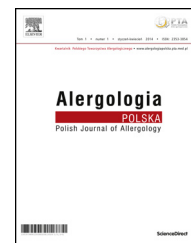


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Review/Praca pogładowa

Bronchial challenge tests with direct and indirect stimuli – Which are more useful?

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ABSTRACT

There is a large selection of bronchial challenge tests. The most commonly employed tests involve provocation with methacholine, histamine, exercise, a hypertonic saline solution, or mannitol. The first two mentioned tests use pharmacological substances acting directly on bronchial smooth muscles causing constriction and increased blood vessel permeability. The remaining tests are considered as indirect stimuli, which by osmotic mechanisms lead to release of endogenous mediators responsible for quick obturation of the respiratory tract. Studies in recent years concerning direct tests have shown interpretation discrepancies when using approved methods and tools to carry out the tests. Bronchial challenge tests with mannitol, which have greater specificity for asthma, are increasingly being promoted. However, negative results of these tests are frequently encountered in patients with well-controlled asthma which is not the case in tests involving methacholine.

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The diagnosis of symptomatic asthma presenting with classic symptoms is not difficult and relies chiefly on a patient's anamnesis, physical examination, and spirometry [1]. Patients with periodic asthma or chronic mild asthma, the most common kind, usually report shortness of breath induced only by exercise, after contact with trigger allergens, or appearing during nighttime. However, these

patients are usually symptom-free during a doctor's visit. A common scenario arises where these patients are referred to an allergy/asthma specialist but never present with any wheezes, rhonchi, or obturation in spirometry results. Provoking characteristic symptoms of asthma or changes in spirometry results seems to be a logical approach to speeding up the diagnostic process in patients suspected of

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having asthma or in patients who have been taking asthma medications for years but do not have reliable documentation clearly indicating a diagnosis.

Bronchial hyperreactivity (also known as hyperresponsiveness) is a core characteristic of asthma. The definition of asthma according to GINA included bronchial hyperreactivity beginning from the acceptance of the inflammatory theory of asthma until 2014 [2]. Although bronchial hyperreactivity has been recently removed from the definition of asthma according to GINA, British Thoracic Society (BTS) 2014 criteria state that a bronchial challenge test is needed to provoke obturation in patients suspected of having the condition. There is still a need for a universal test to aid in the diagnosis of asthma [1, 3]. There has been ongoing research for many years into such a test [4, 5].

Bronchial challenge tests serve to measure the bronchial response to various stimuli that lead to impaired airflow in the respiratory tract. These tests are constantly being studied and perfected. Their development is mainly fueled by physicians' need for a quick test capable of confirming or excluding asthma in patients with periodically appearing symptoms of the disease. Development of spirometry studies allow for increasingly precise measurements of ventilation disorders.

The pioneer work of Curry from the 1940s involving histamine and acetylcholine along with its synthetic derivatives showed that inhalatory administration of these substances result in greater bronchial constriction in patients with asthma than in healthy subjects [4, 5]. Bronchial response to the above-mentioned substances was measured using vital capacity (VC) [4, 5]. Forced expiratory volume in 1 s (FEV_1), introduced at the same time by Tiffeneau and Pinelli allowed for the measurement of dynamic ventilatory changes. The FEV_1 measures the effects of stimuli provoking constriction and medications dilating the respiratory tract [6–9]. Further research into these functional tests resulted in newer techniques of measuring dynamic changes in bronchial challenge tests: assessment of airflow (FEV_1 , peak expiratory flow (PEF)), airway resistance (Raw) [10], conductance (Gaw) [11], reactance [11, 12], fractional exhaled nitric oxide (FeNO) [13, 14], computer analyzed respiratory sounds [15], and changes in arterial oxygen saturation (SaO_2) [12].

This article will attempt to review current knowledge on bronchial challenge tests used in various clinical centers [16–18].

To date, a number of studies have been published on challenge tests using various stimuli causing impaired airflow in the airways [4, 5, 19–28]. Research on measuring bronchial responsiveness in the 1970s was mainly conducted in centers located in Canada, United States, Holland, England, and France. In Poland, studies involving acetylcholine, and later physostigmine, were conducted by Droszcz et al. in the 1960s [19, 20] while Górski et al. studied tests with polymyxin B in the 1970s [22, 23]. Certain challenge tests require specialized tools to administer the provoking stimuli or measure the effects thereof [29–31].

Challenge tests may be classified according to various characteristics such as the mechanism of action of the provoking agent. According to Pauwels, challenge tests may be divided into specific (usage of a specific 'sensitizing' substance) and nonspecific tests (all other substances) [32].

Another classification involves dividing the tests into direct and indirect challenges. Direct challenge tests use stimuli that directly cause constriction of smooth muscles or dilation of blood vessels such as histamine, acetylcholine, methacholine [4, 5], prostaglandin D2 [33, 34], and leukotrienes C4, D4, E4 [35–37]. Indirect challenge tests use various other stimuli which lead to the release of endogenous histamine and/or other mediators responsible for bronchial constriction such as cold air [38–40], physical exertion [41, 42], hyperventilation [43], hypertonic saline solutions [25, 44, 45], polymyxin B [22, 23], mannitol [46], distilled water [24, 25, 47], propranolol [48, 49], adenosine [50, 51], bradykinin [52, 53], or tachykinin [54].

All the above-mentioned provoking stimuli affect airflow in the respiratory tract in patients both healthy and with disease although patients with asthma usually have a more pronounced response. The most commonly assessed parameter has been FEV_1 . A reduction of the FEV_1 of at least 20% (in comparison to the control/initial measurement) after administration of the provoking trigger has been arbitrarily accepted as a positive result.

The control/initial measurement is taken after inhaling the diluting agent used to prepare differing concentrations of the provoking substance. Currently, there is no consensus as to what changes are required in parameters other than FEV_1 in order to obtain a positive result of a challenge test. The most commonly encountered parameter is PC_{100Raw} , the concentration of a provoking substance resulting in a 100% increase of airway resistance from the baseline value. The variability of Raw, sGaw (specific airway conductance), and reactance during challenge tests is substantially greater than that of FEV_1 and are assessed less frequently because of this [28].

The majority of research has focused on histamine and methacholine. There is hope in finding a cut-off value for a histamine or methacholine concentration and a cut-off value for the change in FEV_1 which will allow differentiating patients with bronchial hyperreactivity from those without. A complication is the fact that bronchial hyperreactivity, although a core feature of asthma, is not specific for asthma.

Recommendations by the American Thoracic Society (ATS) from 1999 state that the basic substance for a bronchial challenge is methacholine, however, both methods of administering the aerosol (a 2 min continuous inhalation and a dosimetric 5 breath protocol) are accepted as equivalent [28]. Now, 16 years later, it is well known that the PC_{20FEV_1} measured during tests with methacholine being administered by both routes differ among themselves and especially so in patients with low-grade bronchial hyperreactivity [55]. It is also known that how the aerosol is inhaled affect the PC_{20FEV_1} with deep breaths preventing bronchial constriction. The newest recommendations state that the dosimetric 5 breath method of administration should be performed with calm breathing, i.e. at tidal volume [56]. An important factor affecting bronchial response is the size of the inhaled particles. Nebulizers creating an aerosol mainly with particle sizes $<1\mu m$ in diameter result in less positive results during challenge tests [55]. This theory is supported by results obtained in a study by Lieutier-Colas et al. investigating provocation with cat

allergens. Their experiment found that particle diameters of about 10 μm resulted in the earliest bronchial response [57].

Patient safety is a primary concern and a basic principle of all bronchial challenge tests is a gradual increase in the concentration of the provoking substance and spirometry assessment after each administration. In theory, the administration of a highly provoking substance may lead to life-threatening constriction of the bronchial lumen. However, studies *in vivo* have not confirmed such a situation. We now know that a constrictive bronchial response to methacholine, histamine, or leukotriene D_4 reaches a plateau and further increasing their concentrations or number of doses does not affect the bronchial lumen, however, patients with bronchial hyperreactivity react with greater obturation than healthy patients. Resulting curves with varying slopes in a graph of such data may differentiate between healthy subjects and those with asthma [58, 59]. A much simpler parameter allowing assessment of the intensity of bronchial hyperreactivity is the provocative concentration causing a 20% decrease of the FEV_1 in comparison to the baseline value ($\text{PC}_{20}\text{FEV}_1$ X or $\text{PD}_{20}\text{FEV}_1$ X, where X is the provocative substance). Classification of the degree of bronchial hyperreactivity presented by the ATS in 1999 differ from those published earlier [28]. The previous cut-off value between healthy and hyperreactive bronchi was a $\text{PC}_{20}\text{FEV}_1$ value of 8 mg/ml. This value resulted in 5% of the healthy population having a positive result [21, 60]. This resulted in a high sensitivity of the test but a low specificity. Further research by Cockcroft showed that a $\text{PC}_{20}\text{FEV}_1$ equal to 16 mg/ml resulted in up to 20% of subjects having a positive test result in a group of randomly selected subjects. With this in mind, Cockcroft has proposed changing the cut-off of value to 4-16 mg/ml when using methacholine with low-grade bronchial hyperreactivity being $\text{PC}_{20}\text{FEV}_1 = 1-4$ mg/ml and medium and high-grade bronchial hyperreactivity, characteristic for asthma, being defined as $\text{PC}_{20}\text{FEV}_1 < 1$ mg/ml [61].

It is important to keep in mind the definitions of specificity and sensitivity of tests in assessing bronchial hyperreactivity [62]. Sensitivity of a bronchial challenge test is the proportion of positive results to the sum of positive and negative results in a studied population with bronchial hyperreactivity. A sensitivity of 100% means that the test will identify all subjects with bronchial hyperreactivity. The specificity of a diagnostic test is the proportion of negative results to the sum of negative and false-positive results in a studied healthy population. A specificity of 100% means that all healthy subjects will have a negative result [62].

Bronchial challenge tests are time-consuming there have been numerous attempts to simplify or shorten them [63, 64].

The assessment of bronchial hyperreactivity is without a doubt a useful tool in clinical research or assessing new medications, however, their widespread usage in diagnostics, including that of asthma, has been met with varying enthusiasm.

Methacholine and histamine

The ATS, in 1999, approved two types of tests, the exercise and methacholine challenge tests for the diagnosis of

asthma and two methods of inhaling methacholine, a 2 min continuous inhalation and a dosimetric 5 breath method. However, methacholine in Poland has not been approved for use *in vivo* [28]. Histamine, although approved for use in skin testing and earlier produced and sold in vials as a stimulatory agent for gastric secretions, also has not been approved for bronchial testing [65].

The question then arises, why did the ATS choose methacholine as the first choice in bronchial challenge tests? Could results of animal studies showing large eosinophilic inflammatory infiltration upon conjunctival provocation with histamine have had a role to play [66]? These results have not been reproduced in human populations. A study by Higgins et al. [67] showed that equivalent doses of methacholine and histamine gave similar results in bronchial challenge tests in a general population noting that those challenged with histamine also had hoarseness more frequently [67].

Beckert and Jones, in the textbook "Bronchial Asthma – Emerging Therapeutic Strategies" (2012) wrote that histamine and methacholine practically have very similar effects on bronchial smooth muscles and when used in equivalent doses evoke similar responses. Numerous research and diagnostic centers prefer methacholine in order to avoid symptoms associated with the effects of histamine on sympathetic nerve fibers in the respiratory tract. However, centers performing functional tests which have been using histamine for years continue to do so with no problems. The authors add that histamine is not registered as a medical substance in the United States [68].

A brief outline of indirect stimuli commonly used in bronchial challenge tests will be presented. These include: exercise, hypertonic saline solution, adenosine, mannitol, and eucapnic voluntary hyperventilation.

Bronchial exercise challenge test

A precise description of this procedure is outline in the ATS recommendations from 1999 [28]. A majority of patients with active asthma not treated with anti-inflammatory medications respond with bronchial obturation upon physical exertion. The bronchial exercise challenge test has a fairly low sensitivity but a high specificity in detecting asthma. The test may be conducted using a cycloergometer or a treadmill. The basis of the test is to raise the heartrate to 80-90% of the maximal heart rate and maintain this exertion for 4-6 min. The most common way of calculating maximal heart rate is the equation "220 – age = maximum heart rate". A proper spirometry assessment must precede the physical exercise and be repeated at 5, 10, 15, 20, and 30 min after cessation of the exercise. Changes in FEV_1 are then assessed. A decrease of at least 10% is considered as a positive result. For diagnostic purposes, a decrease of 15% is more reliable.

Bronchial challenge test with hypertonic saline solution

Inhalation of hypertonic saline solutions have long been used as methods to facilitate expectoration of sputum [69].

Solutions of 3%, 4% and 5% NaCl are usually used for sputum induction [70]. A saline concentration of 4.5% is most commonly used in a bronchial challenge test with a high output ultrasonic inhaler [44, 45]. After performing a technically correct spirometry, the test subject inhales a mist of 4.5% NaCl for 30 s after which another spirometry measurement is performed. Subsequent inhalations are then doubled in duration (i.e. 1 min, 2 min, 4 min, and 8 min). The test is considered as positive if a 15% decrease in FEV₁ is measured compared to a baseline value. If after a total inhalation time of 15.5 min the FEV₁ does not decrease 15% or more then the test is considered as negative. It is important to note that the specificity and sensitivity of the bronchial challenge test with hypertonic saline solution is similar to that of the bronchial exercise challenge test. Its advantage over the exercise test is the possibility to collect sputum samples for additional laboratory tests. This test may be administered in any clinical center equipped with a spirometer and a high output ultrasonic inhaler and with access to necessary provocative agents [71]. The procedure is considered safe and may even be performed in pregnant subjects [68].

Bronchial challenge test with adenosine

The mechanism of action of this test takes into account the degranulation of mast cells caused by adenosine and the release of endogenous mediators causing constriction of bronchial smooth muscles. Adenosine monophosphate (AMP) diluted in a phosphate buffer in increasing concentrations doubling from 0.09 to 800 mg/ml are administered through a jet nebulizer with the subject breathing normally for 2 min (similarly as in the methacholine challenge test). This test is considered as positive if there is at least a 20% decrease in the FEV₁. This test was previously called a "universal specific test" due to the fact that inhaled AMP, just like an allergen, causes increased eosinophil count in the respiratory tract even 60 min after administration [50]. Although not a popular test, it has recently been the subject of intensive study [72].

Bronchial challenge test with mannitol

Similarly to the exercise and hypertonic saline tests, the bronchial challenge test with mannitol also depends on a hyperosmotic mechanism. Mannitol is widely used in medicine, for example, in neurology as an agent decreasing intracranial pressure, and recently as an aid in clearing the airways of patients with cystic fibrosis [64, 73, 74]. Bronchial testing with mannitol has been one of the more commonly used and promoted bronchial challenge tests. We also have experience with this test and have published articles outlining the pros as well as cons of a commercially available test kit (Aridol, Pharmaxis) [75, 76]. A huge advantage is the fact that this commercially available test kit includes everything necessary for the test except for the spirometer. This allows for the test be conducted in any doctor's office with personnel who are capable of conducting technically correct spirometry tests.

The kit for mannitol provocation includes a dry powder inhaler (DPI) and capsules containing various dosages of the

substance (0 mg, 5 mg, 10 mg, 20 mg, and 40 mg). The test begins with performing a technically correct spirometry. The studied subject is then administered the 0 mg dose (an empty capsule) through the DPI. A subsequent spirometry assessment is performed after 60 s. The FEV₁ measured at this stage is considered the baseline value to which further measurements are compared. The following stages of the test involve the studied subject inhaling increasing doses (5 mg, 10 mg, 20 mg, 40 mg, 80 mg) with the three last administrations being 160 mg resulting in a cumulative dosage of 635 mg. The 80 mg and 160 mg doses are administered with multiples of the 40 mg capsule. Unfortunately, the inhalation of 4 capsules, one after another through the provided DPI, is quite difficult because of a buildup of an electrostatic charge on the capsules as well as the inhaler [75]. The test is considered as positive if the FEV₁ is decreased by at least 15% of the baseline value or if there is a decrease of at least 10% of the FEV₁ between subsequent stages of the test. The PD₁₅FEV₁ range for the quantitative assessment of the intensity of bronchial hyperreactivity has not yet been determined for this test although attempts have been made [77, 78]. Although the specificity of this test is greater than the methacholine test, patients with well-controlled asthma may produce negative results.

Eucapnic voluntary hyperventilation test

This test is a sort of variation of the exercise test. Bronchial secretions become hyperosmotic due to drying of the airway mucosa caused by hyperventilation. Occasionally, achieving such a stage of hyperventilation in athletes in a classic exercise test is difficult because the exercise intensity is limited by safety precautions involving maximal heart rates. Prolonged hyperventilation leads to hypocapnia which may then result in neurological symptoms including syncope and seizures. Hypocapnia and its consequences may be prevented by administering 5% CO₂ during the hyperventilation [79].

A proper spirometry assessment is performed before the test. During the test the patient breathes a mixture of 5% CO₂, 21% O₂ and 74% N₂ while breathing at a rate of 30–60 breaths for 6 min. A spirometry assessment is performed immediately after cessation of the hyperventilation and then at 5, 10, 15, and 20 min intervals. The test is considered as positive if the FEV₁ decreases by 10% of the baseline value. Although hyperventilation performed during this test is slower than during measurement of the Maximal Voluntary Ventilation (MVV), this test can be quite strenuous for the general population. Decreases in FEV₁ during this test may be significant and exceed 50% with accompanying cardiological symptoms. This test is usually carried out in athletes who have obtained negative results in methacholine or hypertonic saline tests [80].

Conclusions

Based on our own experience and the above presented data from available studies, it can be stated that bronchial challenge tests with methacholine or histamine have a high

sensitivity and are well tolerated by studied subjects. However, quantitative assessment of test results is quite difficult because many factors may affect the PC_{20} FEV_1 values such as the method of administering the provocative agent (2 min continuous inhalation or dosimetric method), the type of nebulizer used, or the technique of inhaling the created mist in the 5 breath method. Widespread use of methacholine or histamine as provoking agents in bronchial challenge tests is limited by their lack of approval in such situations.

A bronchial challenge test with mannitol, despite its disadvantages, could be a potentially useful test in our country if approved due to the availability of a ready kit which contains all that is necessary for its administration and the safety profile of the substance. This test is also highly specific for diagnosing asthma. This commercially available kit with mannitol, in spite of its disadvantages published in our previous papers, seems to present with a greater chance of being approved in Poland than with methacholine.

Authors' contributions/Wkład autorów

EŚ – study design, acceptance of final manuscript version, literature search; MK – literature search; ZS – acceptance of final manuscript version, literature search.

Conflict of interest/Konflikt interesu

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Ethics/Etyka

The work described in this article have been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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