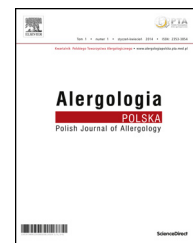


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Review/Praca pogładowa

Bronchial allergen challenge – An old, but still useful tool in research and diagnostics



Prowokacja oskrzelowa z alergenem – stare, ale nadal przydatne narzędzie badawcze i diagnostyczne

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ABSTRACT

A bronchial allergen challenge may serve both as a diagnostic as well as investigative procedure. Its usage in the diagnosis of asthma is infrequent due to its time-consuming nature as well as (in our opinion) an unfounded fear for a patient's safety. It is quite useful in the diagnostics of workplace related illnesses. Due to its ability to produce a controlled, long-lasting allergic-inflammatory reaction in the bronchi, this procedure is commonly used in research settings as well as in the assessment of new substances which may potentially have a place in the treatment of asthma, for ex. through the blocking of late asthmatic reactions. Changes stimulated by a bronchial allergen challenge may be evaluated by many methods using different materials, i.e. samples obtained from direct biopsies, bronchoalveolar lavages, exhaled breath condensates, or measured fractional exhaled nitric oxide. This procedure is a seemingly ideal research and diagnostic tool, however, a common protocol for its execution has not yet been accepted. Recent legal regulations have resulted in difficulties obtaining various allergens for challenge tests. Hopefully this is a temporary and minor setback for this very useful and constantly developing procedure.

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A bronchial allergen challenge (BAC) is a procedure that involves the sequential inhalation of increasing doses of an allergen that is suspected of causing symptoms in allergic patients [1]. This procedure is performed for various reasons, but always during a symptom-free period in patients that have or are suspected of having an allergy to a given allergen.

It was first used as a diagnostic tool to assess whether a given (suspected) allergen was the causative factor of symptoms in a patient. In 1981, Spector and Chai had already proposed that the main indication for a BAC could be the examination of a given allergen's role as the culprit in asthma in certain patients. We currently know that a correlation between clinical symptoms and [positive] skin test results is usually sufficient evidence for this. However, a [concise] medical history and results of the above-mentioned tests are sometimes divergent, and it is in such situations, without a doubt, that the BAC finds its niche [2]. Often enough a BAC may also serve to provide definitive evidence in situations where a patient simply cannot be convinced that the source of their asthma is a beloved pet. The BAC is still considered a valuable diagnostic tool in workplace-related illnesses, especially in baker's or car sprayer's asthma [3].

It's currently known that the BAC is a safe procedure provided that it is carried out by trained personnel in a center equipped with first aid capabilities in case of an anaphylactic reaction [4, 5]. Contraindications to the procedure include: 1) unstable asthma requiring constant use of medications which may affect the test, 2) asthma which required a hospitalization within the last year, 3) infections of the respiratory tract within the last 4 weeks, 5) $FEV_1 < 70\%$ of the predicted value, 6) time immediately after an influenza vaccination (< 1 week), 7) regular usage of beta-blockers, 8) other general contraindications to spirometry (i. e. Recent myocardial infarction, stroke, abdominal or thoracic surgery, aneurysms, seizures, untreated arterial hypertension, pregnancy, breastfeeding, etc.) [6].

The first description of an allergen challenge with fresh grass pollen as a diagnostic tool in patients with hay fever appeared in a textbook by Blackley in 1873 [7]. During the 1940s, inhalation of an aerosolized allergen was introduced and spirometry was used to measure the bronchial response [8]. The 1950s saw Herxheimer and colleagues, taking advantage of the advances in spirometry, present for the first time a biphasic allergic response of the respiratory system after the inhalation of an allergen. They called the first part of this response the 'early asthmatic reaction' (EAR), and the second part the 'late asthmatic response' (LAR) [9].

By measuring the forced expiratory volume in one second (FEV_1), they found that the EAR appears after about 15 min and is characterized by a decrease [in the FEV_1] of at least 20% in comparison to the initial value. Usually the EAR resolves within 60 min, however, the LAR (a second 15–20% decrease of the FEV_1) appears around 3 to 8 h later and may last even more than a day.

We would like to present results of our own study involving a BAC in a group of patients ($n = 32$) allergic to house dust mites. A biphasic asthmatic response (EAR+ LAR+) was observed in 13 (40.6%) of the studied patients, an

isolated early reaction (EAR+ LAR–) in 9 (28.1%), an isolated late reaction in 2 (6.25%), and a negative reaction (EAR– LAR–) in 8 (25%). A positive result was noted in 75% of the patients of which 62.5% had a LAR+ [10]. Our results do not differ fundamentally from those of other authors [11].

A quite large retrospective study assessing the effects of various allergens on the intensity of the LAR was recently published. House dust mites seemed to exacerbate this reaction more severely than pollen, and animal allergens were positioned in-between the two [12].

From our own experience, we can add that not all pollens uniformly stimulate a bronchial response in patients with seasonal allergic rhinitis. In fact, patients allergic to grass pollen exhibited positive reactions substantially more often than patients allergic to birch tree pollen (90% vs 50%) when challenged with their respective allergens [13].

The 1970s saw challenge tests flourish, both non-specific (histamine, methacholine) and specific (with a selected allergen) [14–16]. Different challenge protocols were, and still are, used in various research centers using assorted tools for dosing allergen extracts [16, 17].

A study published in 2015 showed that the different BAC protocols similarly decrease spirometric indices and increase the eosinophil count in the sputum [18].

Aqueous solutions of allergens are most commonly used [19], dry powders less so [10, 20, 21], and then recombinant allergens, with the latter being used solely for nasal challenges, for the time being [22].

Allergens may be administered by (jet) nebulizers, such as the Wright (no longer in production) or DeVilbiss 646, dry powder nebulizers, [allergen] challenge chambers [23], simulated workplace environments, or even by exposure to live animals such as cats [24].

In addition to the article that showed that differing methods of allergen administration give similar results in select parameters, a multitude of other works appeared questioning whether varying bronchial challenge protocols can be considered equivalent. Some of these publications showed that the result of a challenge may be affected by the depth of inspiration before or during inhalation of the administered provoking substance with deep breathing preventing bronchoconstriction which may consequently produce false negative results in patients with clearly hyperreactive bronchi [25, 26]. Similarly, a dosimetric method of administering the provoking substance is less sensitive and does not detect bronchial hyperreactivity in patients which have had positive results when challenged using a method requiring 2 min of calm breathing (at tidal volume). Authors have proposed alternative thresholds of the $PC_{20}FEV_1$ for methacholine used to identify bronchial hyperreactivity based on if it was administered with a 5-breath protocol or 2 min [tidal volume] inhalation [27]. The most recent publications show that only nebulizers approved by ATS guidelines (from 1999) should be used when assessing the degree of bronchial hyperreactivity with methacholine. Newer nebulizers require further studies [28].

The previously mentioned [allergen] provocation chamber is an ideal tool for research studies, however, its diagnostic usefulness in individual patients is limited [29].

Melillo et al, in the year 1997, published a report that summed up then current knowledge on the subject of allergen tests concerning bronchial as well as nasal and conjunctival provocation tests attempting their standardization [30]. The latest publication effort to recap the usefulness of bronchial provocation tests was in 2015 [6].

For diagnostic purposes, increasing doses or concentrations of a provoking allergen are administered during one day beginning with very low values continuing to concentrations/doses greatly exceeding those which are inhaled during natural exposure.

The assessment of a [new] therapy's success involves performing usually at least two challenges; the first being before the commencement of a given therapy, and the second after completion and/or additionally sometime later (even years) such as in allergen immunotherapy in order to compare an organism's sensitivity to an inhaled allergen [31-36].

It would be worthwhile here to mention the results of a study completed in our center on a group of 32 patients with pollinosis, allergic to grass pollen. We assessed the bronchial response to inhaled pollen allergens during the course of a 3-year subcutaneous immunotherapy (all-year exposure method). Initially, a biphasic response (EAR+ LAR+) was noted in 48.3% of patients, in 20.7% of patients after 1 year, in 17.2% after 2 years, and in none after 3 years [36].

Challenges are also used to evaluate various mechanisms of an allergic reaction with the aim to stimulate and gradually increase the intensity of an allergic inflammation without inducing clinical symptoms [37-40]. Inhalational administration of small doses of an allergen in intervals of at least one day reduce the LAR [41].

Many tools have been used in an attempt to measure the response of the bronchi to an allergen provocation. The earliest methods focused on the intensity of clinical symptoms [7]. In regards to nasal challenge tests, this subjective method still seems to be more useful than measurements of nasal airway resistance or flow [42].

A study published in 2015 assessed the effects of a provocation using a well-known phenomenon that patients have difficulty speaking full sentences during a severe exacerbation of asthma. This experiment correlated disorders of ventilation with the ability to fluidly read text [43]. Spirometry is the most widespread method of assessment utilizing parameters such as FEV₁, Raw, PC₂₀, PD₂₀ [19]. An oscillometric technique of measurement was used for this purpose [44]. However, the most consistently observed changes were in terms of the FEV₁ [30].

Hyperreactivity in regards to histamine is observed to increase after an allergen challenge in patients with a positive LAR. Such a reaction has not been observed in individuals with an isolated EAR [19].

It is known that allergic inflammation in the airways induces cytological and biochemical changes in the bronchial walls, secretions, and exhaled air. Various biological materials/methods have been and constantly are being explored to evaluate these changes.

Biopsies (at least two: pre- and post-challenge) are required in order to assess changes in the bronchial walls. Significantly more information can be obtained through

multiple biopsies taken at different time intervals from the provocation [45]. Unfortunately, this requires undergoing repeated bronchofiberscopies, which are unpleasant for the patient.

Bronchoalveolar lavages provide the most material for assessing secretions of the lower respiratory tract. From a researcher's perspective (having much experience with this technique), it is a simple procedure which allows for acquisition of much research and diagnostic material for both cytological and biochemical studies [46-48]. Unfortunately, the simplicity of this procedure is not so obvious from the perspective of the patient nor from an ethical standpoint.

Modern medicine's approach to diagnostics and therapy is focused on minimizing invasive procedures. Stimulation of sputum production by inhalation of hypertonic saline solutions seems to be an ideal way of obtaining valuable diagnostic material. This procedure may be repeated numerous times, even in patients with asthma (after premedication with salbutamol) without harm to the patient [49]. Also, newer research techniques allow for attaining more and more information from sputum samples obtained by this method [50, 51].

Exhaled air also contains various substances, products of an allergic inflammatory process, of which the usefulness in diagnostic and research settings is still the subject of many investigations. Most publications have focused on exhaled nitric oxide [52, 53]. Our center has also acquired much experience in this area [54]. Measuring the fractional exhaled nitric oxide (FeNO) is a non-invasive, easy to perform procedure which allows for significantly longer monitoring of the consequences of a single allergen challenge. Unfortunately, attempts to replace spirometry indices with FeNO measurements have not succeeded due to difficulty in correlating the quite dynamic changes between FEV₁ and concentrations of exhaled NO. Changes in the FEV₁ can be observed in a relatively short time after inhalation of an allergen whereas the FeNO rises gradually. Our experience has shown that a peak concentration is achieved between 75 and 100 h after administration of an allergen with the first 8 h resulting in little change in the FeNO when compared to variations in the FEV₁ [54].

Samples of exhaled breath condensate may also be of use in the evaluation of changes in the lungs after a BAC [55, 56]. Bronchial allergen challenges also influence the cytological, as well as biochemical, composition of blood [57].

A study published in 2011 suggested that every bronchial constriction, regardless of the provoking factor, may be associated with remodeling of the respiratory tract which may discourage some investigators from utilizing a provocation test as a research tool [58]. It would be prudent to take into account that individuals with bronchial hyperreactivity are constantly being exposed within their natural surroundings to substances causing multiple contractions and relaxations of the bronchi. The few repeated contractions induced during a provocation test would seem to be of negligible concern in the development of remodeling.

Recently, the pharmaceutical market has been hit with a shortage (or even complete lack) of many allergens for challenge tests. This is the result of new European Union regulations which classify such allergens as medications.

The production of allergens, their research and marketing are subject to the same regulations as medications [59]. From a marketing angle, these regulations may lead to significantly decreased production, or cessation altogether, of diagnostic allergens less commonly used by investigators.

Authors' contributions/Wkład autorów

ZS – study design, acceptance of final manuscript version. GS – data collection, literature search. MK – literature search, translation into English. EMS – study design, literature search.

Conflict of interest/Konflikt interesu

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None declared.

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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