Allied Health Professional Symposium: Session 1: How to survive in allergy laboratory

New asthma test (FeNO, Bronchial provocation test)

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Introduction

Asthma is one of the most common chronic airway diseases characterized by symptoms such as dyspnea, wheezing, chest tightness, and coughing which are associated with reversible airflow limitation. Asthma is also associated with airway hyperresponsiveness to direct or indirect stimuli and chronic airway inflammation. These features persist even when symptoms are absent or pulmonary function is normal. These pathophysiologic features of asthma can be utilized in diagnostic tests for asthma such as FeNO and bronchial provocation tests.

1. FeNO

Fractional exhaled nitric oxide (FeNO) is a useful indicator of type 2 or allergic inflammation in the airways. Nitric oxide is one of the endogenous regulators, which not only plays a role in normal physiological processes such as vasodilation and bronchodilation but also plays an important role in acute and chronic inflammation by increasing production during inflammation. Nitric oxide is present in all organs of all mammals and is produced in lung. Asthma patients have an increased NO and an increased expression of inducible NO synthase (NOS 2) in bronchial epithelial cells. Nitric oxide was associated with airway eosinophilia which was confirmed by bronchial biopsy and induced sputum analysis. The association between eosinophil inflammation and steroid responsiveness is also well known.

1) Test procedure

The most important advantage of FeNO that the measurement is easy and simple. It is also non-invasive, which makes it easy to repeat measurements and can be used in patients with severe airflow limitation. However, since measurement values can be influenced by test methods and various factors, the procedure

should be standardized.

2) Equipment

The method of measuring nitric oxides can be classified into chemiluminescent detection method and electrochemical detection method. The chemiluminescence method is sensitive and accurate, but the machine is heavy (20-50 kg), large and inconvenient to use. On the other hand, the electrochemical method measuring instrument is slightly less sensitive than the chemiluminescence method, but is widely used because it is light and easy to use. Electrochemical equipments include NIOX MINO®, NIOX VERO® (Aerocrine, Solna, Sweden) and NO breath (Bedfont Scientific Ltd, UK). Although there are relatively small deviation in the result of FeNO according to the measuring device, it is not recommended to compare the results from other device for monitoring in the same patient.

3) Precautions before test

Several factors may influence the nitric oxide measurement. Therefore, educate the patient in advance to control the factors that may affect the test, and postpone the test schedule if it is unavoidable.

① If lung function test or bronchial provocation test is performed on the same day, first perform oximeter nitrogen measurement test.
② On the test day, steroids, leukotriene antagonists, bronchodilators, etc. should be discontinued.
③ Test should be performed after the airway infection has recovered.
④ It is recommended to avoid meals, water or coffee 1 hour before the test. In particular, avoid eating foods containing nitrates such as spinach, carrots, cabbage, lettuce, and celery.
⑤ Stop smoking on the day of test.
⑥ Avoid vigorous exercise just before the test.

4) Measurement

In an upright position, first breathe as much as possible and then inhale air through the mouthpiece to total lung capacity. The difference between the FeNO test and the pulmonary function test is that the breath flow rate should be kept in 50 mL/sec constantly when testing FeNO. It is important to breathe out at a constant rate for 10 seconds, according to the machine's guidance signal. If the expiratory flow rate is not constant, the measurement results are inconsistent. Patients may not be able to maintain exhalation at a rate of 50 mL/sec for more than 10 seconds. In this case, keep the flow rate of 50 mL/sec for more than 6 seconds for 12 years old and more and for more than 4 seconds for under 12 years old. Exhaling at a rate of 50 mL/sec for 6-seconds is the time for adults to secure exhalation of more than 0.3 L, which is a criterion for sufficient sampling of the airway condition. If the patient cannot maintain a certain expiratory flow rate for more than 10 seconds, describe these in the results.

5) Interpretation

FeNO is used to assess whether the patient's respiratory symptoms are related to allergic inflammation (or eosinophilic inflammation) in the airways, or to diagnose allergic diseases such as asthma. It is also used as an index for evaluating symptoms and inflammatory characteristics and the degree of inflammation in patients who have already been diagnosed with asthma. Nitric oxide (NO) is a substance that is also produced by normal people and is affected by various factors such as race, age, sex, smoking, physical condition, and disease. Therefore, it is now more appropriate to use the cut-off value for specific purposes (or for predicting the prognosis in a particular disease group) to diagnose the disease in a group of patients with particular symptom rather than using generalized reference values.

FeNO is more appropriate for rule in, rather than rule out, asthma diagnosis in patients complaining of dyspnea, wheezing or cough. In adult patients with respiratory symptoms, the possibility of asthma is considerably higher when FeNO value is larger than 50 ppb (for children larger than 35 ppb). For adults, 25-50 ppb (20-35 ppb for children and adolescents) should be carefully interpreted considering the test conditions and clinical situation. In 2011, the ATS / ERS standard guidelines recommend that this test be used to supplement diagnosis rather than to confirm asthma. FeNO can be used not only for diagnosis of asthma but also for evaluation of allergic inflammation in asthmatic patients. If respiratory symptoms are not well controlled in patients with asthma under anti-inflammatory therapy, FeNO may help evaluate whether the uncontrolled symptoms is due to the uncontrolled allergic inflammation of the airway.

2. Methacholine bronchial provocation test

The bronchial provocation test consist of specific and nonspecific tests. The former uses specific allergens or occupational substances for the testing and the latter uses nonspecific stimulation such as drugs like methacholine, histamine, mannitol, or exercise or hyperventilation. In addition to evaluating the reversibility of airflow limitation through bronchodilator response, it is important for asthma diagnosis to measure the degree of airway hyperresponsiveness. The types of nonspecific bronchial provocation test can be classified into direct and indirect methods depending on the mechanism by which bronchoconstriction is induced. Direct bronchial provocation test uses substances which induce airway constriction by directly stimulating the airway smooth muscle (methacholine). A direct methods is sensitive but the specificity is low because it can be positive in some conditions other than asthma like other lung disease or rhinitis and even in some normal people. Methacholine is a derivative of acetylcholine and is most widely used for bronchial provocation challenge. Indirect bronchial provocation test is a method using mannitol, exercise, hyperventilation, adenosine, hypertonic saline, etc. It induces airway constriction by inducing the changes in osmotic pressure in the airway, which leads to the release of mediators from inflammatory cells. The sensitivity of indirect test is low compared to direct method, but specificity is high, so it is useful for the

diagnosis of asthma and is also used for diagnosis of exercise-induced bronchoconstriction. Here we only review the most widely used methacholine bronchial provocation test. Methacholine is a parasympathetic stimulant that stimulates muscarinic receptors in the bronchial smooth muscle to increase bronchoconstriction. The methacholine bronchial challenge test assess the airway hyperresponsiveness, an important feature of asthma.

1) Indication and contraindication

(1) Indication

1. Diagnose the asthma if a history of asthma is suspected but the diagnosis is not made with other methods
2. Exclude the diagnosis of asthma if the possibility of asthma on the basis of medical history is low
3. Assess the severity of asthma in patients with a history of occupational asthma
4. Assess the response to the treatment in asthmatic patients

(2) Contraindication

Past medical history should be evaluated to decide whether or not the methacholine bronchial provocation test should be conducted. In the case of absolute contraindication, instead of evaluating bronchial hyperresponsiveness through methacholine bronchial provocation test, it is recommended to perform bronchodilator response test. In addition, if the patient is a relative contraindication to the methacholine bronchial provocation test, the physician must perform a thorough evaluation of the benefits and risks of the test.

(3) Absolute contraindication

1) severe airway obstruction (FEV1 is 1.0 L or less than 50% of the predicted value)
2) If myocardial infarction or stroke occurred during the past 3 months
3) When blood pressure is not properly controlled in patients with hypertension (systolic blood pressure> 200 mmHg or diastolic blood pressure <100 mmHg)
4) If the patient have an aortic aneurysm

(4) Relative contraindication

1) severe airway obstruction (FEV1 less than 60% of 1.5 L or predicted)
2) If the patient cannot perform the test properly
3) Pregnant or lactating women
4) Patient with myasthenia gravis or taking cholinesterase inhibitor

2) Precautions before the test

Attention should be paid as the following drugs or foods may affect airway hyperresponsiveness (table 1). In some cases, so it is needed to stop the drug for a certain period of time.

Table 1. Drugs and foods that affect airway hyperresponsiveness

Factors		Minimum time to hold
Drug		
Short-acting inhaled bronchodilators	salbutamol, albuterol, terbutaline	8h
Medium-acting bronchodilators	ipratropium	24h
Long-acting inhaled bronchodilators	salmeterol, formoterol, tiotropium	48h
Oral bronchodilators	liquid theophylline	12h
	Intermediate-acting theophylline	24h
	Long-acting theophylline	48h
Leukotriene modifiers	montelukast, pranlukast, zarfirlukast	24h
Antihistamine	Hydroxyzine	3d
	Cetirizine	3d
Food		
Coffee, tea, cola drinks, chocolate		Day of study

3) Procedure

(1) Preparation before test

Make sure that there is no contraindication before the test, and check again whether the patient is using any medication that may affect the test. It is advisable to explain to the patient in advance that the procedure may be accompanied by mild coughing and shortness of breath. However, be cautious so that the explanations of the test process do not bias the results. Inhalation of methacholine may cause airway constriction. Therefore, both examinees and examiners should consider this. In case of severe airway constriction, epinephrine injection and short-acting bronchodilator inhaler should be prepared. It should be possible. However, methacholine bronchial provocation testing has been performed safely for a long period of time globally, and delayed or prolonged effects of methacholine rarely occurred.

(2) Facilities

Examiner should have facilities to minimize exposure to methacholine. There are methods to minimize exposure to methacholine such as using a breathing filter, an experimental gas hood, and a high efficiency

particulate filter (HEPA) air purifier. It is recommended that the subject be as far away from the examiner as possible during the breathing of the methacholine by the breathing machine, and the method using the dosimeter is more helpful in reducing exposure of the methacholine. Examiner with asthma may be at greater risk of developing bronchoconstriction. It is also a good idea to perform a methacholine bronchial provocation test to the examiners to see if they are sensitive to methacholine.

(3) Storage and preparation of methacoline

Available in dry crystalline powder form, methacholine is the most widely used drug for nonspecific induction testing. FDA-approved methacholine (Provocholine) is sealed in vials, packaged individually, and does not need to be stored dry or frozen. If packaged or delivered in bulk, it should be dried and frozen. Sterilized saline is used as a diluent and it may contain 0.4% phenol. Buffer solutions are not stable and should not be used as a diluent.

The methacholine solution should be mixed with a pharmacist or a well-trained person using sterilized methods. After mixing, they are kept refrigerated at 4 ° C. Under these conditions, it is known that stability can be maintained for up to 3 months at a concentration of 0.125 mg/ml or more. For concentrations as low as 0.025 mg/ml, they should be mixed or diluted on the day of the test. The methacholine solution should be warmed in room temperature before the test.

(4) Test protocol

There are two methods of testing: the 2-min tidal breathing method and the five-breath dosimeter method. The 2-min tidal breathing method is a method in which nebulized methacholine solution through a compressor and a nebulizer is inhaled by the patient by the tidal breathing for 2 minutes, followed by measuring FEV1 values at 30 and 90 seconds after inhalation. It is recommended to use a rotameter to maintain the compressed air pressure of the atomizer at 50 lb during the test and to maintain the flow rate at 0.13 mL/min ± 10%. The five breath dosimeter method measures FEV1 values after 30 and 90 seconds after total 5 breaths of methacholine spray delivered via a dosimeter. Patient is instructed to breathe in slowly and deeply for 5 seconds to match the signal or sound indicated by the test equipment, and then allow the patient to hold his breath at total lung capacity for approximately 5 seconds. If FEV1 is not decreased by more than 20% over baseline FEV1 value after methacholine inhalation, measure FEV1 while gradually increasing the methacholine concentration to 16 to 25 mg/dL. If the FEV1 is decreased by more than 20%, stop methacholine inhalation. Both methods are generally available in concentrations of 1, 4, 8, and 16 mg/ml, but may be performed at lower concentrations when baseline pulmonary function is low or patient is highly sensitive.

(5) Interpretation

Changes in the FEV1 value are the main measurements in the methacholine bronchial provocation test. The concentration of methacholine at the time point when FEV1 declines to 20% of the baseline value is called PC20 (provocation concentration causing a 20% fall in FEV1) and is used as an index of bronchial

hyperresponsiveness. When the PC20 value is 16 mg/mL or less, it is interpreted to be positive.

When interpreting PC20 values in individual patients, the following factors should be considered.

① Presence of pre-test probability

② Basal pulmonary function and bronchoconstriction and degree

③ whether the patient has performed lung function tests as instructed

④ Whether drugs, food, or environmental factors that may affect lung function and airway hyperresponsiveness are controlled

⑤ How much of the lung function is restored when the bronchodilator is administered after the methacholine challenge test

⑥ Sensitivity, specificity and reproducibility of methacholine bronchodilation test,

Methacholine bronchial provocation test is known to have high diagnostic sensitivity but relatively low specificity. In other words, if the test result is negative, the possibility of diagnosis of asthma is low, but if it is positive the patient may not be asthmatic. However, in a Korean study comparing the diagnostic ability of methacholine and mannitol bronchial challenge, the sensitivity of the methacholine challenge test was similar to or lower than that of the mannitol challenge test. There are several possible causes of false negative in asthmatic patients with methacholine bronchial provocation test. The storage and dilution of methacholine, the volume of nebulized spray, and the size of the sprayed particles may affect the bronchial delivery and activity of methacholine. One of the important factors to consider is the sensitivity difference according to the test methods. It is known that there is no significant difference between the two methods in the presence of severe bronchial hyperresponsiveness. However, in case of mild asthma with mild bronchial hyperresponsiveness or normal pulmonary function, it is known that the five breath dosimeter method is less sensitive than the 2 minute tidal breathing method. Therefore, in case of mild asthma with mild bronchial hypersensitivity or normal pulmonary function, considering the possibility of false negativity in five breath dosimeter method and possibility of false positivity in 2-minute tidal breathing method. It is necessary to interpret the results considering the inspection conditions and methods.

Conclusion

As with other diseases, the most important factor in the diagnosis of asthma is medical history and physical examination, but it is also essential for the confirmative diagnosis to show the objective evidence of the asthma. Although various test methods have been developed, there is still room for improvement in accuracy and simplicity. In addition, efforts to standardize these tests in Korean should be continued.

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