



Provocation studies in the diagnosis of occupational asthma

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The list of occupational allergens grows longer each year as new products and substances are reported by industries, ranging from pharmaceutical to automobile manufacturing companies, to be causative agents of occupational asthma (OA). The identification of an agent that causes OA has medical, legal, and financial consequences for employers and employees. The diagnosis of OA and identification of the offending agent should be undertaken in a careful, methodical, and objective manner. Evaluation includes (1) a careful history and physical examination, (2) testing of skin or serum for specific immunoglobulin E (IgE), (3) nonspecific bronchial challenge testing to determine airway hyperresponsiveness, (4) serial monitoring of peak expiratory flow rate (PEFR) or forced expiratory volume in 1 s (FEV₁) within and outside of the workplace, and (5) provocation testing with the suspected agent [1–3].

In nonspecific bronchial challenge testing, bronchoconstrictor agents, exercise, or hyperventilation is used to detect the presence of airway hyperresponsiveness. In the evaluation of suspected work-related symptoms, nonspecific challenge testing is indicated if the diagnosis of asthma is unclear after the history is taken and the physical examination and spirometry are done. Some protocols suggest nonspecific challenge testing to establish the degree or severity of asthma before administering specific challenge testing.

Provocation studies using specific agents, also known as specific bronchial challenge (SBC) testing, specific bronchoprovocation testing, occupational challenge testing, or specific inhalation challenges, are considered to be the gold standard for diagnosing OA [4]. In most patients encountered in clinical practice, a diagnosis of OA can be made with reasonable certainty with only the history,

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physical examination, and a comparison of serial peak flow rates or spirometry taken within and outside of the workplace. SBC testing more commonly is used as a research tool in tertiary medical centers and often is used as a last resort for OA diagnosis in situations involving significant legal and financial implications for the patient. A survey of academic training programs in the United States and Canada found that SBC testing is performed infrequently because of the lack of available facilities with the proper equipment, inability to locate a site for referral, concerns about reimbursement, and lack of an appropriate diagnostic reagent [5].

This article focuses on nonspecific bronchial challenge and SBC testing for identification of OA triggers. The pathophysiology, clinical manifestations, other diagnostic testing and management of OA are discussed elsewhere in this issue.

Nonspecific bronchial challenge testing

General considerations

When a worker first reports new respiratory symptoms at work and a thorough clinical evaluation with spirometry is equivocal, a nonspecific bronchial challenge can be performed to find out if the symptoms are caused by asthma. The nonspecific bronchial challenge is used to determine the presence of nonspecific airway hyperresponsiveness, a hallmark of asthma, by exposing subjects to inhaled stimuli in a controlled setting and measuring their response by pulmonary function testing. Bernstein and colleagues have recommended performing the bronchial challenge at the workplace [6], because resolution of nonspecific hyperresponsiveness within a few days after the cessation of workplace exposure has been reported [7]; however, this approach may not be practical. In a laboratory setting, obtaining a negative result several days after workplace exposure has ended provides useful information to help rule out active asthma at that time. Nonspecific bronchial challenge testing should not be used for pre-employment screening, because there is no evidence that a positive test predicts whether a worker is more likely to develop occupational allergies or asthma [8].

To identify an occupational cause, results from nonspecific bronchial challenge testing that have been obtained within and outside of the workplace can be compared, if practical [9]. In most cases, repeat challenge testing can be performed at least 1 week after cessation of workplace exposure. Because bronchial hyperresponsiveness can linger for prolonged periods after exposure has ended, the absence of a difference in the results does not rule out OA.

Nonspecific bronchial challenge and SBC testing should be performed with certain precautions (Box 1). In general, challenge testing should be done when the patient is the least symptomatic. The patient's baseline FEV₁ should be at least 70% of the predicted value. Symptomatic asthma is a contraindication to the procedure. Anxiety or anticipation may influence the bronchial response. A patient may be told that the result of the test worsens, improves, or has no effect on lung function [10]. Circadian rhythms also may have a role in the response to

Box 1. Guidelines for methacholine bronchial challenge**Indications**

To determine the presence and severity of airway hyperresponsiveness

Contraindications

Symptomatic asthma or FEV₁ less than 70% of predicted value

Preparation

No recent exercise or recent or ongoing respiratory tract infection

No ingestion of coffee, chocolate, and cola drinks and no smoking for at least 6 hours.

Medications to be stopped before the challenge

Short-acting *C*-agonists, short-acting theophylline preparations, *B*-adrenergic agents, and anticholinergic agents for at least 8 hours

Sustained release *C*-agonist and theophylline preparations for at least 12 hours

Antihistamines for at least 48 hours (astemizole for at least 3 weeks)

Allowed medications

Inhaled cromolyn and nedocromil

Inhaled or oral corticosteroids

Procedure

1. Baseline FEV₁ set by challenge with saline control
2. Initial methacholine concentration should be less than 0.1 mg/mL, which is increased progressively to a maximum of 25 mg/mL; five breaths given at each concentration
3. FEV₁ measured after five breaths of each concentration
4. Challenge ends when fall of 20% or more in FEV₁ is seen or when maximum concentration is reached

Interpretation

Provocative concentration that produces a fall in FEV₁ by 20% (PC₂₀FEV₁) is measured

Cutoff value for PC₂₀FEV₁ that is diagnostic for asthma varies. Many centers use 25 mg/mL or less, and others require 8 mg/mL or less.

challenge, so repetitive testing should be done at the same time of the day on different days. Challenges should not be performed immediately after exercising or during or after a viral infection, which can increase bronchial hyperresponsiveness and affect test results. Coffee, chocolate, cola drinks, and smoking should be avoided at least 6 hours before testing. Short-acting β -agonist agents, short-acting

theophylline preparations, α -adrenergic agents, and anticholinergic agents should be stopped at least 8 hours before testing. Long-acting β -agonists and sustained-release theophylline preparations should be stopped at least 12 hours before testing. Antihistamines should be avoided for 48 hours or longer, depending on their half-life. Mast cell stabilizers, such as cromolyn and nedocromil, can be continued before methacholine or histamine challenges but should be avoided 24 hours before allergen, occupational agent, and exercise challenges (Table 1) [11]. The American Thoracic Society Guidelines for Methacholine and Exercise Testing do not require the withholding of systemic or inhaled steroids before challenges, because these drugs do not block the bronchoconstriction that is induced by methacholine or histamine. Bronchial challenges for patients taking corticosteroids usually are done to follow changes in airway hyperresponsiveness. Systemic and inhaled steroids may decrease chronic airway hyperresponsiveness and may be withheld, depending on the purpose of the challenge [12,13].

The FEV₁ is the most widely used pulmonary function value to assess bronchial response, because it is easy and convenient to perform. It is less variable than other pulmonary function values, such as forced vital capacity (FVC) or maximum midexpiratory flow rate (MMFR, or FEF_{25%–75%}). Other tests that have been used to evaluate bronchial response are vital capacity, PEFR, functional residual capacity, specific airways resistance, and specific airway conductance. A fall from baseline FEV₁ of 20% is considered a significant response to bronchial challenge.

Methacholine and histamine are the two most widely used bronchoconstrictor agents, because the responses that they elicit are usually short-lived, making them ideal for testing. Methacholine is preferred, because histamine can cause headache and tachycardia, and tachyphylaxis can develop with repeat challenges. Responsiveness to these bronchoconstrictor agents often correlates well with asthma severity. Because positive and negative changes in airway mucosa

Table 1
Withholding medications before challenge

Medication	Withholding period	
	Nonspecific challenge (h)	Specific challenge
Short-acting β -agonists	8	8h
Short-acting theophylline	8	8h
α -Adrenergic agents	8	8h
Anticholinergic agents	8	8h
Long-acting β -agonists	12	12h
Sustained release theophylline	12–24 ^a	12–24h ^a
Antihistamines	48 ^b	48h ^b
Inhaled cromolyn/nedocromil	—	1 mo ^c
Inhaled steroids	—	1 mo ^c

^a depends on preparation (eg, 12-hour sustained release versus 24-hour sustained release).

^b Astemizole and similar preparations withheld for 3 weeks.

^c One month often recommended to wash out long-term anti-inflammatory effects; if asthma is unstable without maintenance therapy, give dose at most 8 hours before challenge.

osmolarity are believed to lead to airflow limitation, nonisotonic aerosols (eg, water, hypotonic or hypertonic saline solutions) can be used for bronchial challenges. Although testing with aerosols are considered to be less sensitive than methacholine testing, it can be helpful for physicians who prefer to use nonpharmacologic alternatives when evaluating airway hyperresponsiveness. Bronchial challenge testing with nonisotonic aerosols is cheap, easy to perform, and relatively free of side effects [14]. Exercise and hyperventilation testing also can demonstrate the presence of nonspecific airway hyperresponsiveness but requires more equipment and usually is reserved for diagnosing suspected exercise-induced asthma when the methacholine challenge results are negative.

Procedures

Several standard protocols differ in the length of the procedure, the sequence for increasing the dose, and the units for expressing concentration or dose. Among the most widely used methods are those described by Cockcroft [15], Chai [16], and Yan [17]. Most protocols start with five breaths of saline solution. A 15% or greater drop in the FEV₁ mitigates against further testing. If a negative saline response occurs, the patient is given five breaths at a time of progressively increasing concentrations of methacholine or histamine (Table 2). For safety reasons, the initial concentration of both agents is usually less than 0.1 mg/mL. Spirometry is performed after each set of five breaths. The response to provocation is measured by the concentration (PC₂₀ FEV₁) or cumulative dose (PD₂₀FEV₁) of methacholine or histamine that is required to decrease the patient's baseline FEV₁ by 20%. The concentration is expressed in mg/mL, whereas the cumulative dose is expressed as cumulative μ mol or cumulative breath units. Each breath unit is equivalent to one breath of a 1-mg/mL concentration.

Interpretation of bronchial challenge testing results varies among different centers. Approximately 85% to 100% of asthmatics have a PC₂₀FEV₁ of 8 mg/mL or less of methacholine or histamine [15,18]. Less than 5% of normal patients have a 20% decrease in FEV₁ after five inhalations of the usual maximal concentration of 10-mg/mL histamine and 25-mg/mL methacholine [16]. Some practitioners consider a PC₂₀FEV₁ of 25 mg/mL or less to be a positive result for asthma, whereas others require a PC₂₀FEV₁ of 8 mg/mL or less. Cockcroft classified a PC₂₀FEV₁ to methacholine of 2.0 to 8.0 mg/mL as an indicator of

Table 2
Suggested dilution schedule for methacholine using five-breath dosimeter protocol

Methacholine dose	Addition of NaCl (mL)	Dilution (mg/mL)
100 mg (powdered)	6.25	A: 16
3 mL of dilution A	9	B: 4
3 mL of dilution B	9	C: 1
3 mL of dilution C	9	D: 0.25
3 mL of dilution D	9	E: 0.0625

Data from American Thoracic Society. Guidelines for methacholine and exercise challenge testing 1999. *Am J Respir Crit Care Med* 2000;141:309–29.

mild asthma; 0.25 to less than 2.0 mg/mL as moderate asthma; and less than 0.25 mg/mL as severe asthma [15]. Studies have shown that a $PC_{20}FEV_1$ of 8 mg/mL or less has a sensitivity of 85% and a specificity of 95% for asthma [18]. Although the sensitivity of a $PC_{20}FEV_1$ of 25 mg/mL or less may be similar to that of 8 mg/mL, its specificity is lower (79%). A cutoff level of 25 mg/mL is less specific and has a lower positive predictive value than does a level of 8 mg/mL or less [19]. Persons with allergic rhinitis but no history of asthma symptoms may have $PC_{20}FEV_1$ values of 8 mg/mL or less but may exhibit a plateau in the dose–response curve, whereas persons with asthma continue to show a decline in FEV_1 as the dose of methacholine or histamine is increased [20].

The relationship between the patient history, methacholine challenge, and SBC testing has been examined. In this study, patients with suspected latex-induced OA, isocyanate-induced OA, and OA induced by hairdressing-related substances were evaluated. The authors found that the combination of a positive history and methacholine challenge had a specificity of 62% to 80% and a sensitivity of 52% to 83%. There was good correlation between a positive history and methacholine challenge and subsequent confirmation by a positive SBC test. Some authors suggest that a negative methacholine challenge, even with a positive history, can rule out OA and obviate the need for a time-consuming SBC test [21].

Serial monitoring of pulmonary function

After a diagnosis of asthma is made, serial monitoring of pulmonary function at and away from the workplace is a convenient way of evaluating whether the asthma is related to the occupation. The PEFR is defined as the greatest expiratory flow that is produced with forceful expiration after maximal inspiration, measured in L/min. The peak-flow meter device, which ranges from a simple tube to computerized models, is a portable, easy-to-use, inexpensive way to monitor lung function outside of the doctor's office. The PEFR only measures the most effort-dependent part of the FVC and is similar to the maximum expiratory flow rate. It is more useful in patients with moderate-to-severe asthma, because it can miss subtle airflow limitation with mild episodes. Numerous peak-flow devices are available, and only those that conform to the criteria set by the American Thoracic Society (ATS) should be used [22]. The ATS recommends that devices should provide for readings between 60 to 400 L/min for children and 100 to 850 L/min for adults. Patients should be instructed on the proper technique for using the peak-flow meter. The patient usually is standing and is asked to inhale fully, place the meter in the mouth, close lips around it, and exhale with maximum force. The PEFR can be obtained with an exhalation time of 1 to 2 s.

Malo and co-workers has recommended checking PEFR every 2 hours at and away from the workplace [23]. Monitoring at and away from the workplace for 2 weeks at a time has been recommended [24]. The sensitivity and specificity of PEFR in OA has been reported to be between 81% to 89% and 74% to 89%, respectively [25,26]. The European Academy of Allergy and Clinical Immunology has issued guidelines for self-monitoring of PEFRs [27]. A mean daily

PEFR that is below the normal predicted value or a diurnal variability (difference between the highest and lowest PEFs) that is greater than 20% at the workplace, when compared with normal values away from the workplace, strongly suggests the presence of OA. OASYS-2, a computer-aided system for the diagnosis of OA that uses visual analysis of serial PEFs is apparently reproducible, specific, and sensitive and can be used without an experienced interpreter [28]. The dependence on effort is one of the shortcomings of the PEF. Depending on the motivation of the patient and the possible consequences of a diagnosis of work-related illness, the patient may not report accurate PEF results. This potential for inaccurate reporting is an important limitation of this method.

The FEV₁ reflects the volume that is expired in the first second of the FVC (expressed in liters) and is considered to be superior to PEF as a measure of airway patency. Performing spirometry to compare daily FEV₁ values before and after work for at least 1 week has been used to detect work-related bronchial deterioration. Because serial monitoring of pulmonary function has been shown to be more sensitive than measurement before and after work, serial FEV₁ monitoring would seem to be the ideal diagnostic tool; however, in a study comparing serial monitoring of PEF and FEV₁ using the ventilometer (a device that allows self-monitoring of both values), the authors concluded that unsupervised FEV₁ measurement is not more accurate in diagnosing OA than is unsupervised PEF monitoring [29].

SBC testing

General considerations

Specific bronchial challenge testing can be done to confirm one suspected agent or to narrow down a list of possible agents. Testing most often is performed when there is diagnostic uncertainty or diagnostic dispute or for research [8]. New OA triggers can be investigated and confirmed with SBC testing [30–32]. In general, the precautions and preparation that are recommended for SBC testing are identical to those for nonspecific testing. Nonspecific testing, however, often is performed in subjects who have not started asthma medications; in contrast, specific testing is often done in patients already following a drug regimen for treatment of asthma. Short-acting β_2 -agonists should be stopped 8 hours before the challenge, whereas long-acting β_2 -agonists and sustained-release theophylline should be withheld 48 hours before testing. It is preferable to discontinue inhaled cromolyn and inhaled steroids 1 month before the challenge [8]. If medications cannot be withheld without a worsening of symptoms, the minimum amount should be given at the end of each challenge day but no sooner than 8 hours before the next challenge [33]. Instability of asthma during the cessation of medication can lead to false-positive results. The patients' baseline FEV₁ should be at least 70% of the predicted value, and testing should be done when the patient is the least symptomatic. Acute symptomatic asthma is a contraindication

to the procedure. Ideally, workplace exposure should have ended for at least 1 week before SBC testing. This testing should be performed only in a medically supervised setting, usually a hospital or research facility, that has resuscitation equipment available to treat possible life-threatening anaphylactic reactions.

The American Conference for Governmental Industrial Hygienists sets the threshold limit values and short-term exposure limits for many occupational agents [34]. These limits should not be exceeded in any SBC testing. If possible, the level of the suspected agent is measured in the workplace, and this level is used to guide the dose used in testing. The duration and concentration that are used in the challenge is determined by the investigator based on the patient's clinical history, the patient's airway hyperresponsiveness on nonspecific bronchial challenge testing, and the nature of the agent. If the subject has a history of a severe, immediate reaction, exposure should be shorter and more incremental. High-molecular-weight allergens (eg, animal or vegetable proteins) usually cause immediate, IgE-mediated reactions and an isolated early or biphasic reaction and often can be tested during 1 day of active challenge testing [2]. Low-molecular-weight allergens (eg, isocyanates, plicatic acid) can have non-IgE-mediated mechanisms of action and can produce isolated late or biphasic reactions, necessitating progressive incremental testing over several days [33]. The PC₂₀ derived from the baseline nonspecific bronchial challenge is useful in planning SBC testing. The lower the PC₂₀, the greater the baseline airway hyperresponsiveness and the more likely that an immediate significant reaction will occur. A shorter starting duration of exposure is used if the PC₂₀ 0.25 mg/mL or less; a longer duration used if the PC₂₀ is greater than 0.25 mg/ml [33].

Procedures

Because there are many possible forms of workplace exposure to a suspected agent, there are many different protocols for exposing a patient during a challenge. No standardized or universally accepted protocols exist for SBC testing. In 1989, the American Academy of Allergy, Asthma and Immunology released the Guidelines for Bronchoprovocation on the Investigation of Occupational Asthma, which reviewed general principles for SBC testing [8]. The Canadian Thoracic Society also released guidelines on the diagnosis and management of OA [35].

Cartier and Malo recommend a general sequence, which is used by many centers for SBC testing (Boxes 2,3) [33]. Regardless of the method used, they first suggest 2 control days. On the first control day, the patient is followed for 8 hours outside of the workplace to make sure that the underlying asthma is stable. Baseline spirometry is obtained, and FEV₁ is measured every 10 minutes for the first hour, every 30 minutes for the second hour, and every hour for up to 8 hours. A nonspecific bronchial challenge is performed at the end of the day to determine the level of baseline airway hyperresponsiveness. The baseline PC₂₀ can be used to determine the starting exposure dose of the active occupational agent. If there are signs of unstable asthma, such as a baseline FEV₁ of less than 2 L and fluctuations in FEV₁ of more than 10%, further testing should be postponed.

Box 2. Performance of specific inhalation challenges on the control days**Day 1****Aim**

To make sure asthma is stable

Prerequisites

Stop use of inhaled β_2 -agonists 8 hours before the test; stop use of oral sustained-release theophylline 48 hours before the test; and stop use of inhaled or oral anti-inflammatory preparations on the morning of the test.

Procedure

1. Obtain baseline spirometry, oral temperature, and white blood cell count.
2. Measure FEV₁ every 10 minutes in the first hour, every 30 minutes in the second hour, and every hour for 7 to 8 hours. Take oral temperature hourly.
3. Measure PC₂₀ (nonspecific bronchial challenge) at the end of the day.
4. Monitor peak expiratory flow rate in the evening at home.
5. If baseline FEV₁ is less than 2 L or if FEV₁ fluctuates more than 10%, improve asthma treatment and postpone tests.

Day 2**Aim**

To make sure subjects do not react to a control agent

Prerequisites

Same as for Day 1

Baseline FEV₁ should be $\pm 10\%$ of baseline FEV₁ for Day 1.

Procedure

1. Exposure to a control agent (eg, lactose if causal agent is flour, diluent for isocyanates, other wood dusts for a wood dust agent) lasts for 30 to 120 minutes.
2. FEV₁ should be measured as outlined in Day 1
3. Measurement of PC₂₀ is not needed.
4. If baseline FEV₁ is less than 2 L or if FEV₁ fluctuates more than 10%, improve asthma treatment and postpone tests.

Adapted from Cartier A, Malo J-L. Occupational challenge tests. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, editors. *Asthma in the workplace*. 2nd edition. New York: Marcel Dekker; 1999. p. 211–33; with permission.

Box 3. Performance of specific inhalation challenge tests on the active days**Day 3 and subsequent days****Aim**

To verify if patient shows a significant reaction after exposure to suspected agent

Prerequisite

Same as Day 2 (control day; see Box 2)

Procedures

Progressive exposure with in-between functional assessments

1. For high-molecular-weight agents (expect immediate reaction)
One breath, 10 s, 20 s, 30 s, 2 min, 5 min, 30 min, 60 min, etc for a total exposure of about 2 hours
2. For low-molecular-weight agents
One breath, 10 s, 20 s, and 30 s for a total exposure of 1 minute on the first active day
1 min, 2 min, 2 min for total exposure of 5 minutes of the second active day
5 min, 10 min, and 15 min for total exposure of 30 minutes on the third active day
15 min, 15 min, 30 min, and 30 min for total of 2 hours on the fourth active day
3. Assess FEV₁ immediately and 10 min after each exposure period (eg, check FEV₁ twice between the one-breath and 10 second exposure for high molecular weight agents)
4. If FEV₁ changes less than 10%, continue with protocol.
5. If FEV₁ changes more than 10%, repeat the previous period of exposure.
6. If FEV₁ changes 20% or more, stop exposure.

Results

For positive reaction

Repeat PC₂₀ at end of the day or the following morning provided FEV₁ is back to baseline value.

If PC₂₀ is significantly lower than that from Day 1 (control day; see Box 2), treat with anti-inflammatory preparation; repeat PC₂₀ until it is back to baseline value.

For late reaction

Administer inhaled β_2 -agonist.

If FEV_1 is back to $\pm 10\%$ of its baseline value 15 to 20 min later, patient may be discharged with instructions to use inhaled β_2 -agonist on an as-needed basis

If FEV_1 is not back to its baseline value, patient should be given oral steroids, discharged, and followed-up.

For negative reaction

Repeat PC_{20} at the end of the day.

If there is no significant change from the baseline PC_{20} , no further exposure is needed.

If the PC_{20} is significantly lower than the baseline value, increase duration of exposure to a maximum of 4 hours the following day.

Adapted from Cartier A, Malo J-L. Occupational challenge tests. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, editors. *Asthma in the workplace*. 2nd edition. New York: Marcel Dekker; 1999. p. 211–33; with permission.

On the second control day, the patient is exposed to a placebo (eg diluent aerosol or lactose powder). Consultation with an industrial hygienist may be helpful in identifying the appropriate agent to be used. The duration of exposure and the concentration of this agent depends on the nature of the test agent. Commonly, exposure can last up to 2 hours if no reactions are seen. FEV_1 is measured at regular intervals during and after exposure for up to 8 hours as on the first control day. Immediate and late reactions are detected during this period of monitoring. If a decrease in FEV_1 occurs after placebo exposure, it should be less than 10% from the baseline value. A higher decrease indicates bronchial lability that can affect test results, and further testing should be postponed until the underlying asthma is stabilized.

After the 2 control days, testing with the specific occupational agent begins and can last from 1 to 4 days, depending on the expected severity of the reaction. In general, the duration of total exposure per day builds up to a maximum of 2 hours on the last day (eg, 1 minute on day 1 of testing, 5 minutes on day 2, 30 minutes of day 3, and 2 hours on day 4). The total duration of exposure per day is broken down in increments (eg, 10, 20, and 30 seconds to a total of 1 minute). If an immediate severe reaction is anticipated based on the patient's history or a low baseline PC_{20} , the initial exposure should be short and the duration should increase over the following days. Agents that are not expected to produce severe immediate reactions may be administered for the maximum of the 2 hours on the first test day.

The FEV₁ is determined just before and 10 minutes after every exposure. A sustained decrease in FEV₁ of 20% or more from the baseline value at any time is considered to be a positive response; the testing is stopped if this event occurs. Inhaled β_2 -agonist should be administered to restore FEV₁ to within 10% of baseline. A course of systemic steroids may be used if the FEV₁ is not restored with inhaled β_2 -agonist treatment. If FEV₁ does not diminish by 20% or more after 8 hours of observation, the SBC generally is deemed a negative test. Patients are instructed to continue peak-flow monitoring for the rest of that evening; however, significant drops are unlikely [33].

A repeat nonspecific bronchial challenge is performed at the end of the last testing day regardless of whether a positive or negative result has been observed. If the PC₂₀ after a negative specific challenge is significantly lower than the baseline PC₂₀ from the first control day, increased airway hyperresponsiveness may be present, and an extra day of testing may be performed (with exposure to the agent for up to 4 hours). The significant change in PC₂₀, even after a negative SBC test, may indicate sensitization to the agent, and a longer exposure may produce a positive result [36].

False-positive SBC testing results most often are caused by irritant reactions to components other than the test agent or when the patient's asthma is uncontrolled; therefore, ensuring that asthma is stable and performing a challenge with the proper control agent are necessary. False-negative results can occur if exposure is too short, the wrong agent is tested, or the patient has been away from the workplace for too long [37,38]. In the latter case, peak-flow monitoring should continue once the patient returns to the workplace, and SBC testing should be repeated when peak-flow readings start to decrease [33].

Dust challenge

Exposure to dust (eg, grain and feed dust) can be simulated in the laboratory by having the subject pour dust or powder back and forth between two trays, dispersing the agent into the air. This classic method first was performed by Pepys and Hutchcroft to investigate occupational dusts [39]. A well-ventilated room is required. Dry lactose powder is used on the control day. On the subsequent test days, measured amounts of the test agent are added to the lactose.

A dust generator, a closed-circuit apparatus that delivers the test agent through a breathing port, minimizes contamination of the air in the laboratory. Precise amounts of particles can be released for measured amounts of time, increasing the accuracy and reproducibility of challenges [40]. The dust challenge has been used to test for reactions to flour, wood dust, penicillin, platinum salts, guar gums, and piperazine [41,42].

Aerosol challenge

Many water-soluble occupational agents can be aerosolized and delivered in a controlled manner with a nebulizer. The test extracts often are prepared and

stored in the same way as conventional allergenic extracts (eg, animal dander, pollen); concentrations are expressed as wt/vol or protein nitrogen units/mL. An intradermal skin test endpoint titration can be done first to determine the initial challenge dose. In general, the initial challenge dose concentration is between 10- to 100-fold higher than the concentration that produced a wheal more than 5 mm in diameter (eg, an initial concentration of 0.05 $\mu\text{g/mL}$ if the skin titration endpoint was 0.005 $\mu\text{g/mL}$) [43]. The test dose (concentration and duration of challenge) is increased progressively on separate test days. The protocol for the number of doses can differ for each agent, depending on the nature and amount of exposure at the workplace; the testing generally takes several days. Various types of nebulizer can be used, including the DeVilbiss jet nebulizer (Sunrise Medical, Somerset, PA), the Wright nebulizer, or an ultrasonic nebulizer [44]. A drop in FEV_1 of 20% or more indicates a positive result.

Vapor challenge

The most common agents that patients are exposed to in vapor or gas form are isocyanates, glutaraldehyde, and formaldehyde. The isocyanates have been one of the most studied agents in OA [45,46]. Toluene diisocyanate (TDI) and diphenylmethane diisocyanate (MDI) are delivered as vapor, whereas hexamethylene diisocyanate can be delivered in a nebulized solution. A well-ventilated room is required. Measured amounts of TDI and MDI are placed in the room. MDI can be heated to release the vapors [33,47]. In a simpler form of exposure to TDI, the patient paints the test agents on cardboard for specific periods of time [48]. Control challenges with appropriate chemical components should be performed to rule out sensitivity to other agents. This rule varies according to the product being tested (eg, paints, plastics). Because isocyanate-induced asthma can be associated with severe reactions, challenge exposure is done progressively over several days (see Table 2).

A closed-circuit apparatus for isocyanate challenge testing has been developed [49]. This device generates liquid aerosol and vapor for inhalation through a breathing port. As with devices used for dust and powder challenges, the closed-circuit nature minimizes contamination of the laboratory and sensitization of personnel.

Challenge chamber

Challenge chambers offer a controlled environment in which the delivery of the test agent into the atmosphere can most closely approximate natural exposure and the concentration can be controlled rigorously. These chambers range from a simple enclosed space to a sophisticated room with capabilities for monitoring variables such as humidity and temperature. Chambers are useful for assessing vapors, fumes, and gases, such as formaldehyde and TDI, and can be used for aerosols and dusts. Most challenge chambers are located in academic medical centers and research facilities.

Workplace challenge

If the patient's symptoms are not debilitating and if he or she continues to work, serial monitoring of PEFr can be done at and away from the workplace to establish a diagnosis. Some patients may have stopped going to work because of their symptoms. If laboratory facilities for specific challenge are not available, bringing the patient back to the workplace may help determine the possible offending agent. A protocol for exposure that is similar that for laboratory challenge should be followed, and emergency equipment should be available in the event of a serious reaction. Limitations of a workplace challenge include exposure to several possible agents, an uncontrolled environment, and possible nonspecific triggers such as stress, exertion, cold air, or pollution [50].

Animal exposure challenge

Laboratory workers performing research experiments on mice, rats, guinea pigs, and rabbits can develop OA symptoms from this exposure [51]. One third of laboratory animal workers have allergy to animals, and one third of these workers have asthma [52]. The diagnosis is made by a suggestive history, positive skin test responses to the relevant allergens, and serial pulmonary-function monitoring at and away from the workplace. Specific challenge testing is rarely necessary [53]. As with other occupational allergens, certain situations may make SBC testing useful. Wood and Eggleston have described rat exposure challenges for laboratory personnel [54]. Air sampling is used to determine exposure levels to the rat antigen Rat n 1. Exposure levels are highest with cleaning and feeding and are lower with surgery or sacrifice. The challenge is performed in the workplace (the existing animal vivarium), and exposure lasts for about 1 hour. Air sampling was done throughout the exposure to ensure correlation with usual working levels. Spirometry and nasal lavage for levels of histamine and toxoid–antitoxoid mixture esterase are performed approximately every 15 minutes. Exposure chambers or facilities similar to those used for cat challenges in pharmaceutical clinical research are not used widely but would provide a more controlled environment.

Summary

Specific and nonspecific provocation studies, although not always essential for diagnosing OA, help confirm the diagnosis and identify the offending agent. Nonspecific bronchial challenge testing is used to detect airway hyperresponsiveness and to clarify the nature of the patient's symptoms. Pharmacologic bronchoconstrictor agents (eg, methacholine, histamine) most commonly are used for the challenge, but nonisotonic aerosols, exercise and hyperventilation also can show airway hyperresponsiveness. Nonspecific challenges usually are done in the laboratory, but can be done at the workplace if emergency equipment is available. A comparison of results obtained at and away from the workplace (at least 1 week apart) may be helpful in diagnosing OA.

Specific bronchial challenge testing is considered the gold standard for OA diagnosis. It can be crucial in helping physicians, employers, and employees make decisions about continued employment, compensation, career changes, and treatment. Testing can pinpoint new industrial agents that cause OA, enabling dissemination of information on its hazards to the public and within the industry. The nature of the agent determines the type of protocol that is used for testing. Agents can be in the form of dusts, powders, aerosols, vapors gases, and animal dander. Exposure can be as simple as having patients simulate their work activities, or as complicated as using special challenge chambers with controlled environments and precise delivery of agents. Performing control challenges with a component that is separate from the test agent is essential to avoid false-positive results. The timing, duration, and dosing of exposure depend on the type of reaction that has been experienced previously, the nature of the agent, and the patient's baseline airway hyperresponsiveness. Serial spirometry and observation often are done for up to 8 hours to monitor early and late reactions. SBC testing should be performed in the proper medical setting in which emergency equipment available and should be administered only by healthcare personnel who are trained and experienced in the procedures. Safety of the patient is the primary consideration.

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