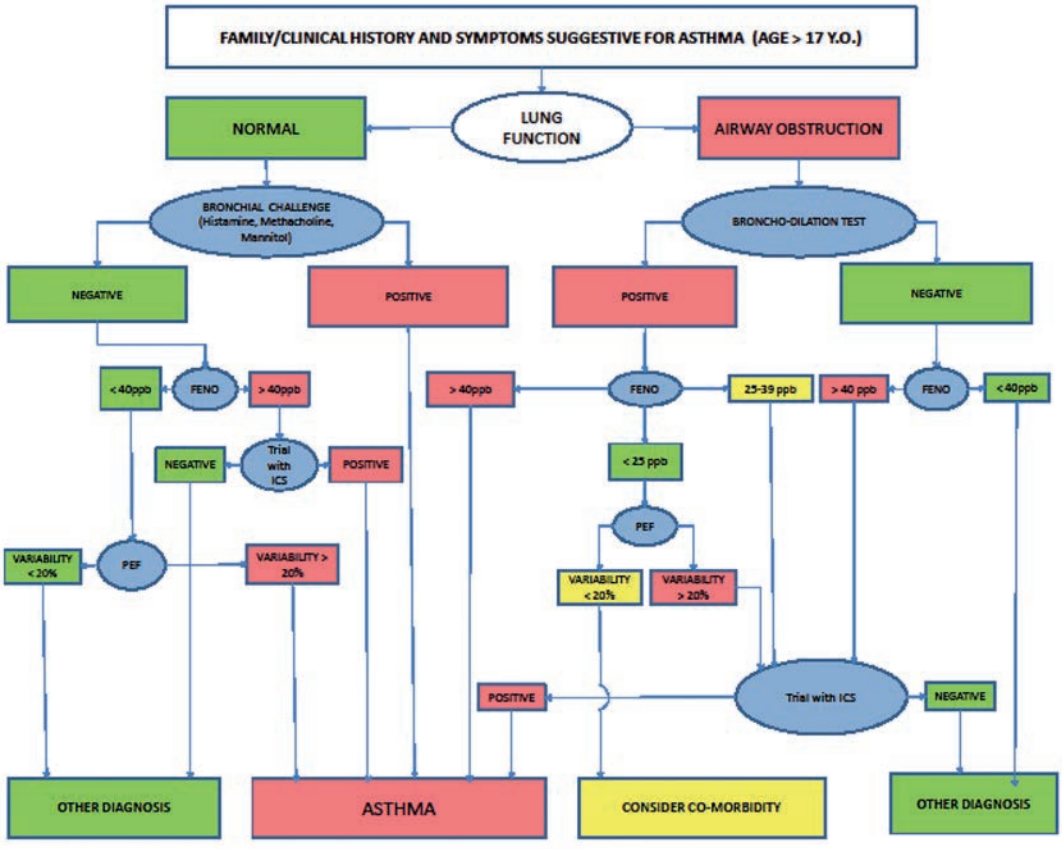

Figure 3. Proposed diagnostic algorithm for asthma including FE_{NO} in assessment of airway inflammation.

mend integrating FE_{NO} testing in the diagnostic flowchart for asthma. We also recommend to use FE_{NO} for diagnostic purpose in combination with lung function assessment and trials with ICS (Figure 3).

- in the **assessment of response to ICS treatment**: high FE_{NO} values are associated with an increased probability to achieve improvement of asthma symptoms after having started (or increased) ICS treatment [99, 129-131];
- in the **evaluation of adherence to ICS treatment**: non-adherent patients tend to have high FE_{NO} levels despite the given treatment, and the so-called "FE_{NO} suppression test" [130] should be done in all patients not properly responding to the asthma therapy, particularly in difficult-to-treat asthmatics;
- in the **phenotypization process of severe asthmatics and as a biomarker for biologic treatments**: FE_{NO} is one of the key biomarker of type-2 inflammation and high levels are suggestive of a type-2 inflammatory pathway underlying the asthma pathogenesis; moreover, patients with high levels of FE_{NO} are those who have the highest probability to respond to anti-IgE and anti-IL4-receptor-alpha biologic treatments [163-166,169], while it seems not to be a good response-biomarker for anti-IL5 agents [167,168].

The use of FE_{NO} is suitable and recommendable in both adults and children, and it should be implemented and encouraged as it proved to be cost-effective when applied to the management of patients with (suspect) asthma [257-259]. For these reasons, we believe that this position paper, like other recently published [260], can be useful for clinicians taking care about asthmatic patients as a guide in the interpretation of FE_{NO} results.

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