

# Diagnostic approaches in asthma

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**Key words**

- Asthma
- Spirometry
- Provocation test
- Impulse oscillometry
- Markers of inflammation

**SUMMARY.** Asthma is a common chronic respiratory disease characterized by paroxysmal or persistent respiratory symptoms associated with variable airflow limitation and airway hyperresponsiveness. The early diagnosis and treatment of asthma is important for improving the health of the patient and minimizing the social and economic burden of the disease. No single symptom or diagnostic test defines asthma; it is a heterogeneous disease with a variety of symptoms, including wheezing, cough, shortness of breath, and chest tightness. International guidelines specify that asthma diagnosis should be based on both symptoms and objective evidence of variable airflow obstruction and/or airway hyperresponsiveness. The main diagnostic features are an obstructive pattern on spirometry, a positive bronchodilation test and evidence of reversibility or variability in peak expiratory flow (PEF) or spirometric results after treatment. Direct and indirect methods of revealing bronchial hyperresponsiveness (BHR) and markers of inflammation, such as differential eosinophil count in induced sputum, exhaled nitric oxide (NO) and pH in exhaled breath condensate, are also considered key points in asthma diagnosis. Recently, small molecules generated from cellular metabolic activity, known as metabolomics, have been investigated as a potential diagnostic tool. The diverse features and phenotypes of asthma add complexity to the diagnosis, which should be made with caution using a reliable approach, in order to reduce the possibility of over- and under-diagnosis. *Pneumon* 2014, 27(1):74-80.

## INTRODUCTION

Although asthma is a worldwide problem, with an estimated 300 million affected individuals, there is not a gold standard definition of the disease. According to the recent Global Initiative for Asthma (GINA) report, asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable airflow obstruction within the lung that is often reversible either

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spontaneously or with treatment<sup>1</sup>. The cornerstones of the current definition of asthma are inflammation, hyperresponsiveness, reversible airway obstruction, and respiratory symptoms<sup>2-4</sup>.

## MEDICAL HISTORY AND CLINICAL EXAMINATION

It is apparent that the central feature of the definition of asthma is the combination of symptoms (mainly wheezing, dyspnoea and cough) along with evidence of variable airflow obstruction. There is poor correlation between symptoms and measurement of function, which means that they represent different mechanisms<sup>5</sup>. The triad of wheezing, cough and shortness of breath constitutes the major clinical feature of asthma, and one or more of these symptoms are reported by more than 90% of patients, but even this symptom complex is nonspecific<sup>6</sup>. In many cases a careful clinical history will allow a reasonably certain diagnosis of asthma, or an alternative diagnosis, to be made. Symptoms after allergen exposure or viral upper respiratory infections, seasonal variability of symptoms and a positive family history of asthma and/or atopy are also helpful diagnostic indications. In the European Community Respiratory Health Survey II, for example, an appropriate questionnaire was used as screening test for asthma in a large number of subjects in the general population of European countries<sup>7</sup>. Recently, a simple pre-interview screening questionnaire, the Asthma Screening Questionnaire (ASQ), consisting of 6 questions, has been developed to help physicians to diagnose asthma (Table 1). A cutoff point in the ASQ total score of  $\geq 4$  was associated with the highest combination of sensitivity (96%) and specificity (100%)<sup>8</sup>. The physical examination is useful when polyphonic expiratory wheezing is detected, as this is considered the most characteristic finding in asthma, reflecting airflow turbulence due to airflow limitation, although in many cases wheezing may be absent or only detected when the subject performs forced exhalation.

## MEASUREMENT OF LUNG FUNCTION

Measurement of lung function estimates the severity of airflow limitation and the presence of reversibility and variability, and therefore provides confirmation of the diagnosis of asthma. Spirometry is the recommended method of measuring airflow limitation and reversibility. A useful assessment of airflow limitation is the ratio of FEV1 to FVC, and values  $< 0.70$  are indicative of asthma. The

**TABLE 1.** Scoring system of the Asthma Screening Questionnaire

Questionnaire	Score
1. Do you cough more than the average person?	2
2. Do you have a cough that comes mainly from your chest and not from your throat?	2
3. Do you have worsening of the following symptoms when you lie down to sleep?	
Cough	1
Chest tightness	1
Wheeze	1
Shortness of breath	1
4. Do you have worsening of the following symptoms after exercise or physical activity?	
Cough	1
Chest tightness	1
Wheeze	1
Shortness of breath	1
5. Do you have worsening of the following symptoms after laughing or crying?	
Cough	1
Chest tightness	1
Wheeze	1
Shortness of breath	1
6. Do you have worsening of the following symptoms after talking on the phone?	
Cough	1
Chest tightness	1
Wheeze	1
Shortness of breath	1

FEV1/FVC ratio is normally greater than 0.75 to 0.80, but the predicted values are less reliable in young adults and in the elderly. The degree of reversibility in FEV1 which indicates a diagnosis of asthma is generally accepted as 12% and 200ml from the pre-bronchodilator value 10 minutes after the inhalation of 400mcg of salbutamol<sup>9,10</sup>. This test has reduced sensitivity as some patients with asthma do not exhibit a positive bronchodilation test (e.g., patients with mild asthma or asthma under treatment) and may require retesting several times over time for the changes to become apparent. Reversibility of FEV1 can also be assessed after a trial of inhaled corticosteroids (equivalent of beclomethasone 200mcg twice daily for 6-8 weeks) or oral prednisolone (30mg once daily for 14 days)<sup>9,10</sup>. An alternative but less reliable method is the measurement peak expiratory flow (PEF). PEF is effort dependent and

as it is determined largely by the diameter of the central airways it underestimates the FEV1. Improvement of pre-bronchodilator PEF by  $>60\text{L}/\text{min}$  or  $>20\%$  after inhalation of a bronchodilator, or diurnal variation in PEF of more than 20% (i.e., the difference between the morning and afternoon values, before treatment intake, divided by the mean of the two) suggests a diagnosis of asthma. Another easy method for assessment of the variability of PEF is the minimum morning pre-bronchodilator PEF over one week, expressed as percent of the best (Min% Max).

An additional benefit in asthma diagnosis can be provided by the impulse oscillometry technique, for which the least cooperation of the patient is required. Impulse oscillometry measures the total respiratory resistance by connecting the mouth of the patient with a pneumotachograph and a source of air waves of very low volume, just like a loudspeaker. With this method, pressure and airflow in the mouth are simultaneously recorded while the subject is breathing normally. The oscillations of air flow artificially generated by the loudspeaker at frequencies from 2 to 30 Hz, are superimposed on the natural flow of the tidal volume. The resulting pressure and flow rates are subjected to Fourier analysis to determine the mechanical behaviour of the respiratory system<sup>11</sup>. A 20-25% decrease in total respiratory resistance (i.e., the respiratory resistance at 5Hz, R5) is considered equivalent to a 12% increase in FEV1 after bronchodilation, while a 35% increase in R5 is considered equivalent to a 20% decrease in FEV1 during the methacholine bronchial challenge test<sup>12-14</sup>. The ease of conducting impulse oscillometry makes it a good alternative method for investigating reversibility or bronchial hyperresponsiveness (BHR) in adults and even in children, when there is failure of good cooperation for spirometry. Its relatively high cost and the fact that it is available in only a few centres are the main disadvantages.

### MEASUREMENT OF BRONCHIAL HYPERRESPONSIVENESS (BHR)

BHR accounts for the abnormal response of the airway to various agonists, resulting in bronchoconstriction. It is assessed by delivering progressively increasing doses of a provocative stimulus until a chosen index of airway calibre changes by a fixed amount. BHR supports the diagnosis of asthma, especially in cases where asthma is a serious possibility but spirometry performed before and after administration of a bronchodilator has not established the diagnosis. BHR, however, may vary over time, often

increasing during exacerbations and decreasing during treatment with antiinflammatory medications. Direct airway challenges cause airflow limitation predominantly via a direct effect on airway smooth muscle. Indirect airway challenges induce airflow limitation by an action on cells other than the smooth muscle cells. On stimulation, such cells release mediators that provoke smooth muscle contraction. Methacholine and histamine are the drugs most often used as stimuli for direct challenges, delivered via nebulizer or dosimeter in doubling concentrations. The results are expressed as the provocative concentration (PC), or the dose (PD) causing a 20% fall in FEV1 (PC<sub>20</sub> and PD<sub>20</sub> respectively)<sup>15</sup>. PC<sub>20</sub>  $\leq 8\text{mg}/\text{ml}$  or PD<sub>20</sub>  $\leq 800\mu\text{g}$  are characteristic of asthma, with increased sensitivity but limited specificity. This means that a negative test can be useful to exclude a diagnosis of asthma, especially in steroid naive subjects, while a positive test does not always mean that the patient has asthma, as BHR has been described in patients with diseases other than asthma, such as allergic rhinitis, chronic obstructive pulmonary disease (COPD), bronchiectasis and cystic fibrosis (CF)<sup>14</sup>.

Indirect challenges are performed using physical stimuli (e.g., exercise, hypertonic distilled water, mannitol, eucapnic voluntary hyperpnoea of dry air) or pharmacological stimuli (e.g., adenosine, aspirin, allergen)<sup>16</sup>. Exercise causes airway narrowing by the loss of water via evaporation from the airway surface stimulating the release of mediators<sup>17</sup>. In the exercise protocol a treadmill or ergometric bicycle is used until the heart rate reaches 80-90% of the maximum predicted value. The highest FEV1 is measured before and after 5, 10, 15 and 30 minutes of exercise. A fall in FEV1  $\geq 10\%$  is considered positive and indicative of asthma<sup>18</sup>. Nebulised hypertonic saline causes bronchospasm in susceptible individuals by increasing the osmolality of the surface of the airways. A solution of 4.5% is inhaled initially for 30 sec followed by spirometry after 60 sec. This process is repeated with progressively increasing exposure times until a fall in FEV1 of  $\geq 15\%$  is effected. Inhalation of mannitol powder acts in the same way as hypertonic saline. The initial dose is 5mg and this is gradually doubled to reach 160mg. One minute after each inhalation spirometry is performed and the test is considered positive when a 15% fall in FEV is achieved (or a 10% fall between consecutive doses) or a cumulative dose of 635mg has been administered<sup>19</sup>. In eucapnic voluntary hyperpnoea the patient breathes frigid air for 4 minutes at specified minute ventilation calculated as prechallenge FEV1 $\times 25$ . In order to maintain eucapnia, the inflow of carbon dioxide is calculated. Spirometry

is performed before the challenge and at 3, 5 and 10 minutes after the end of the challenge. The response is calculated as prechallenge FEV1 minus the lowest value of FEV1 measured after the challenge, divided by the prechallenge FEV1, and expressed as a percentage. A cut-off value for a positive response is defined as a 9% fall in FEV1<sup>20</sup>. Adenosine is administered in gradually doubling doses ranging from 0.09 to 800mg/ml and the test is considered positive with a 20% fall in FEV1. The current reference standard method for diagnosing occupational asthma is a specific inhalation challenge with the suspected agent<sup>16</sup>.

## MARKERS OF AIRWAY INFLAMMATION

Eosinophilic airway inflammation is a major feature of asthma. This can be assessed non-invasively using the induced sputum differential eosinophil count or the fractional exhaled nitric oxide (NO) concentration (FENO)<sup>21,22</sup>. The induced sputum eosinophil percentage has been shown to be a good aid in the diagnosis of mild to moderate asthma with normal baseline lung function<sup>23</sup>. Under these circumstances the sputum eosinophil count with a cut-off of 1% clearly performed better than the variation in peak flow, the bronchodilation test or the blood eosinophil count, but slightly less well than the methacholine challenge. In another study using the same type of patients, sputum eosinophils with a cut-off of 3% performed equally as well as FENO, but much better than peak flow variation<sup>24</sup>. According to BTS a raised sputum eosinophil count of >2% is seen in 70-80% of patients with untreated asthma, and although this is not specific to asthma it is considered suggestive<sup>4</sup>. The most recent GINA guidelines do not suggest the routine use of induced sputum in the diagnostic approach to bronchial asthma, as sputum eosinophilia has not been evaluated prospectively as an aid in asthma diagnosis<sup>1</sup>. Patients with asthma were found to have high FENO levels in their exhaled breath<sup>25,26</sup> and this quickly prompted the evaluation of FENO as a potential noninvasive method for diagnosing asthma and monitoring the response to antiinflammatory therapy. The predictive value of FENO is higher than that of conventional measurements such as peak flow and spirometry<sup>24</sup>, and similar to that associated with bronchial challenge tests<sup>27</sup>. FENO has been investigated as screening tool for asthma in young adults in whom it was found that values of >19ppb had 85.2% specificity and 52.4% sensitivity for the diagnosis of asthma, with better diagnostic performance in non smokers, as values of

>25ppb had specificity >90% for the diagnosis of asthma in both in smokers and non smokers<sup>28</sup>.

In general, in patients presenting with asthma-like symptoms, an increased FENO of >25ppb provides supportive rather than conclusive evidence for asthma diagnosis<sup>29</sup>. In one study, FENO >34 ppb had high predictive value of PC<sub>20</sub> <16mg/ml in patients with suspected asthma in whom the bronchodilation test failed to demonstrate reversibility or was not indicated<sup>30</sup>. It has recently been reported that FENO >32ppb was associated with a sensitivity of 0.47 and a specificity of 0.85 for the identification of the PD<sub>20</sub> <800mcg. In smokers, FENO >11ppb was associated with a sensitivity of 0.85 and a specificity of 0.5 for the identification of PD<sub>20</sub> <800mcg, while in atopic subjects FENO >26ppb was associated with a sensitivity of 0.55 and a specificity of 0.85<sup>31</sup>. Currently, however, international guidelines do not recommend FENO or sputum eosinophils as diagnostic methods for asthma but rather as tools for the follow up of patients with diagnosed asthma<sup>1,29</sup>.

Exhaled breath condensate (EBC) pH has been found decreased in patients with asthma<sup>32</sup>. Recently, a decrease in EBC pH of greater than 0.4 units during the period at work compared to the off-work period was shown to achieve the most satisfactory sensitivity and specificity for diagnosing occupational asthma<sup>33</sup>.

## METABOLOMICS

Metabolomics is the study of small molecules (< 1kDa) generated from cellular metabolic activity<sup>34</sup> and currently there is no corresponding Greek term. Basically this is a kind of cellular "signature" that characterizes each individual and it can be investigated with noninvasive methods in a variety of biological samples. New techniques with increased sensitivity and improved statistical analysis are currently available for the study of multiple biomarkers in each sample. For respiratory diseases, mass spectrometry, nuclear magnetic resonance and the electronic nose have been used. Metabolomics-based biomarkers have recently been investigated for potential use as diagnostic tools in asthma<sup>35</sup>. Mass spectrometry, based on the electrical charge to mass ratio, is the most sensitive of the metabolomic approaches. Using mass spectrometry on urine samples Mattarucchi and colleagues<sup>36</sup> described a small cohort of children, differentiating between those with and without asthma. eNose-based models for asthma vs no asthma have been created<sup>37</sup>. Nuclear magnetic resonance analysis has been applied on the metabolome of

exhaled breath condensate in children with and without asthma<sup>38</sup>. Finally, the application of nuclear magnetic resonance spectrometry in urine detecting 70 metabolites in children contributed to the diagnosis of asthma with 94% accuracy<sup>39</sup>, while in exhaled breath condensate this method can successfully differentiate subjects with asthma from healthy individuals<sup>40</sup>.

## CONCLUSIONS

Asthma is a heterogeneous syndrome with many clinical classifications based on symptoms, lung function and response to therapy. The diagnosis of asthma is more difficult than that of most other chronic illnesses. The

gold standard remains the combination of the history of compatible symptoms with evidence of reversibility or variability in airflow limitation. In several cases of suspected asthma without reversibility of airflow limitation further investigation is needed, using direct or indirect bronchial challenges and/or estimation of airway inflammation (Table 2). Despite the improvements in knowledge about asthma and the development of new non-invasive methods for assessing the underlying inflammation, the definitive diagnosis of asthma remains a challenge. Careful evaluation and objective measurements of lung function and/or airway inflammation are needed, as early diagnosis and treatment of asthma is important for improving the health of the patient and minimizing the social and economic burden of the disease.

**TABLE 2.** Summary of the methods used in the diagnosis of asthma: advantages and disadvantages

Method	Advantages	Disadvantages
Physical examination	Easy in clinical practice	Normal in many cases
Questionnaires	Useful to non specialists	Further investigation with objective methods is required
Spirometry	Reveals obstructive pattern	Normal spirometry does not exclude the diagnosis of asthma
Bronchodilation test	Helpful in diagnosis when spirometry is normal	Negative bronchodilation test does not exclude the diagnosis of asthma
Peak flow measurement	Performed by the patient him-/herself	- Less reliable than spirometry - May underestimate the degree of obstruction
Bronchial challenge	Helpful in diagnosis when bronchodilation testing is negative	- Special equipment is required - May be positive in diseases other than asthma
Impulse oscillometry	Least cooperation from the patient is needed	- Special equipment is required - Not widely available
Eucapnic hyperventilation	Helpful in diagnosis of exercise induced asthma	Special equipment is required
Exhaled nitric oxide	Non invasive method	- Special equipment is required - The results are influenced by atopy and smoking - More useful in follow up of asthma
Induced sputum	Non invasive method	- Time consuming - Not recommended in the international guidelines
Exhaled breath condensate	Non invasive method	- Special equipment is required - Has not been applied in clinical practice
Metabolimcs	Non invasive method	- Expensive and complicated equipment is required - Still under research

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