

Chinese expert consensus on clinical use of non-invasive airway inflammation assessment in bronchial asthma

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Introduction

The most essential pathological change of bronchial asthma (hereinafter referred to as asthma) is chronic airway inflammation. The severity of airway inflammation is closely associated with airway hyperresponsiveness (AHR), which is the origin of the clinical symptoms (1). Therefore, the assessment of airway inflammation, particularly by noninvasive methods, is of great significance to understand the pathophysiological characteristics of asthma, to guide the management and to achieve better overall control of asthma (2).

The Chinese Society of Chest Physicians and the China Asthma Alliance have convened a panel of experts in related fields to discuss and develop this consensus, referring to relevant international guidelines, and important literatures published within and outside China in recent years (3,4). This consensus aims to guide non-invasive airway inflammation assessment, especially the fractional exhaled nitric oxide (FeNO) measurement, including the methodology, quality control and results interpretation, in order to achieve better outcomes of clinical practice. In light of many aspects on the clinical significance and value of FeNO measurement remaining to be explored, this consensus also deals with current problems and proposes further research directions. Clinical studies, especially those with multi-center collaborations, are in dire need to improve the clinical practices and research in this field.

The relationship among airway inflammation, airway responsiveness and asthma control

Asthma is a chronic inflammatory disease of the airways, involving various inflammatory cells, structural cells, and cellular mediators. The chronic inflammation leads to development and progression of AHR, widespread but variable and reversible airflow obstruction. The clinical manifestations include recurrent episodes of wheezing, breathlessness, chest tightness, coughing and other symptoms which frequently occur at night and/or early in the morning, and are often reversible either spontaneously or with treatment. Eosinophils (EOS) are the dominant inflammatory cells in the asthmatic airways. Increased number of EOS in the airways is the main pathological characteristic of asthma. EOS-released toxic proteins [e.g., major basic protein, eosinophil cationic protein (ECP)], inflammatory mediators (e.g., platelet activating factors, leukotrienes C4, D4), cytokines (e.g., IL-5), oxygen free radicals (e.g., superoxide anions, hydrogen peroxide and singlet oxygen) play essential roles in the pathogenesis of asthma.

AHR refers to the unduly strong and long-lasting contractile response of trachea and bronchi to endogenous and exogenous (including a variety of physical, chemical and immunological) stimuli. Over 99% of asthma patients present with varying severity of AHR, which is vital pathophysiological feature of this disorder. The airway

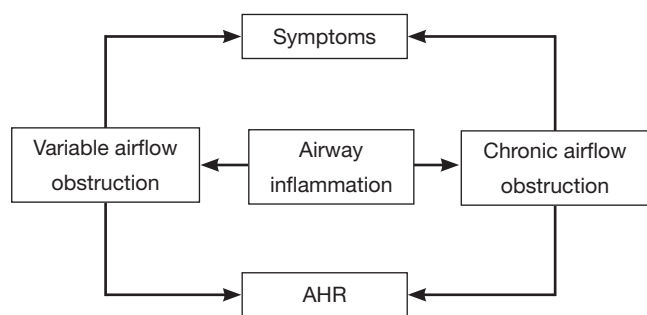


Figure 1 The relationship among airway inflammation, AHR and asthma symptoms. AHR, AHR airway hyperresponsiveness.

response to stimuli in asthma patients can be 100-1,000 times higher compared with normal individuals. Therefore, airway responsiveness is not only an important measure for diagnosing or excluding asthma, but also for assessing asthma severity and treatment efficacy. Numerous studies have demonstrated that, controlling chronic airway inflammation is solely the effective approach to alleviate AHR, achieve and maintain asthma control, avoid acute exacerbations, improve pulmonary function and quality of life, reduce emergency visits and hospitalization, and ultimately cut down the mortality.

Thus, objective assessment of the airway inflammation makes a great sense for the diagnosis of asthma, selection and adjustment of treatment strategy, and achievement of asthma control.

The relationship among airway inflammation, AHR and asthma symptoms is illustrated in *Figure 1*.

The assessment methods and evaluation of airway inflammation

The assessment of airway responsiveness (5-7)

Airway responsiveness can be measured by bronchial provocation test (BPT). First introduced in 1873, BPT is used to induce airway constriction by inhalation of antigens or non-specific irritants. Since 1980s, this technique has been widely recognized and its methodology has been gradually refined to become more and more standardized. International and Chinese respiratory societies have issued guidelines on airway responsiveness measurement for better use of this technique in research and management of asthma. Depending on various irritants in use, airway responsiveness measurement is categorized as nonspecific and specific approaches. The non-specific approach involves

irritants including histamine, methacholine, propranolol, distilled water, dry cold air, mannitol, leukotriene E₄, adenosine monophosphate and ozone, among which, histamine and methacholine are commonly used. Specific measurement refers to the inhalation of liquid allergen extracts as the irritant, aiming at clarifying the relationship between AHR and specific allergens (8).

Methods

BPTs include direct and indirect provocation tests. The most preferred irritants used in direct provocation tests are histamine phosphate and methacholine. The test begins with measurement of baseline pulmonary function, followed by the provocation only if the baseline FEV₁ is greater than 70% predicted. The dosage of the inhaled irritant escalates from the lowest concentration until the test becomes positive or asthmatic symptoms appear. Inhaled bronchodilator is then given to render pulmonary function back to or near baseline. If the test remains negative with maximum concentration of the irritant, the irritant inhalation should be discontinued and inhaled bronchodilators should be given.

Chiefly, two methods are available for the testing: (I) Using FEV₁ as the indicator, such as Chai protocol (inhalation through five breathes of a fixed duration), Yan protocol (inhalation with a hand bulb nebulizer), and Cockcroft protocol (inhalation during tidal breathing). First, the subjects are determined for baseline FEV₁ with forced expiration, and then they inhale histamine or methacholine in stepwise incremental concentrations. FEV₁ is measured at each dose. When FEV₁ drops by $\geq 20\%$ from baseline or, physical examination reveals wheezing, or the maximum dose has been reached, the subjects should be given inhaled bronchodilator (salbutamol). Then the computer automatically calculates the PD₂₀ to show a positive or negative BPT, as well as the degree of airway responsiveness. (II) Using forced oscillation technique for consecutive tracings of respiratory resistance, e.g., Astograph protocol. Subjects breathe calmly and inhale normal saline for recording of baseline resistance value. Thereafter, the subjects inhale stepwise incremental concentrations of methacholine while the respiratory resistance is continuously monitored. The entire nebulization system comprises of 12 serially arranged nebulizers. The first one contains normal saline and the last one contains bronchodilators. Nebulizers No. 2 to No. 11 deliver 2-fold incremental concentrations of methacholine (49 to 25,000 mg/L). The subjects inhale each dose of the

Table 1 Severity of airway hyperresponsiveness (AHR) (PD20-FEV₁)

Grade	Histamine		Methacholine	
	mg	μmol	mg	μmol
Normal	>2.400	>7.8	>2.500	>12.8
Borderline	1.013-2.400	3.3-7.8	1.076-2.500	5.5-12.8
Mild	0.276-1.012	0.9-3.2	0.294-1.075	1.5-5.4
Moderate	0.031-0.275	0.1-0.8	0.035-0.293	0.18-1.4
Severe	<0.031	<0.1	<0.035	<0.18

irritant for 1 min. While the subjects inhale normal saline, the resistance is recorded as baseline resistance. Then, the device continuously records changes in respiratory resistance while the subjects continuously inhale the two-fold incremental concentrations of methacholine. When the respiratory resistance reaches 2 times the baseline level or, physical examination reveals wheezing, or the maximal dose has been reached, the subjects should be given inhaled bronchodilator (salbutamol). Finally, the computer automatically calculates Dmin and PD35 to show a positive or negative BPT, as well as the degree of airway responsiveness (9).

Evaluation indicators

(I) Qualitative analysis: (i) If FEV₁ or peak expiratory flow (PEF) drops $\geq 20\%$ from the baseline values, specific airway conductance (sGaw) drops $\geq 35\%$, or wheezing occurs, the test can be evaluated as positive. (ii) If FEV₁ drops by 15.9% to 20% after the inhalation of the maximum concentration of irritants with no shortness of breath or wheezing, the test is evaluated as probably positive. (iii) If the indicators do not reach the positive criteria above after the inhalation of the maximum concentration irritant, the result is evaluated as negative. (II) Quantitative analysis: PD20 refers to the cumulative dose of inhaled irritant when FEV₁ drops by 20% from its baseline value. PC20 refers to the concentration when FEV₁ drops by 20% from the baseline value. AHR is quantitatively graded by PD20 or PC20. For instance, the severity grading of AHR based on PC20-FEV₁ (methacholine) is described as: <1.0 g/L, moderate to severe AHR; 1.0-4.0 g/L, mild AHR; 4.0-16 g/L, borderline AHR; >16 g/L, normal airway responsiveness (Table 1).

Clinical significance

(I) To help the diagnosis of asthma: positive airway responsiveness indicates a significant probability of asthma. If the positive result is accompanied with asthmatic

symptoms, a diagnosis of asthma can be determined for most of the times. (II) To assess the severity and prognosis of asthma: the grade of AHR is positively correlated with the severity of asthma. Greater AHR indicates more severe asthma and a worse prognosis. (III) To guide the treatment of asthma and assess the efficacy. The higher airway responsiveness, the more necessity for active anti-inflammatory treatment is needed. If AHR is reduced after anti-inflammatory treatment in asthma patients, which indicates airway inflammation is controlled, the treatment can be stepped down. If airway responsiveness does not decrease after treatment, the patients probably need step-up treatment. (IV) To study the pathogenesis of asthma: since AHR is characteristic of asthma, investigating the mechanisms of AHR helps to understand the pathogenesis of asthma and contributes to the treatment of this disorder. (V) To indicate the airway responsiveness in other diseases associated with AHR. (VI) To clarify the causes (or allergens) of asthma by specific BPT.

Important tips

(I) The patient should be clinically stable, without asthma attack for at least 1 week. There is no obvious apnea or wheezing during the test. (II) The test is started by conducting a baseline spirometry to record the initial FEV₁. For eligibility of BPT, FEV₁ should mandatorily be $\geq 70\%$ predicted value. (III) Patients with severe cardiopulmonary dysfunctions or hypertension, or women in pregnancy, are not eligible for this test. (IV) There might be a certain degree of risks during the BPT and rescue therapy should be available. The concentration of the inhaled drug should start from low dose, and increase gradually. Rescue agents include short acting β_2 agonists, epinephrine, dexamethasone, oxygen and tracheal intubation equipment. An experienced clinician should be present during the test. The patient should be observed until FEV₁ is back to or near baseline.

The measurement of airway responsiveness can indirectly reflect the severity of airway inflammation, and evaluate the efficacy of treatment and the prognosis of asthma. However, it demands sophisticated devices, and is also complex and time-consuming. BPT is not suitable for patients with poor pulmonary function ($FEV_1 < 70\%$ predicted value) and those with asthma exacerbations. Moreover, BPT is rather sensitive more than specific, and therefore a positive test can be seen in a variety of non-asthmatic conditions, like allergic rhinitis, cystic fibrosis, and viral infections. Finally, BPT may lag behind development of airway inflammation and may also trigger the exacerbation of asthma.

Induced sputum cytology

Sputum induction is a non-invasive procedure to induce sputum specimen by inhalation of hypertonic saline administrated by nebulisation in order to study the features and extent of airway inflammation by analyzing the cellular components and the soluble mediators. In addition to its value in understanding the mechanisms of airway inflammation in asthma, determining and differentiating diagnosis of many other respiratory disorders, induced sputum can be also of great value in investigating the treatment efficacy of drug and how they work. Induced sputum sample is easy to obtain. The sample can reflect the concentration of airway secretions in the natural (undiluted) state. Relevant inflammatory cells, mediators and cytokines can be directly detected. This can serve as a relatively direct reflection of airway inflammation and provide the quantitative information of airway inflammation, similar to those from BALF. The sensitivity and specificity of induced sputum cytology are superior to those of peripheral blood test. The procedure is non-invasive, repeatable and safe (10).

Test method

(I) Measure FEV_1 at baseline. (II) The patient inhales salbutamol 200 μg /time for 2 times. (III) Re-measure FEV_1 after 20 min. If FEV_1 is $>60\%$ predicted, the following procedures can be performed. (IV) Instruct the patient to clear up nasal secretions, breathe calmly and inhale 3% hypertonic saline by an ultrasonic nebulizer for about 15 min. At 5, 10, 15 and 20 min of the induction, respectively, instruct the patient to gargle vigorously, cough forcefully and expectorate into a sputum collection container. (V) After each sputum induction, re-measure FEV_1 . If FEV_1 declines by less than 10%, the test can be continued. If

FEV_1 decreases by 10% to 20%, the subjects should inhale salbutamol 200 μg again. If FEV_1 did not improve after the inhalation of salbutamol, the test should be terminated. Otherwise, sputum induction can be continued. (VI) If the amount of induced sputum is not satisfactory, the above steps can be repeated with 4% and 5% hypertonic saline sequentially. Inhalation of sodium chloride solution in each concentration should be at least for 7 min. Cumulative nebulization duration should be no more than 30 min. (VII) End the induction, if the collected volume of sputum specimen is sufficient for the test (sputum volume should be ≥ 0.7 mL or 0.3 g, and percentage of mouth epithelial cells should be $<5\%$), or the total inhalation duration reaches 30 min. (VIII) Discontinue the induction, if the patients present with chest tightness, cough, wheezing, and dyspnea, or if FEV_1 decreases by over 20% from the baseline value, and FEV_1 is $<60\%$ predicted. The subjects should be given inhaled salbutamol 200–400 μg until the symptoms are relieved.

Improved induction method: start by inhaling nebulized normal saline for 1 min, followed by 3%, 4%, and 5% of hypertonic saline for 1–2 min; collect the sputum specimens in accordance with the method described above; measure FEV_1 before each procedure. If FEV_1 declines by over 10%, or sufficient volume of sputum for a test has been collected, stop the procedure.

Evaluation indicators

Theoretically, the levels of all cells and mediators involved in airway inflammation, like EOS, neutrophils, ECP, NO^3^-/NO^2^- , and cytokines can be detected in induced sputum. Currently, induced sputum is mainly used for cell count in clinical practice and to determine the type of airway inflammation. The normal percentage of induced sputum EOS is $<3.0\%$ according to the international criteria (11), and it is $<2.5\%$ in Chinese cough guidelines (12). If the EOS count in induced sputum is above the normal range, the existence of eosinophilic airway inflammation can be determined.

Clinical significance

EOS in induced sputum, as one of the markers of airway inflammation, can timely reflect the level of airway inflammation, and sensitively indicate the responsiveness to treatment of glucocorticosteroids (13). In addition, a substantial proportion of patients present mixed or non-eosinophilic asthma. It is also of some clinical significance to observe the changes of other cell counts in the induced sputum.

Clinical application: (I) To differentiate asthma from other respiratory diseases. (II) To identify the types of

airway inflammation in asthma and other respiratory diseases. (III) To study the causes for the exacerbation of airway inflammatory diseases. (IV) To predict the effect of glucocorticosteroids and guide medication options. Increased EOS in asthmatic sputum usually indicates that the patients can benefit more from glucocorticosteroids. (V) To evaluate the drug actions on airway inflammation. It is helpful to note the changes of cellular components and inflammatory mediators in induced sputum before and after the treatment for mechanistic study of drugs.

Important tips

(I) The inhalation of hypertonic saline is a non-specific stimulation on the airway. This can induce airway smooth muscle contraction and reduce the ventilation function, particularly in asthmatic patients. Therefore, the indications and contraindications of induced sputum test must be strictly followed. Lung function or PEF must be measured before the procedure. Sputum induction should not be considered for subjects who are not accessible to a pulmonary function test. (II) During the process of sputum induction, the operator must closely observe the response of the patients. Necessary rescue facilities should be provided at the site. (III) Inhaled β_2 receptor agonist should be given before and after the induction. (IV) The sputum samples induced by the inhalation of hypertonic saline should be processed for the test as soon as possible. Because the "sputum" may contain saliva, the collected sample should be screened for quality. First, we need to identify whether the specimen is obtained from the upper respiratory tract or lower respiratory tract, which can be judged under a microscope by the mucus content and squamous cell counts. Few squamous cells are observed in the sputum from the subglottic airway. (V) The sticky part of the induced sputum or the sputum without squamous cell observed via an inverted microscope should be selected for examination. The total cell counts and cell viability, EOS and supernatant ECP levels in selected sputum specimens are higher than those in the unselected specimens. Probable dilution of induced sputum by saliva before the analysis may interfere with the results of tests. Another approach is to examine all the spit-ups including saliva and sputum. In comparison of these two methods, the latter may lead to a higher proportion of neutrophils in the classification count of non-squamous cells and a higher concentration of supernatant ECP. Whichever method being used, addition of 0.1% dithiothreitol (DTT) is needed to homogenize the specimen. DTT breaks up the disulfide bonds crosslinking the glycoprotein filaments and thereby dissolves the mucus

and disperses the cells.

It is generally safe to carry out induced sputum examination in strict accordance with the procedures. The main complication is asthma episodes induced by bronchial spasm. Therefore, standard operating procedure (SOP) should be developed and appropriate rescue facilities should be configured to manage adverse reactions.

Absolute contraindications: (I) Any patients with $FEV_1 < 1$ L. (II) Recent massive hemoptysis. (III) Moderate to severe acute exacerbation, acute or chronic respiratory failure, pneumothorax or mediastinal emphysema, massive pleural or pericardial effusion, and severe heart failure. Relative contraindication: active tuberculosis.

Although induced sputum examination is simple to perform, it is time-consuming and work-consuming. The lack of standardized procedures and poor quality control of the operation may hamper the accuracy of the results and limit the widespread application of this technology in clinical practice.

Fractional exhaled nitric oxide (FeNO) measurement

Nitric oxide, a gas molecule, is attracting widespread attention for its presence in the airways. Nitric oxide can be produced by various structural cells and inflammatory cells on the surface of airways under oxidation of nitric oxide synthase (Figure 2). The physiological roles of nitric oxide in the airways include neurotransmission, the dilation of blood vessels, the relaxation of airways and the regulation of inflammatory mediators. Although the roles of nitric oxide in asthmatic airways are not completely clear, a large number of studies have demonstrated that FeNO increases in asthma and declines after glucocorticosteroids therapy. In 1997, European Respiratory Society (ERS) developed the recommendations for FeNO measurement for the first time (14). The recommendations were later jointly revised by the American Thoracic Society (ATS) and ERS in 2005 (15). In 2011, ATS issued the guidelines for the clinical application of FeNO (3). In 2014, British National Institute for Health and Care Excellence (NICE) also promulgated relevant guideline documents online (16).

Allergens and other stimulating factors induce airway inflammation, with the release of cytokines by EOS and other cells, which results in the activation of nitric oxide synthase and the increase of nitric oxide levels in the airways.

Methods

Currently, the electrochemical detection method is

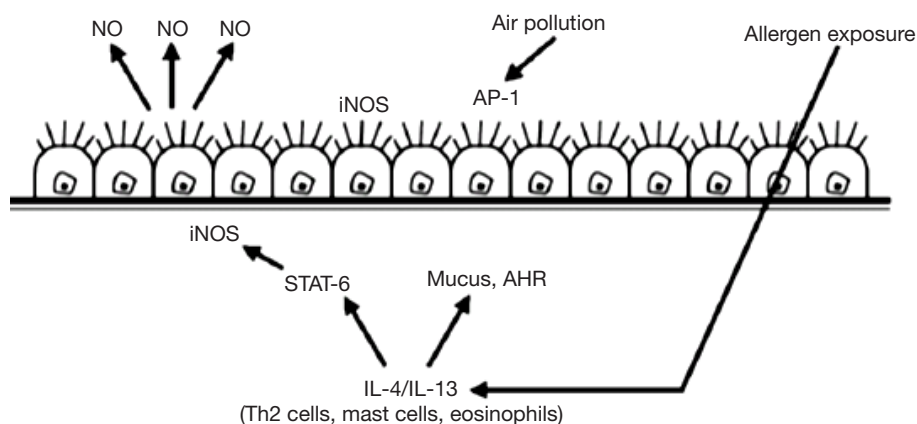


Figure 2 Schematic representation of the formation of nitric oxide (NO).

recommended. Subjects generally take seats, rest for 5 min, and then try the best to exhale the air. Then, keep the mouthpieces (including filter/bacteria-filtering mouthpiece) of the nitric oxide analyzer tight in mouths. Inspire to reach the total lung capacity (TLC). Then, steadily and slowly expire at a constant flow rate of 50 mL/s ($\pm 5\%$) for 10 s (at least 4 s for children under 12 years of age, at least 6 s for adults and children over 12 years of age). The results are presented in ppb units.

Evaluation indicators

For American children and adults, the normal ranges of FeNO are 5-20 and 5-25 ppb, respectively; and the recommended cutoff values are 20 and 25 ppb, respectively (3,17). A multi-center clinical study in China showed the reference values of FeNO for healthy children (<15 years) and adults were 5-24 and 5-30 ppb, respectively, and suggested the cutoff values should be 24 and 30 ppb, respectively.

Clinical significance

(I) FeNO measurement is recommended to (i) assist the diagnosis and differential diagnosis of asthma; (ii) differentiate airway inflammation types and evaluate the severity; (iii) predict the responsiveness to inhaled corticosteroid (ICS) therapy; (iv) evaluate the adherence to ICS treatment; (v) assess the level of asthma control and predict the acute exacerbations; (vi) guide the adjustment of asthma treatment regimen (3). (II) FeNO is related to eosinophilic airway inflammation and of high diagnostic value for eosinophilic asthma rather than for non-eosinophilic asthma. As far as this is concerned, the diagnosis should be made cautiously. Recent study showed

that in elderly patients with clinically stable asthma, the level of FeNO is not high and is not related to sample-size of study population, complications, treatments, symptoms and spirometry of subjects, suggesting that routine FeNO test is clinically relevant in elderly asthmatic patients (18). Few studies on cutoff values of FeNO have been reported in China. Further epidemiological data and more rigorously designed randomized controlled clinical studies are needed.

Important tips

(I) FeNO should be measured in the room. The indoor air concentration of nitric oxide can affect the test results (nitric oxide in ambient air is required <5 ppb). Before the test, subjects should have been kept on abstinence of alcohol for 4 h, of nitrate-containing food for 2 h, of water and other food for 1 h. Smoking and strenuous exercise are also prohibited for 1 h before the test. Inspiration and expiration during the test should be continuous. Do not hold and pause breath (14-16). (II) The following factors can up-regulate FeNO expression: food and medicines containing nitrates, L-arginine, inhaled β -agonists, atopy and viral infections. The following factors can down-regulate FeNO expression: smoking, drinking, inhaled or oral corticosteroids, leukotriene receptor antagonists, pulmonary function test, induced sputum test, BPT and omalizumab. As for the effects of gender, pregnancy, menstrual period and caffeine on FeNO, the current results are controversial (16-19).

In conclusion, FeNO is a novel, non-invasive and easy-to-use biomarker that relates prominently to allergic airway inflammation. FeNO test may contribute to the typing of airway inflammation, diagnosis, evaluation and treatment of asthma. Currently, FeNO test has been wide-spreading in clinical practice.

Exhaled breath condensate (EBC) analysis

EBC analysis is a new method to evaluate the inflammation and oxidative stress of airways by measuring the biochemical markers in EBC.

Measurement methods

Patients directly exhale the air into a collection tube. Then the tube is immersed in an ice bath or other condensing apparatus (below 4 °C). As the temperature decreases, the exhaled gas condenses into EBC. It usually takes 10-15 min to collect 2-4 mL of EBC. Special tests are always needed due to the generally low concentration of liquid medium in EBC.

Evaluation indicators

Nowadays, commonly recognized EBC biomarkers include hydrogen peroxide (H₂O₂), 8-iso-prostaglandin, products of nitric oxide, leukotrienes and pH value. Few studies focus on other EBC indicators such as prostaglandin, glutathione, aldehydes, cytokines, adenosine, endothelin 1, interferon α , cations, macrophage-related media and growth factors. The conclusions are also controversial. (I) H₂O₂: H₂O₂ is an important product of the oxidation cascade, and is usually measured with spectrophotometry or fluorescence spectrometry. Studies have showed that children with asthma have significantly increased H₂O₂ levels (20). A meta-analysis reported that the H₂O₂ level in non-smoking asthma patients is negatively correlated with FEV₁, and dramatically declines after glucocorticosteroids therapy (21). (II) 8-iso-prostaglandin: this is a prostaglandin-related analogue compound that can be measured by enzyme immunoassay (EIA), gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/mass spectrometry (LC/MS). A majority of studies showed that the level of 8-iso-prostaglandin was elevated in asthmatic children (20,22-27), and correlated with the severity of asthma (22). (III) Products of nitric oxide: 3-nitro-tyrosine and nitrogen oxides (NOX) produced by nitric oxide pathway can be detected in EBC and measured by EIA. Several studies showed that the level of nitrite and the ratio of nitrite/nitrate significantly increased in children with asthma (28,29). (IV) Leukotrienes: leukotriene pathway is involved in the asthmatic airway inflammation. Leukotrienes can be detected by EIA, GS/MS or LC/MS. Most studies reported significantly higher levels of leukotrienes in children with asthma (23,25-27,30,31). (V) pH: the pH value of EBC can be measured with a pH meter or blood gas analysis. It is

significantly low in patients with asthma, and even lower in severe cases (32,33).

Clinical significance

Literatures (meta-analysis) reported the correlation between the levels of EBC indicators and asthma control. The severity of asthma is related to the levels of 8-iso-prostaglandin, nitrite and nitrate. The level of asthma control is related to the level of 8-iso-prostaglandin, interferon γ and IL-4 (21). Further studies are needed to determine whether EBC biomarkers can be used as independent predictors for evaluating the onset and control of asthma (34).

The biomarkers in EBC, which can reflect the severity of airway inflammation, have been widely used for asthma research in recent years. Unfortunately, the very low and unstable level of mediators in EBC render sophisticated instruments needed for the detection, and therefore deters the widespread use of EBC test in clinical practice.

Other assessments

Since various inflammatory cells, cell components and inflammatory mediators interact in airway inflammation, novel indicators for evaluation have attracted increasing interest in recent years.

EOS count in peripheral blood is a reliable indicator of eosinophilic asthma phenotype (35). Increased EOS indicates acute exacerbation of eosinophilic asthma. In addition, peripheral EOS count can be used to monitor the effects of asthma medication and immune intervention. For example, peripheral EOS count >150/ μ L suggests that the asthma patient is suitable for mepolizumab and other anti-IL-5 therapy (36). Moreover, a significant increase in the daily variation of peripheral EOS count can also indicate an episode of asthma (37).

Urinary leukotriene E4 (ULTE4) can be significantly increased in acute exacerbation of childhood asthma. The concentration of ULTE4 may reflect the severity of airflow obstruction in asthmatic children and predict the risk of asthma onsets triggered by second-hand smoking and air pollution (38). Meanwhile, ULTE4 level or ULTE4/FeNO ratio may be used to predict the response to leukotriene receptor antagonists in children with mild to moderate asthma (39).

The serum levels of periostin, an extracellular matrix protein, may be used to determine whether the patients have Th2-driven airway inflammation and to screen suitable candidates for anti-IL-13 therapy (40).

Bromotyrosine (BrY) is a highly specific indicator for EOS activation. Urinary BrY level quickly increases during the acute exacerbation of asthma (41).

S-nitrosoglutathione (GSNO) is an important bioactive factor against AHR *in vivo*. FeNO decreases rapidly in asthma patients after the inhalation of GSNO. FeNO decrement rate can be detected with GSNO provocation test. This can be used to identify individuals with high EOS counts and high concentration of GSNO reductase (42).

The level of urinary F2-isoprostanes (F2IsoPs) significantly increases in asthma patients and it is closely related to the severity of asthma (43).

The increase in serum arginase is closely related to the acute exacerbation and poor control of asthma (44). The arginase activity is inversely associated with FeNO levels in patients with severe asthma (45). Serum arginase level and its activity may both be important indicators of airway inflammation, particularly for patients with glucocorticosteroid-resistant asthma.

Clinical significance of FeNO measurement

Diagnosis and differential diagnosis of asthma

The diagnosis of typical asthma is based on the symptoms and the medical history. For individuals with atypical symptoms and needs for differential diagnosis, laboratory tests should be carried out. Currently, the most commonly used tests in clinical practice include BPT, bronchodilation test and PEF monitoring. The above tests mainly reflect ventilation function but could not confirm on the existence of airway inflammation. A large number of clinical studies have been conducted on the diagnostic value of FeNO, but with mixed conclusions. In several studies from the western hemisphere, where clinical symptoms and positive BPT or bronchodilator test were set as the gold standard for the diagnosis of asthma, the diagnostic cutoff values varied among investigators (7, 12, 16 and 20 ppb). Overall, those results linked the diagnostic performance of FeNO measurement for asthma to a sensitivity ranging from 32% to 88%, a specificity from 79% to 93%, a positive predictive value from 70% to 80%, and a negative predictive value from 61% to 92% (44-47). A Japanese study reported that allergic rhinitis and smoking can significantly interfere with the measurement results. Therefore, the optimal diagnostic cutoffs for smokers and individuals with rhinitis should be adjusted to 18 and 28 ppb (48). A clinical study in China demonstrated that with symptoms and positive BPT or

bronchodilator test as the gold standard for the diagnosis of asthma, and FeNO ≥ 36.5 ppb as the cutoff value, the diagnosis of asthma with FeNO measurement was linked to a sensitivity of 79.2%, a specificity of 94.3%, a positive predictive value of 92.7%, a negative predictive value of 83.3%, and an accuracy rate of 87.1% (49). Another Chinese study on 358 cases of childhood asthma reported a favorable negative predictive value of FeNO when 17.9 ppb was set as the cutoff value; however, the positive predictive value was only 56.33% (50).

For individuals with atypical asthmatic symptoms, it is an important diagnostic method to determine AHR by BPT. Negative BPT results usually help to exclude the diagnosis of asthma. However, not all individuals can complete BPT in clinical practice, such as children who are unable to cooperate for a pulmonary function test and patients who have ventilatory dysfunction. Relevant clinical studies reported conflicting results on whether FeNO could be used as an alternative marker for AHR. In 2005, Berkman *et al.* reported that the diagnostic value of FeNO was equivalent to that of BPT when the cutoff value was 7 ppb (47). However, other studies showed the extent of correlation between FeNO and AHR were not consistent, suggesting that the relationship between nitric oxide metabolism and AHR may be very complex (51).

Although the results of the above studies showed certain sensitivity and specificity of FeNO for the diagnosis of asthma, current guidelines have not recommended use of FeNO alone for asthma diagnosis (3), based on following considerations: (I) The FeNO levels in asthma patients overlap with the ranges in healthy individuals. Thus, it is difficult to set a normal predicted value of FeNO. The value of the reference range obtained from healthy populations should be inferior to that obtained from the patients. Therefore, ATS guidelines recommend using cutoff values, rather than the reference ranges, for the interpretation of FeNO. (II) The airway inflammation in asthma may include different phenotypes, such as eosinophilic phenotype, neutrophilic phenotype, mixed cellular phenotype and paucigranulocytic asthma phenotype. Eosinophilic phenotype accounts for approximately 50-60% of asthma cases. FeNO mainly reflects the eosinophilic airway inflammation. For other phenotypes of airway inflammation or for patients who already received glucocorticosteroid therapy, FeNO test results may be negative, which is a major setback for FeNO in the diagnosis of asthma. (III) The diagnostic cutoff values in use differed across studies. Some of them which focused on determining the optimal cutoff values of FeNO measurement

in diagnosing asthma, the results varied from 20 to 26 ppb (52,53). While the higher cutoff value corresponds to higher specificity and lower sensitivity of the asthma diagnosis, and vice versa, the best cutoff value for diagnosing asthma has not been recognized yet. (IV) Genetics, age, gender, atopic status, body weight, height, current smoking status and diet can interfere with FeNO levels (54). In some patients with chronic obstructive pulmonary disease (COPD) or eosinophilic bronchitis, FeNO levels may also increase, which might lead to false-positive results in the diagnosis. (V) The levels of FeNO may suggest a likelihood of having asthma, but are not sufficiently robust to confirm or exclude asthma. The diagnostic values of high versus low levels of FeNO are interpreted in different scenarios. For patients with typical symptoms, increased FeNO level is supportive of the diagnosis of asthma; for patients with atypical symptoms, a low FeNO level is valuable for excluding asthma. Meanwhile, other diseases that may cause similar symptoms need to be differentiated, such as reactive airway dysfunction syndrome, COPD, bronchiectasis, cystic fibrosis, primary ciliary dyskinesia, bronchial hyperresponsiveness syndrome after long-term viral infection, vocal cord dysfunction, anxiety-hyperventilation, gastroesophageal reflux disease, heart disease and pulmonary hypertension.

As mentioned above, in view of various confounding factors in FeNO test and the overlap between healthy subject and asthma patients, we conclude that: (I) Current evidence is insufficient to recommend FeNO as a routine diagnostic tool in clinical practice. (II) FeNO detection is still of high diagnostic value for individuals with certain clinical characteristics. For early onset and atopic individuals, high level of FeNO strongly suggests the diagnosis of asthma. (III) The diagnostic significance of negative predictive value of FeNO is superior to that of the positive predictive value. The lower FeNO levels can be more robust for excluding the diagnosis of asthma, in particular, an extremely low level of FeNO may suggest the least possibility of asthma. However, clinical manifestations and other laboratory investigations are still needed for a comprehensive evaluation. (IV) A complete diagnosis of asthma includes not only the identification of asthma, but also the evaluation of the severity and control of asthma. In the future, the clinical and inflammatory phenotypes might also be included in asthma diagnosis. Therefore, from a broader perspective, FeNO test will enrich the diagnostic tools of asthma. Especially in identifying the phenotypes of airway inflammation for further prediction of glucocorticoid responsiveness, FeNO test may be advantageous, which will be discussed in details in the text below.

Differentiation of airway inflammation phenotypes and assessment of airway inflammation

The main phenotypes of airway inflammation in asthma are described as eosinophilic, neutrophilic, mixed and paucigranulocytic. Different airway inflammation phenotypes are associated with different clinical features and treatment responses, and ultimately, different clinical outcomes. Identification of these phenotypes and assessment of the airway inflammation may guide clinicians in the selection and adjustment of treatment strategy.

FeNO mainly reflects eosinophilic inflammation of airways (3). Many studies showed that in glucocorticoid-naïve adults or children with mild to moderate asthma, FeNO is positively correlated to EOS counts in the sputum, BALF, lung tissue and peripheral blood (55-62). The levels of FeNO are significantly related to ECP in peripheral blood and BALF (55,63). In nonsmoking asthma patients, the sensitivity and specificity of FeNO (>36 ppb) are 78% and 72% respectively for the identification of EOS >3% in induced sputum (60). In determining the presence or absence of eosinophilic inflammation, the negative predictive value of low levels of FeNO is more significant (64,65). But for asthma patients with a history of smoking or glucocorticoid therapy, FeNO level is less valuable for the determination of eosinophilic airway inflammation (66). ATS guidelines [2011] (3) recommended FeNO as the marker of eosinophilic airway inflammation and classified FeNO levels into low level (<25 ppb in adults, <20 ppb in children), intermediate level (25-50 ppb in adults, 20-35 ppb in children) and high level (>50 ppb in adults, >35 ppb in children) (Table 2). By ATS recommendations, FeNO <25 ppb in adults (<20 ppb in children) can be used to initially exclude eosinophilic inflammation and glucocorticoid therapy is not recommended (strong recommendation, high level of evidence); FeNO >50 ppb in adults (>35 ppb in children) indicates eosinophilic inflammation and glucocorticoid therapy is recommended for symptomatic patients (strong recommendation, moderate quality of evidence); FeNO between 25 and 50 ppb in adults and (20-35 ppb in children) indicates eosinophilic inflammation but it should be interpreted in the clinical context (strong recommendation, low level of evidence).

FeNO levels can be used to identify patients with severe asthma and persistent eosinophilic airway inflammation. Studies suggest that compared with those in mild-moderate asthma group, patients in severe asthma group have higher FeNO levels (medians, 38 and 27 ppb) and sputum EOS

Table 2 FeNO for the differentiation of the airway inflammation types of asthma and prediction of treatment response

FeNO (ppb)	Determination of eosinophilic inflammation type	Prediction of response to ICS treatment
>50 ppb in adults and children ≥ 12 years; >35 ppb in children <12 years	Probable	Likely to benefit from ICS
25-50 ppb in adults and children ≥ 12 years; 20-35 ppb in children <12 years	Possible; comprehensively evaluate in clinical context	Comprehensively evaluate response to ICS in clinical context
<25 ppb in adults and children ≥ 12 years; <20 ppb in children <12 years;	Unlikely	Unlikely to benefit from ICS

FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroid.

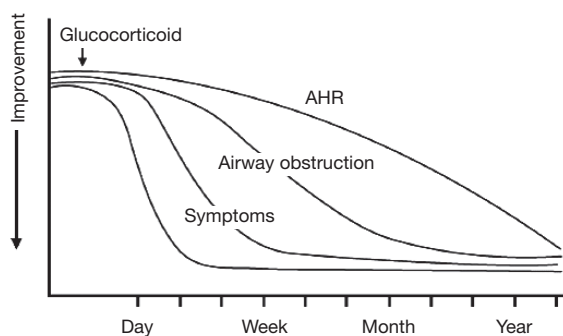


Figure 3 Schematic representation of the response durations of FeNO and other evaluation indicators of asthma to steroid therapy. AHR, airway hyperresponsiveness; FeNO, fractional exhaled nitric oxide.

counts (medians, 11.8% and 0.8%) (67). Even in patients with severe asthma being on ICS or oral steroid therapy, FeNO is still related with the eosinophilic inflammation in bronchial mucosa biopsies. FeNO cut point greater than 72.9 ppb demonstrates a sensitivity of 56% and a specificity of 100% for the identification of persistent eosinophilic airway inflammation (56,68).

Evaluation of the response to inhaled corticosteroid (ICS) therapy

The response to ICS treatment is heterogeneous in patients with asthma. It is critical to select the optimal dose of ICS in individualized treatment to reduce adverse reactions, and meanwhile to maintain asthma control and reduce the risk of exacerbations. Given the correlation between the presence of eosinophilic inflammation and the response to corticosteroid therapy in airways disease (69), the monitoring of FeNO levels helps identify those who might

benefit from corticosteroid therapy, and evaluate individual's response to corticosteroids. The improvement in FeNO levels appears earlier compared with pulmonary function, bronchodilator responsiveness, PEF variability and AHR (70-72) (Figure 3). In steroid-naïve asthmatic adults or children, FeNO levels and anti-inflammatory effects of ICS exhibit a significant dose-response relationship (73), and the decline in FeNO levels was accompanied by the improvement of inflammation, lung function, airway responsiveness and the level of asthma control (74,75). In steroid-naïve asthma patients, FeNO cutoff point of 47 ppb or greater yields a high positive predictive value for ICS responsiveness; and a FeNO cutoff point within the range of 20-30 ppb yields a higher negative predictive value for the responsiveness to ICS therapy (76). Even when patients do not demonstrate EOS in the sputum, FeNO is still highly predictive of steroid response (at a cutoff point of 33 ppb) (77). On the other hand, FeNO monitoring contributes to screening asthma patients with "uncontrolled inflammation" who, however, have received corticosteroid (78). For patients with well-controlled asthma and FeNO level above 25 ppb (ACT score >20), continuous ICS therapy may reduce FeNO level, significantly improve FEF₂₅₋₇₅ and other indicators of small airway function (79). ATS guidelines [2011] (3) suggest that high levels of FeNO usually indicate a significant response to ICS therapy. The criteria for steroid response (see Table 3) is a reduction of >20% in FeNO for values >50 ppb, or >10 ppb for values <50 ppb as the cut point (weak recommendation, low level of evidence). ATS guidelines point out that FeNO can provide important information for clinicians to decide who might benefit from steroid treatment. However, these outcomes may differ depending on the target population and epidemiology of eosinophilic inflammation.

The decline in FeNO levels is related to the duration

Table 3 Evaluation of the efficacy of steroid therapy according to FeNO changes

FeNO baseline	Change amplitude	Steroid response
>50 ppb in adults and children \geq 12 years; >35 ppb in children <12 years	Reduction >20% from baseline	Good response
<50 ppb in adults and children \geq 12 years; <35 ppb in children <12 years	Reduction >10 ppb from baseline	Good response

FeNO, fractional exhaled nitric oxide.

of steroid therapy. A study showed that FeNO gradually decreased after regular inhalation of budesonide for 1 week, and significantly fell after 3–4 weeks (80).

FeNO is also significant to evaluate the response to treatment in patients with severe asthma. In patients with refractory asthma, the sensitivity, specificity, positive predictive value and negative predictive value of FeNO (\geq 30 ppb) to evaluate the response to high-dose ICS or systemic corticosteroid treatment were 87.5%, 90.6%, 87.5 % and 90.6%, respectively (81).

Evaluation of patient compliance to inhaled corticosteroid (ICS) therapy

The patient compliance to ICS treatment is a prerequisite for good control of asthma. However, the overall compliance to ICS treatment is relatively low. The reasons may include: patient concerns about drug-related adverse reactions, under-estimation of the disease severity and inadequate patient-physician communications (4). A study with continuous monitoring of FeNO levels after ICS therapy in asthmatic children and synchronous assessment of their compliance to treatment showed that, unlike pulmonary function test, FeNO levels were significantly correlated with patient compliance (82); they usually increased with in subjects with poor compliance (83). The authors believe raising parents awareness of therapeutic agents and FeNO monitoring help improve medication compliance in children, so as to achieve better asthma control. In a study on the FeNO monitoring in patients with ICS refractory asthma, FeNO levels decreased significantly in those with good adherence. Therefore, under certain conditions, FeNO can also be used as an evaluation indicator for treatment compliance in patients with refractory asthma (84). After ICS treatment, FeNO level changes more rapidly than other inflammatory markers. Therefore, it is more pragmatic to be used for monitoring patient adherence and therapeutic response.

Assessment of asthma control and prediction of acute exacerbation

The levels of asthma control are classified as controlled, partially controlled and uncontrolled. The evaluation indicators consist of daytime/night-time symptoms, limitations of activities, the need for rescue medications, pulmonary function, and acute exacerbations (85). Asthmatic children with lower FeNO levels (<20 ppb) have better pulmonary function and asthma control, when compared with those with higher FeNO levels (\geq 20 ppb) (86). FeNO is correlated to TLC. The increase in FeNO levels is positively correlated with the elevation of TLC, which is related to the acute exacerbations and poor control of asthma (87). High levels of FeNO (>50 ppb for adults, >35 ppb for children) or elevation of FeNO levels by >40% from the levels at remission suggests uncontrolled asthma or aggravation of eosinophilic airway inflammation (88).

In summary, FeNO as a direct indicator of airway inflammation, in combination with clinical indicators, can comprehensively reflect the profile of asthma control. When FeNO is used to assess asthma control, clinical workers should be prompted for exclusion of other factors (such as sinusitis, anxiety, gastro-esophageal reflux, obesity or persistent allergen exposure) which can also affect the levels of FeNO. Anyway, these factors reflect certain limitations with FeNO measurement (3).

In children and adults with allergic asthma, FeNO levels above 300% predicted value may predict a greater need for SABA and increased risk of acute episodes (89) in the following year. The likelihood of acute exacerbation in the future is 85% in clinically stable asthma with FeNO \geq 28 ppb and FEV₁ \leq 76%. However, the likelihood of acute exacerbation in the future is nearly zero in asthma patients with FeNO \leq 28 ppb and FEV₁ \geq 76% predicted (90).

Guiding adjustment of treatment strategies

The management strategy of asthma recommended by

GINA and relevant guidelines are mainly based on the clinical symptoms and pulmonary function instead of airway inflammation as the nature of asthma. Treatment strategy for asthma based on the detection of airway inflammation has been expected over a long time. In several previous studies, ICS dose was adjusted according to EOS count in induced sputum. The results proved this strategy was more effective to control asthma than that simply based on symptoms or pulmonary function (91). The level of FeNO is related to the eosinophilic inflammation of airways. Theoretically, the asthma treatment strategy based on FeNO should be an ideal solution. In recent years, dozens of relevant clinical studies on adults and children with asthma have been reported, wherein the dose of ICS was usually adjusted based on a combination of clinical assessment and FeNO level, stepping up or down when FeNO reached a certain cut point. Most of the studies showed treatment strategy based on FeNO can maintain favorable asthma control with the same or a lower dose of ICS (92), compared to the adjustment of therapeutic strategies simply based on clinical symptoms. In addition, some studies demonstrated the treatment strategy could help improve airway inflammation and reduce airway responsiveness (93). A meta-analysis of clinical trials on whether FeNO-based management can reduce the risk of acute episodes of asthma showed that the rates of acute exacerbation declined by 47% and 30% in adults and children, respectively, without increasing the ICS dose (94,95). In 2011, Powell *et al.* (96) completed a double-blind, randomized controlled study in which 242 pregnant women with asthma were managed based on FeNO. The treatment was up- or down-stepped according to asthma test score (ACQ), or a combination of ACQ and FeNO level (29 bpb as cut point). The results showed the rate of acute exacerbation was 25% in the FeNO group *vs.* 41% in the control group. Moreover, the ICS dosing was lower and neonatal hospital stay was significantly reduced in FeNO group. In view of the special significance of drug safety in pregnant women with asthma, the results have been widely noted and published in *Lancet* (96). However, some studies turned out negative results (97). In a study on childhood asthma, the patients had daily FeNO test at home using a portable FeNO monitor, while the dose of ICS was adjusted with telemonitors. There was no significant difference in improvement of asthma control between this approach and simply symptom-based treatment adjustment (98).

A recent systematic assessment of published randomized controlled clinical trials on FeNO-guided asthma treatment

in the past years showed that the inconsistency among these results are mainly due to different methodologies (99) including disparities in FeNO cutoff values/cut points, patient compliance, LABA and other confounding factors. Therefore, it is necessary to apply a uniform adjustment value and more precise studies should be designed in order to further evaluate the values of asthma treatment strategies guided by traditional methods and biomarkers like FeNO. FeNO measurement is technically mature, easy to operate, and well reproducible; however, these advantages can be outweighed by the increased financial burden of patients if routine FeNO monitoring is proposed, given the current situation in China. In addition, routine therapeutic dose of ICS is safe for most asthma patients. Therefore, the adjustment of asthma treatment should still rely on strategies based on the clinical context, as recommended in GINA guidelines. As for those with potential safety concerns about ICS, such as childhood asthma, asthma in pregnancy and refractory asthma that requires large doses of glucocorticoids, FeNO-based treatment strategies contribute to more accurate adjustment and least adverse reactions of glucocorticoid load and other drugs, under the premise of maintaining asthma control. Future research should focus more specifically on the above asthma populations. For instance, in a recent study, 102 patients with suboptimal asthma control underwent stepwise incremental dosing of ICS therapy within one month, based on FeNO levels; for those whose asthma remained uncontrolled, oral corticosteroid therapy for another month was given. Finally, asthma control was achieved in 53 patients (52%) (81).

In addition, dynamic FeNO monitoring helps to decide on the time for tapering or withdrawal of ICS. A study on childhood asthma showed that high FeNO levels (>47 ppb) may predict failure of asthma control after tapering or withdrawal of ICS, with the sensitivity and specificity being 71% and 93%, respectively (100). Meanwhile, low FeNO levels (<22 ppb) can predict the successful tapering or withdrawal of ICS in 92.5% of the times (101).

Since the advent of FeNO testing, unique advantages of this technique have been shown in the diagnosis and differential diagnosis of asthma, determination of airway inflammation types, prediction and assessment of treatment response, assessment of asthma control and guidance of treatment adjustment. Along with the growing experience in clinical practice and accumulation in understandings, we look forward to a greater role of FeNO technology in the management of asthma. The use and significance of FeNO testing in asthma management are summarized in *Table 4*.

Table 4 The significance of FeNO test for asthma management during steroid therapy

FeNO levels	Controlled inflammation (<25 ppb in adults; <20 ppb in children)	Uncontrolled inflammation (25-50 ppb in adults; 20-35 ppb in children)	Uncontrolled inflammation (>50 ppb in adults; >35 ppb in children)
Symptoms present	<ul style="list-style-type: none"> Poor response to ICS Alternative/combined diagnosis 	<ul style="list-style-type: none"> Poor adherence or inhaler technique Inadequate ICS dose Untreated small airway disease Persistent allergen exposure Rare: steroid resistance 	<ul style="list-style-type: none"> Poor adherence or inhaler technique Inadequate ICS dose Untreated small airway disease Persistent allergen exposure Rare: steroid resistance
Symptoms absent	<ul style="list-style-type: none"> Adequate ICS dosing Good adherence ICS taper 	<ul style="list-style-type: none"> Potential uncontrolled airway inflammation Monitor change in FeNO level and symptoms Do not reduce steroid dosing, unless the patient is asymptomatic with a constant FeNO level Controlled inflammation 	<ul style="list-style-type: none"> Poor adherence or inhaler technique Uncontrolled airway inflammation, potential risk for symptoms and acute exacerbation ICS withdrawal or dose reduction may result in relapse, and FeNO needs to be evaluated Persistent allergen exposure

FeNO, fractional exhaled nitric oxide; ICS, inhaled corticosteroid.

Future directions and prospects of research

Over the past decades, based on in-depth understanding of asthma as a special chronic airway inflammatory disorder, non-invasive assessment of airway inflammation has been widely recognized and used by respiratory physicians in China. A great deal of achievements has been obtained, and experience accumulated. However, the strength of research varies. There is still a lack of convincing data from large-sample clinical trials. Opinions about the non-invasive laboratory techniques for detection of airway inflammation, interpretations and clinical evaluation of test results remain disputed. Chinese experts in the relevant fields developed the present consensus on the commonly concerned issues in clinical practice, based on currently available evidence and their decades of clinical expertise. The Committee acknowledges that this consensus is just an interim statement based on the current body of evidence. The purpose of publishing this document is to call for attention of Chinese respiratory physicians to the detection of airway inflammation, to inspire clinical research in this field in China and promote the use of this technique in clinical practice.

BPT is an important tool for the diagnosis of asthma. AHR revealed by BPT is closely related to airway inflammation. However, airway inflammation is not the only mechanism underlying AHR, and thus the both are not to be mentioned exchangeably. BPT does not directly reflect the airway

inflammation. Further studies are warranted to elucidate on the diagnostic value of BPT and its relationship with airway inflammation under special conditions, for example, the probable existence of BPT-negative airway inflammation. For airway responsiveness, large-sample clinical trials with longer follow-up duration are in dire need to determine its role in the pathogenesis and development of asthma, the regularities and significance of dynamic variation after treatment, the predictive value for the prognosis and risk of asthma.

Induced sputum cytology is a proven, simple method not only useful for counting of inflammatory cells, but also for detecting various components in the supernatant. It is of high value in the differentiation of airway inflammation types and the prediction of the ICS treatment response. Although induced sputum cytology has been currently conducted in many hospitals in China, a SOP for specimen collection, operation and smear examination has not yet been established in most laboratories, which greatly hampers the inter-laboratory recognition of test results in this country. Therefore, we appeal for establishment of the national SOP for this technique, training centers and quality control system so that induced sputum would become a routine examination in clinical practice in the near future.

Nearly 20 years after its first introduction, FeNO testing has hallmarked a major improvement in the field of asthma

research. Recently, a surge of data further broadens the application of FeNO and heralds new challenges. The results of a multi-center study on the predictive value of FeNO in normal Chinese population have been released. However, the optimal cut points for the diagnosis and treatment adjustment in asthma patients are still in expectation. To this end, large-sample clinical trials need to be carried out to obtain more reliable data in different geographic regions, ethnic and age groups. Meanwhile, professional training of clinicians should be reinforced to enable them properly interpret the results of FeNO test based on clinical findings. We also expect miniaturized, budget-friendly detectors to be developed in the future to make possible the use of FeNO test in daily management of asthma. The stability, repeatability of non-velocity-dependent FeNO measurement technique, and whether it reflects the NO concentrations in segment air stream, are still need to be verified by rigorous clinical trials.

The technique to detect inflammatory cells and mediators in peripheral blood and body fluid is mature, and allows easy collection of specimens; however, the drawback is that the indicators can hardly reflect the real condition of airway inflammation timely. The findings of detection are less correlated to what is happening in the lungs. Therefore, it would be inappropriate to overestimate their roles in the evaluation of airway inflammation. Meanwhile, exploratory efforts for novel indicators with higher specificity and sensitivity seem indispensable.

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Footnote

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