SERIES "CONTROVERSIES IN OCCUPATIONAL ASTHMA"
Edited by O. Vandenplas and J-L. Malo
Number 2 in this Series

Diagnosing occupational asthma: how, how much, how far?

G. Moscato*, J-L. Malo#, D. Bernstein¶

Diagnosing occupational asthma: how, how much, how far? G. Moscato, J-L. Malo, D. Bernstein. ©ERS Journals Ltd 2003.
ABSTRACT: Diagnosing occupational asthma is still a challenge because it is based on a stepwise approach in which the depth of investigative means may vary depending on resources. The authors herewith review the existing investigative means from the approach of outlining controversies and queries.

There is no validated clinical questionnaire for diagnosing occupational asthma. Immunological investigation is limited by the lack of standardised extracts for skin-prick testing and specific immunoglobulin E assessments. Criteria for interpretation of changes in peak expiratory flow rates and bronchial responsiveness to pharmacological agents are still open to discussion.

It is worth improving the methodology of specific inhalation challenges, either in the laboratory or in the workplace, to facilitate more extensive use of these tests. Validation of new means that assess airway inflammation, such as exhaled nitric oxide and induced sputum, needs to be performed. There is a need to increase the use of these diagnostic tests because the diagnosis is still too often based on "clinical impression".
Eur Respir J 2003; 21: 879–885.

*Servizio Autonomo di Allergologia e Immunologia Clinica, Fondazione "S. Maugeri", Clinica del Lavoro e della Riabilitazione, IRCCS, Istituto Scientifico di Pavia, Italy. #Dept of Chest Medicine, Sacré-Cœur Hospital, Montreal, Canada. ¶Division of Immunology and Allergy, University of Cincinnati, OH, USA.

Correspondence: J-L. Malo, Dept of Chest Medicine, Sacré-Cœur Hospital, 5400 West Gouin, Montreal, H4J 1C5, Canada.
Fax: 1 5143383123
E-mail: malojl@meddir.umontreal.ca

The diagnostic process aims at determining the presence of a disease based on its symptomatology. Originally, medical diagnoses were based on history and physical examination. With time, diagnostic tools have considerably broadened. Because the most widely accepted definition of asthma [1] is based not only on symptoms but also on functional status (*i.e.* reversible airway obstruction and airway hyperresponsiveness), recommendations have been made to confirm the diagnosis with objective testing. Experienced physicians may over- or underdiagnose asthma [2] and the use of the case definition "physician-based diagnosis of asthma" often employed for population surveys, is subject to criticism. Even simple spirometric tests are insufficiently used to assess asthmatic subjects despite the availability of published and widely disseminated practice guidelines. The eosinophilic inflammatory process involved in asthma has been widely investigated in recent years and has led to the diagnostic practice of identifying eosinophils in sputum specimens, at least in selected or difficult cases. It has been shown that methacholine airway responsiveness and sputum differential eosinophil counts are the most useful objective tests for identifying patients with mild asthma [3]. As with asthma, the diagnosis of occupational asthma (OA) should be confirmed by objective testing, as recommended in recently issued practice guidelines [4]. OA has considerable medical and social consequences. Giving wrong advice based on a medical impression without objective testing could cause workers to remain exposed to the offending agent(s), leading ultimately to a worsening of asthma and death [5]. Conversely, unnecessary removal from a job can have considerable adverse financial consequences for a worker not affected with OA.

In the case of OA with a latency period, identification of the causal relationship between exposure at work and onset of disease is crucial (see Definitions section). Moreover, there are agents for which the immunological mechanism responsible for the disease has been identified (especially high molecular-weight (HMW) agents whose mechanism is immunoglobulin (Ig)E-mediated). In these situations, it is therefore essential to gather information not only to confirm the relevant mechanism but also to identify the causative substance.

The primary objective of using the various tools being reviewed here is to make a valid and precise diagnosis. However, the same tools can be used for other purposes, for example, in research or in epidemiological studies. For these, the methodology may be slightly different. For example, questionnaires are designed differently when they are used for field studies. In population studies, a closed questionnaire is desirable, preferably one that has been validated. The European Community Respiratory Health Survey (ECRHS) questionnaire is probably the most widely used questionnaire for epidemiological studies in asthma. Extra questions have been proposed for OA, but have not been validated. In clinical evaluation of workers, the questionnaire is open. The physician listens to the patient, adds specific questions and formulates a general impression based on the plausibility of the diagnosis.

When dealing with OA, the two key questions to be addressed are as follows. 1) Does the subject suffer from asthma? 2) Is asthma caused by conditions in the workplace (see Definitions section)? Therefore, much of the work is concerned with ascertaining whether the subject has asthma while at work or when exposed to agents usually present at the workplace.

Finally, another crucial issue is the practicality of applying these different tools in varying circumstances. In many countries, the existing workers' compensation systems are not sufficient to facilitate the satisfactory management of OA. In the absence of substantial compensatory benefits, workers with OA are unlikely to leave the workplace. In other countries, OA is managed and remedied in ways that could motivate workers to leave work. In these circumstances, falsification of self-conducted peak expiratory flow (PEF) assessments [6] and overestimation of symptoms are possible. Investigations can also be hindered if workers are illiterate. In some countries, as is unfortunately the case in the USA, physicians' fear of being sued for damages resulting from specific inhalation challenges has inhibited the general use of these most informative tests. Finally, specific inhalation challenges are relatively expensive (though probably cost-effective) and time consuming, and call for medical supervision; these are other possible limitations.

Review of means used for diagnosis and their controversial issues

Table 1 lists controversies/queries and proposals for studies according to the following diagnostic tools.

Questionnaire

As with many other medical conditions, the most commonly used introductory tool for investigating the possibility of OA is the questionnaire. Key elements of the occupational history have been suggested by BERNSTEIN [7]. These include: employment history and job description; a listing of all processes and substances used in the employee's environment; prior jobs. Symptoms are explored with regard to their nature, duration, temporal pattern and relationship to the workplace (e.g. improvement while away from work). In addition, preceding or concomitant rhinoconjunctivitis symptoms should be sought [8, 9]. Hyperventilation symptoms often blur the picture and merit being addressed. However, none of the key points suggested by BERNSTEIN [7] have been tested for sensitivity or specificity by comparison with final diagnosis confirmed by objective testing. Such validation is needed as for an epidemiological questionnaire.

It is critical to determine whether symptoms improve while away from work. Constructing appropriate questions is challenging. Should they be worded as "Are your symptoms less severe on weekends?" or "Are your symptoms worse at work?" Or are these two formulations equivalent? A weekend might not be a sufficient period in which to judge improvement. Bakers often work 6 days a week. Vacations allow for cessation of exposure for longer intervals, but these occur only once or twice a year. Almost everybody feels better while away from work. Worsening of symptoms at work depends entirely on exposure. Some workers are not necessarily constantly exposed to the offending agent. Based on safety data sheets, workers may believe they are being exposed to agents that are without risk. However, these sheets do not necessarily include the content of those constituents found in low concentrations. Symptoms may show very different patterns (abrupt symptoms after Monday's exposure, progressive symptoms with cumulative exposure over the week etc.).

Notwithstanding all these pitfalls, medical questionnaires have to be sensitive but they cannot be expected to be specific. Good sensitivity and poor specificity of medical questionnaires have been found in at least two studies [10, 11]. VANDENPLAS et al. [11] also showed that skin-prick tests (SPT) and assessment of nonspecific bronchial responsiveness were even more sensitive than a questionnaire for workers exposed to latex, although both tools were equally nonspecific. One might propose that every adult-onset asthmatic should be investigated for possible OA if they are exposed at work to an agent known to cause OA.

Immunological assessment

Conventional SPT and assessment of serum-specific IgE antibodies in OA are useful to detect specific IgE responses to HMW allergens [12]. In addition, performing SPT or

Table 1. – Summary of controversies/queries and proposals of studies according to diagnostic tools

Diagnostic tool	Controversies/queries	Proposals for studies
Questionnaire	No validated questionnaire for epidemiological or clinical purposes. Key questions such as: "Are your symptoms less severe on weekends?" or "Are your symptoms worse at work?" not validated	Validation of questionnaires Put emphasis on satisfactory sensitivity
Immunological assessment	No satisfactory extract for skin testing and specific IgE/IgG assessments	Standardised antigens New tests: e.g. MCP-1
Lung-function tests	Can PC20 be "negative" while at work in a case of OA? How can PEF recordings and interpretation be improved?	Validity of a single PC20 assessment while at work
Specific inhalation challenges	Best means to assess the response? Improve methodology and increase use	Assess the validity of combining PEF changes and bronchial hyperresponsiveness at work Assess validity of various functional and inflammatory means to assess the response and not only FEV1 Improve inhalational methodology Quality control of centres
Assessment of inflammation	Sensitivity and specificity of inflammatory testing?	To be assessed in larger studies

Ig: immunoglobulin; PC20: provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (FEV1); OA: occupational asthma; MCP-1: monocyte chemotactic protein-1; PEF: peak expiratory flow.

demonstration of serum IgE antibodies for common inhalants confirms or excludes the atopic status of the patient. SPT are quick, inexpensive, simple to perform and safe [13]. Moreover, they can be carried out in patients with impairment of lung function. However, their use is limited by the lack of commercially available or standardised extracts with known allergen content for most occupational allergens [14].

The sensitivity and specificity of SPT vary appreciably, depending on the diagnostic gold standard used. The quality of the extract used for SPT is critical. It should be emphasised that extracts used for SPT should also be tested for irritant effects and the nonirritant threshold concentration must be predetermined in nonallergic volunteers. In the case of latex allergy, HAMILTON and ADKINSON [15] found that a commercial SPT preparation of nonammoniated natural rubber latex (NRL) at two different dilutions had a sensitivity and specificity between 95–100% in relation to latex symptoms [15]. Most importantly, the negative predictive value was 96%, suggesting that OA can be excluded using this NRL reagent. Similar figures were found by TURJANMAA et al. [16] with a new NRL extract in relation to latex symptoms. By contrast, using a commercial extract of nonammoniated NRL, VANDENPLAS et al. [11] found that SPT had high sensitivity (100%), but lower specificity (21%) for identifying workers with OA confirmed by specific inhalation challenges (SIC), thus bearing out the lack of a satisfactory correlation between immunological tests and SIC or PEF records found in epidemiological surveys for HMW substances [14]. The validity of the combination of SPT with other tools in the diagnosis of OA due to HMW has hardly been evaluated. VANDENPLAS et al. [11] found that the combination of SPT and bronchial hyperresponsiveness was more sensitive than questionnaire alone for workers exposed to latex, although equally nonspecific.

The demonstration of specific antibodies by means of radioallergosorbent test (RAST) or enzyme-linked immunosorbent assay (ELISA) in OA due to HMW allergens is also limited by the lack of commercially available standardised allergen extracts. In general, in-vitro assays of specific IgE are less sensitive but more specific than SPT in identifying cases of OA confirmed by specific provocation challenge [15, 17]. The role of immunological tests assessing an IgE response in the diagnosis of OA due to low molecular-weight chemicals (LMW) is more limited. First, for most chemicals the pathogenetic mechanism of OA is not clear and an IgE mechanism could not be involved. Secondly, LMW compounds are haptens and can act as allergens only after binding with one or more serum protein: in many cases, serum-specific IgE to the hapten-protein conjugate is detectable, but its significance is often questionable, reflecting either exposure or specific sensitisation. Generally, there is a lack of standardised antigens for performing immunological tests for LMW chemicals [12], as well as significant inter-laboratory variability in immunoassay protocols. There are a few exceptions in which the presence of specific serum IgE and/or a positive SPT are considered a sensitive diagnostic aid, e.g. to complex (unconjugate) platinum salts, trimellitic and acid anhydrides [18, 19]. In the case of LMW compounds, particularly some of the most common causes of OA, like isocyanates, the sensitivity and specificity of tests assessing the IgE response is variable, being good for some [20] and less satisfactory for others [21, 22]. It is also worth noting that results of both skin and in-vitro tests of IgE-mediated sensitivity may fall to a level below detection when workers are removed from exposure [12]. Therefore, it is essential to take exposure into account when interpreting results.

In the case of OA due to HMW agents or LMW compounds, evidence of specific sensitisation by skin tests and/or positive IgE associated with a suggestive history is not enough for a diagnosis of allergic OA due to the given agent. The diagnosis requires objective confirmation of asthma and of work-related functional changes, together with a suggestive history and exposure to an agent known to cause OA (see above). The role played in OA by different classes of immunoglobulins, e.g. specific IgG4 antibodies, is not clear [23]. Thus, there is currently no place in clinical practice for tests assessing these responses. In asthma due to LMW chemicals, cell-mediated immunity can be of some importance. In toluene 2,4-diisocyanate (TDI)-induced asthma, activation of CD8+ T-helper cell type 2 (Th2) lymphocytes has been demonstrated in bronchial biopsies and analysis of bronchoalveolar lavage [24]. An increased CD69 expression on peripheral blood eosinophils after specific inhalation challenge with LMW compounds has been described [25]. Recently, an in-vitro assay measuring production of the C-C chemokine monocyte chemotactic protein (MCP)-1 by peripheral mononuclear cells after 48 h of stimulation with diisocyanate/human serum albumin (HSA) reported 79% sensitivity and 91% specificity in identifying workers with SIC-confirmed OA [22]. At present, however, such techniques are used as research tools and not for clinical practice.

Spirometry, assessment of bronchial responsiveness and peak expiratory flow rates

Random performance of spirometry on a routine clinic visit is seldom adequate to establish the presence of asthma. On occasion, routine screening spirometry testing may identify new cases of OA in at-risk worker populations, although this strategy is far less sensitive than screening by questionnaire alone [26, 27]. In a population of red cedar workers, CHAN-YEUNG et al. [28] reported no difference in routine spirometry between red cedar workers and unexposed controls, even though OA was diagnosed in 1% of workers. A subsequent cross-sectional study by the same group of investigators showed that workers exposed to red cedar wood dust had lower lung function and provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC_{20}, FEV_1) compared with office workers [29]. Thus, reduced lung function and airway hyperresponsiveness identified in screening studies can result from chronic exposure, but they are not necessarily useful in identifying new cases of OA.

Cross-shift spirometry and measurement of FEV_1 have commonly been used to screen for OA. A 10% decline in cross-shift FEV_1 is generally considered significant. However, there is little evidence that measuring cross-shift changes in FEV_1 is a sensitive screening method for discriminating workers with OA from exposed workers without the condition [30]. This is probably due to the small number of lung-function measurements that can be made and the unlikelihood of obtaining measurements during active exposure to the causative agent. For this reason, it is advisable to obtain spirometric measurements on multiple days, at more frequent intervals and, if possible, to concurrently measure exposure to suspect causative agents.

Methacholine- and histamine-challenge tests, methods for measuring nonspecific bronchial hyperresponsiveness, have been proposed for the evaluation of OA. Due to the high sensitivity and specificity of these tests in identifying subjects with asthma, a negative test can help to exclude asthma and OA in those workers who remain exposed in the workplace and who have active symptoms [3, 31]. However, this method of ruling out asthma and OA is not infallible. There have been rare cases in which recently exposed individuals with OA had negative methacholine tests. It is widely accepted that a

negative methacholine or histamine test does not exclude OA in a remotely exposed, asymptomatic worker. In such cases, returning to the workplace for lung-function monitoring or evaluation by a specific inhalation challenge test are the only options. Methacholine testing has been successful when used as a screening tool for ruling out OA in diisocyanate-exposed workers reporting lower respiratory symptoms (positive test was $PC20 \leqslant 15$ mg·mL^{-1}). No false-negative tests or "missed cases" of OA were subsequently recognised among methacholine-negative workers who continued to be exposed [26].

Once the presence of asthma or airway hyperresponsiveness is confirmed, workers are instructed on serial self-measurements of PEF rates [4]. This process must be initiated while the worker is still exposed to the suspect agents and should be performed for suitable periods at work (e.g. 2–4 weeks) and away from work. It has been determined that at least four measurements per day are needed. These methods have the following definite and well-documented limitations: 1) they are effort-dependent and rely on optimal compliance of the patient; 2) collected data can be subject to falsification [32]; 3) asthma medications can potentially yield false-negative results; and 4) data obtained from PEF recordings are often not useful in establishing the causative agent. It has also been recognised that because of the aforementioned limitations, there is a need for internal validation of PEF results with serial histamine or methacholine testing. Therefore, guidelines for OA have recommended that serial quantitation of airway responsiveness be conducted concurrently with PEFs and be performed shortly after finishing work (i.e. within 24 h) and repeated after 3–4 weeks away from the workplace. PEF data showing decrements at work and subsequent increases on leave, together with bronchial hyperresponsiveness while at work, support OA. Problems arise if there is disagreement between the results of PEF and methacholine studies. When this occurs, specific inhalation challenge studies conducted in a laboratory or a closely supervised workplace challenge may be necessary to make a final determination.

Workplace and laboratory challenges

The most convincing way to diagnose OA is to monitor the clinical and functional status of a worker during and after exposure to the offending agent either at work or in the laboratory. If this monitoring is carried out at work, it must be performed under usual working conditions. The idea of reproducing the working environment in the hospital laboratory originated from PEPYS [33]. Originally, these tests were done in a "realistic way" by simply asking workers to do their usual work. Later on, environmental monitoring was suggested, specifically in the case of isocyanate exposure, because these chemicals can cause irritant reactions if the level of exposure is too high. More recently, thorough on-line monitoring of exposure conditions using special closed-circuit equipment has been proposed [34, 35]. It is important to ensure that the worker is exposed to low and stable concentrations of the relevant agent, so that the tests, if positive, will result in not-too-pronounced changes in functional status. It is also important to expose subjects for sufficiently long periods. In one of the author's (J-L. Malo) laboratory, it is standard to prolong the exposure for up to 2 h and, in some cases, for up to 4 h, in order to rule out the diagnosis. Thirty minutes is clearly not sufficient. Monitoring should not only include airway calibre (FEV1 is the standard for this) but also possible changes in airway responsiveness to methacholine, as reviewed [36], and changes in induced sputum (see below) [37]. Whether specific inhalation challenges in the laboratory rule out a diagnosis of OA remains to be determined. The prevalence of false-negative tests has not been assessed prospectively by returning the worker to the workplace and examining changes in functional status. It is indeed conceivable that laboratory exposure is not always to the right agent. Exposure methodology has to be further improved, especially with LMW agents, in order to extend the exposure to all chemicals and ensure exposure to the lowest possible concentration. The ideal situation would be to design referral centres in each country, so testing can be carried out by physicians with sufficient expertise. Cost-effectiveness studies also remain to be carried out to further validate this diagnostic approach.

New methods for assessing airway inflammation: induced sputum and exhaled nitric oxide

Induced sputum. The objective confirmation of a history suggestive of OA is usually carried out by means of functional and immunological methods. Indeed, exposure to allergens and chemical sensitisers induces airway inflammation which, as with nonoccupational asthma, is one of the hallmarks of the disease. New, noninvasive tools for measuring airway inflammation in asthma have recently been introduced, and their use has been proposed in the assessment of OA. Standardised and reliable methods for inducing and examining sputum have recently been made available as well [38, 39]. Sputum can be obtained by inhalation of hypertonic saline; the type of inflammatory cells and soluble markers of cell activation can be assessed. The normal and asthmatic cellular profiles of induced sputum are now well described [40, 41] and sputum eosinophilia occurs in most subjects with asthma [39]. The cellular profiles of induced sputum in OA can differ. Several data seem to indicate a central role of eosinophils in OA induced by both LMW compounds and HMW agents. MAESTRELLI et al. [42] first reported the presence of eosinophilic bronchitis following laboratory exposure to isocyanates. OBATA et al. [43] observed a significant change in sputum eosinophils at 6 and 24 h after plicatic-acid challenge among responders; this change was inversely related to the fall in FEV1 at 6 h. LEMIÈRE et al. [44] reported a significant difference in sputum eosinophils and eosinophil-derived mediators in patients with confirmed OA due to LMW compounds or unknown materials by comparing periods at work and periods away from work. These results were confirmed in subsequent studies by the same group after laboratory exposure to occupational agents in subjects with OA due to LMW or HMW agents [37, 45].

In contrast with these data, FRANCO et al. [46] showed a higher degree of neutrophilic inflammation and a lower degree of eosinophilic inflammation in the airways of subjects with asthma due to LMW compounds and still exposed to the occupational sensitiser, in comparison with subjects with OA due to HMW agents or nonoccupational asthmatics. Quite recently, ANEES et al. [47] examined a group of workers with asthma related to LMW compounds and a low degree of diurnal variability in PEF rate while at work and found that only some of them had sputum eosinophilia. Eosinophilia was associated with more severe asthma and greater bronchodilator reversibility, but not with PEF response to work exposure. In eosinophilic and noneosinophilic groups, neutrophils were found to be present in similar proportions in each group. These different findings could be related partly to the different diagnostic inclusion criteria used in the studies and partly to the methodological differences in interpreting the results (different cut-off points were used to define a significant eosinophilia in the studies by LEMIÈRE et al. [44] and ANEES

et al. [47]). Indeed, the cellular profile of sputum in OA needs to be further elucidated.

Some studies have looked at the changes in soluble mediator levels in sputum after exposure to LMW and HMW sensitisers in subjects with OA. Results of these studies reflect the controversies in inflammatory cell counts discussed above. LEMIÈRE *et al.* [44] found a significant change in sputum inflammatory mediators associated with eosinophilic infiltration, *e.g.* sputum eosinophil cationic protein (ECP), interleukin (IL)-5 and eotaxin, in patients with OA confirmed by comparing periods at work and periods away from work and in subjects with OA after SIC with occupational sensitisers [45]. In contrast, PARK *et al.* [48] found a significant increase in sputum of inflammatory mediators associated with neutrophilic infiltration, IL-8 [48, 49] and myeloperoxidase levels (MPO) [49], and in neutrophil chemotactic activity in the serum [49], following grain dust or TDI bronchoprovocation test subjects with OA, thus supporting the view that activated neutrophils may contribute to grain dust- and TDI-induced asthma.

The contribution of induced sputum to the diagnosis of OA in clinical practice in relation to other diagnostic tools is also under evaluation. The findings of LEMIÈRE *et al.* [44] appear to indicate that in cases where SIC cannot be performed, induced sputum can be considered a promising tool for investigating subjects still at work who have OA due to LMW compounds or unknown sensitisers. Moreover, since the same study found no variation in the control group of asthmatics with worsening symptoms at work but without any significant functional change, the authors suggested that sputum is particularly helpful in differentiating between work-aggravated asthma and superimposed OA due to a workplace sensitiser. In contrast, the data by ANEES *et al.* [47] discussed above seem to indicate that analysing the type of inflammatory cells in sputum does not help to confirm a diagnosis of OA in workers with borderline features of the disease.

Increase in airway responsiveness after exposure to occupational agents has been proposed as an early marker of bronchial response to occupational agents [50]. Since changes in sputum have been found to precede functional changes in both FEV_1 and PC_{20} methacholine after laboratory exposure to occupational agents in subjects with OA due to LMW or HMW agents [37], it has been suggested that induced sputum may be a useful tool in the early diagnosis of OA, possibly more sensitive than increased airway responsiveness. The sensitivity of changes in sputum in comparison with airway responsiveness as an early marker of airway inflammation in exposed workers should be evaluated in studies with a larger number of participants.

The time/course of changes in sputum cell count observed during SIC by OBATA *et al.* [43] and LEMIÈRE *et al.* [37] suggest that sputum can be helpful in interpreting the results of an SIC by: 1) supporting evidence of a late reaction during a negative SIC with an LMW compound; and 2) being a satisfactory predictor of a positive response to the SIC when associated with significant changes in airway responsiveness in subjects with OA due to LMW or HMW agents. The sensitivity and specificity of the combination of induced sputum analysis and airway responsiveness and/or PEF in relation to diagnostic gold standard in OA is unknown and needs to be evaluated. Indeed, the majority of available data suggest that sputum analysis could be particularly useful in OA due to LMW compounds, where the objective confirmation of diagnosis often relies only on SIC results, due to the lack of availability of immunological tests. The place of measurement of inflammatory indices derived from sputum in clinical practice of OA needs further evaluation with larger numbers of subjects [51]. Finally, due to its sensitivity to change in occupational exposure, monitoring sputum could play a role in the follow-up of OA, comparing the time/course of inflammatory indices during cessation of exposure to findings obtained during exposure and their variations in relation to pharmacological treatment.

Exhaled nitric oxide. Measurement of nitric oxide (NO) gas in exhaled air is a noninvasive tool and may be a good marker of airway inflammation in asthma [52, 53]. To date, only two studies have examined the role of exhaled NO in OA, comparing the results with those obtained from sputum. In patients with western red cedar asthma, OBATA *et al.* [43] found an increase in levels of exhaled NO after challenge with plicatic acid in both responders and nonresponders, reaching significance only in nonresponders. No correlation was found between the increase in NO and the magnitude of the functional changes. In the same group of patients, the late asthmatic reaction induced by plicatic acid was associated with an increase in sputum eosinophils that was inversely related to the change in functional parameters. In workers with suspected sensitisation to latex or to 4.4′-diphenylmethane diisocyanate, ALLMERS *et al.* [54] found no clear relationship between bronchial response, serum-specific IgE antibodies and increase in exhaled NO levels. Therefore, the usefulness of analysis of exhaled NO in the clinical evaluation of patients with suspected OA and its relation to monitoring sputum in assessing airway inflammation has yet to be determined.

How often is objective testing carried out?

Schemes for objective confirmation of OA have been proposed [14]. However, depending on the resources, the country, the specialty of the treating physician and medicolegal requirements, there is undoubtedly a huge variability in the way objective testing is used. In some countries, the presence of a "suggestive" history and skin reactivity to flour may be sufficient to accept a diagnosis of OA to flour. However, it is well known that sensitisation to flour and the appearance of symptoms do not necessarily add up to a case of OA. In Quebec, Canada, medical boards who examine claims addressed to the Commission de la Santé et Sécurité du Travail (Workers' Compensation Board) ask for referral to a specialist in OA, who almost always relies on several tools, the essential one being the workplace or laboratory exposure challenge. In a recent French study, carried out by collecting data from 559 cases reported on a voluntary basis: PEF rates were assessed in only approximately one-third of the cases; specific immunological sensitisation was evaluated in just under one-half of the cases; and specific inhalation challenges were carried out in only ~10% of the cases [55]. By comparison, in Italy, challenges either at the workplace or in the laboratory, seem to be used more often [56].

Conclusion

Investigation of occupational asthma should still be based on a stepwise approach in which multiplication and judicious use of objective means contribute to improving the quality of the diagnosis. New cost-effective means are worth being validated and regularly introduced to facilitate diagnosis.

References

1. American Thoracic Society. Chronic bronchitis, asthma, and pulmonary emphysema. Statement by the committee on

diagnostic standards for nontuberculous respiratory diseases. *Am Rev Respir Dis* 1962; 85: 762–768.

2. Adelroth E, Hargreave FE, Ramsdale EH. Do physicians need bjective measurements to diagnose asthma? *Am Rev Respir Dis* 1986; 134: 704–707.

3. Hunter CJ, Brightling CE, Woltmann G, Wardlaw AJ, Pavord ID. A comparison of the validity of different diagnostic tests in adults with asthma. *Chest* 2002; 121: 1051–1057.

4. Tarlo SM, Boulet LP, Cartier A, *et al.* Canadian Thoracic Society Guidelines for occupational asthma. *Can Respir J* 1998; 5: 289–300.

5. Ortega HG, Kreiss K, Schill DP, Weissman DN. Fatal asthma from powdering shark cartilage and review of fatal occupational asthma literature. *Am J Ind Med* 2002; 42: 50–54.

6. Malo JL, Trudeau C, Ghezzo H, L'Archevêque J, Cartier A. Do subjects investigated for occupational asthma through serial PEF measurements falsify their results? *J Allergy Clin Immunol* 1995; 96: 601–607.

7. Bernstein DI. Clinical assessment and management of occupational asthma. *In*: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the Workplace. 2nd Edn. New York, Marcel Dekker Inc., 1999; pp. 145–157.

8. Malo JL, Lemière C, Desjardins A, Cartier A. Prevalence and intensity of rhinoconjunctivitis in subjects with occupational asthma. *Eur Respir J* 1997; 10: 1513–1515.

9. Gautrin D, Ghezzo H, Infante-Rivard C, Malo JL. Natural history of sensitization, symptoms and diseases in apprentices exposed to laboratory animals. *Eur Respir J* 2001; 17: 904–908.

10. Malo JL, Ghezzo H, L'Archevêque J, Lagier F, Perrin B, Cartier A. Is the clinical history a satisfactory means of diagnosing occupational asthma? *Am Rev Respir Dis* 1991; 143: 528–532.

11. Vandenplas O, Cangh F, Binard-Van, Brumagne A, *et al.* Occupational asthma in symptomatic workers exposed to natural rubber latex: Evaluation of diagnostic procedures. *J Allergy Clin Immunol* 2001; 107: 542–547.

12. Grammer LC, Patterson R. Immunologic evaluation of occupational asthma. *In*: Bernstein IL, Chan-Yeung M, Malo JL, Bernstein DI, eds. Asthma in the Workplace. 2nd Edn. New York, Marcel Dekker Inc., 1999; pp. 159–171.

13. Moscato G, Perfetti L, Galdi E. Clinical evaluation of occupational asthma. *Monaldi Arch Chest Dis* 1997; 52: 469–473.

14. Malo JL, Chan Yeung M. Occupational asthma. *J Allergy Clin Immunol* 2001; 108: 317–328.

15. Hamilton RG, Adkinson NF. Diagnosis of natural rubber latex allergy: Multicenter latex skin testing efficacy study. *J Allergy Clin Immunol* 1998; 102: 482–490.

16. Turjanmaa K, Palosuo T, Alenius H, *et al.* Latex allergy diagnosis: *in vivo* and *in vitro* standardization of a natural rubber latex extract. *Allergy* 1997; 52: 41–50.

17. Blanco C, Carrillo T, Ortega N, Alvarez M, Dominguez C, Castillo R. Comparison of skin-prick test and specific serum IgE determination for the diagnosis of latex allergy. *Clin Exp Allergy* 1998; 28: 971–976.

18. Cromwell O, Pepys J, Parish WE, Hughes EG. Specific IgE antibodies to platinum salts in sensitized workers. *Clin Allergy* 1979; 9: 109–117.

19. Howe W, Venables KM, Topping MD, *et al.* Tetrachlorophthalic anhydride asthma: evidence for specific IgE antibody. *J Allergy Clin Immunol* 1983; 71: 5–11.

20. Tee RD, Cullinan P, Welch J, Burge PS, Newman-Taylor AJ. Specific IgE to isocyanates: A useful diagnostic role in occupational asthma. *J Allergy Clin Immunol* 1998; 101: 709–715.

21. Cartier A, Grammer L, Malo JL, *et al.* Specific serum antibodies against isocyanates: association with occupational asthma. *J Allergy Clin Immunol* 1989; 84: 507–514.

22. Bernstein DI, Cartier A, Cote J, *et al.* Disocyanate antigen-stimulated monocyte chemoattractant protein-1 synthesis has greater test efficiency than specific antibodies for identification of diisocyanate asthma. *Am J Respir Crit Care Med* 2002; 166: 445–450.

23. Cullinan P. Occupational asthma, IgE and IgG. *Clin Exp Allergy* 1998; 28: 668–670.

24. Maestrelli P, Del Prete GF, DeCarli M, *et al.* CD8 T-cell clones producing interleukin-5 and interferon-gamma in bronchial mucosa of patients with asthma induced by toluene diisocyanate. *Scand J Work Environ Health* 1994; 20: 376–381.

25. Pignatti P, Perfetti L, Galdi E, *et al.* Increased CD69 expression on peripheral blood eosinophils after specific inhalation challenge. *Allergy* 2002; 57: 411–416.

26. Bernstein DI, Korbee L, Stauder T, *et al.* The low prevalence of occupational asthma and antibody-dependent sensitization to diphenylmethane diiosocyanate in a plant engineered for minimal exposure to diisocyanates. *J Allergy Clin Immunol* 1993; 92: 387–396.

27. Kraw M, Tarlo SM. Isocyanate medical surveillance: Respiratory referrals from a foam manufacturing plant over a five-year period. *Am J Ind Med* 1999; 35: 87–91.

28. Chan-Yeung M, Ashley MJ, Corey P, Willson G, Dorken E, Grzybowski S. A respiratory survey of cedar mill workers. I. Prevalence of symptoms and pulmonary function abnormalities. *J Occup Med* 1978; 20: 323–327.

29. Chan-Yeung M, Vedal S, Kus J, Maclean L, Enarson D, Tse KS. Symptoms, pulmonary function, and bronchial hyperreactivity in Western Red Cedar workers compared with those in office workers. *Am Rev Respir Dis* 1984; 130: 1038–1041.

30. Bardy JD, Malo JL, Séguin P, *et al.* Occupational asthma and IgE sensitization in a pharmaceutical company processing psyllium. *Am Rev Respir Dis* 1987; 135: 1033–1038.

31. Cockcroft DW, Murdock KY, Berscheid BA, Gore BP. Sensitivity and specificity of histamine PC20 determination in a random selection of young college students. *J Allergy Clin Immunol* 1992; 89: 23–30.

32. Quirce S, Contreras G, Dybuncio A, Chan-Yeung M. Peak expiratory flow monitoring is not a reliable method for establishing the diagnosis of occupational asthma. *Am J Respir Crit Care Med* 1995; 152: 1100–1102.

33. Pepys J. Inhalation challenge tests in asthma. *New Engl J Med* 1975; 293: 758–759.

34. Cloutier Y, Lagier F, Lemieux R, *et al.* New methodology for specific inhalation challenges with occupational agents in powder form. *Eur Respir J* 1989; 2: 769–777.

35. Vandenplas O, Malo JL, Cartier A, Perreault G, Cloutier Y. Closed-circuit methodology for inhalation challenge test with isocyanates. *Am Rev Respir Dis* 1992; 145: 582–587.

36. Vandenplas O, Malo JL. Inhalation challenges with agents causing occupational asthma. *Eur Respir J* 1997; 10: 2612–2629.

37. Lemière C, Chaboillez S, Malo JL, Cartier A. Changes in sputum cell counts after exposure to occupational agents: What do they mean? *J Allergy Clin Immunol* 2001; 107: 1063–1068.

38. Fahy JV, Liu J, Wong H, Boushey HA. Analysis of cellular and biochemical constituents of induced sputum after allergen challenge: A method for studying allergic airway inflammation. *J Allergy Clin Immunol* 1994; 93: 1031–1039.

39. Pizzichini MMM, Popov TA, Efthimiadis A, *et al.* Spontaneous and induced sputum to measure indices of airway inflammation in asthma. *Am J Respir Crit Care Med* 1996; 154: 866–869.

40. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears M, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000; 161: 475–478.

41. Spanevello A, Confalonieri M, Sulotto F, *et al.* Induced sputum cellularity. *Am J Respir Crit Care Med* 2000; 162: 1172–1174.

42. Maestrelli P, Calcagni PG, Saetta M, *et al.* Sputum eosinophilia after asthmatic responses induced by isocyanates in sensitized subjects. *Clin Exp Allergy* 1994; 24: 29–34.

43. Obata H, Cittrick M, Chan H, Chan-Yeung M. Sputum eosinophils and exhaled nitric oxide during late asthmatic reaction in patients with western red cedar asthma. *Eur Respir J* 1999; 13: 489–495.

44. Lemière C, Pizzichini MMM, Balkissoon R, *et al.* Diagnosing occupational asthma: use of induced sputum. *Eur Respir J* 1999; 13: 482–488.

45. Lemière C, Chaboilliez S, Trudeau C, *et al.* Characterization of airway inflammation after repeated exposures to occupational agents. *J Allergy Clin Immunol* 2000; 106: 1163–1170.

46. Franco A Di, Vagaggini B, Bacci E, *et al.* Leukocyte counts in hypertonic saline-induced sputum in subjects with occupational asthma. *Respir Med* 1998; 92: 550–557.

47. Anees W, Huggins V, Pavord ID, Robertson AS, Burge PS. Occupational asthma due to low molecular weight agents: eosinophilic and non-eosinophilic variants. *Thorax* 2002; 57: 231–236.

48. Park HS, Jung KS, Hwang SC, Nahm DH, Yim HE. Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. *Clin Exp Allergy* 1998; 28: 724–730.

49. Park HS, Jung KS, Kim HY, Nahm DH, Kang KR. Neutrophil activation following TDI bronchial challenges to the airway secretion from subjects with TDI-induced asthma. *Clin Exp Allergy* 1999; 29: 1395–1401.

50. Vandenplas O, Delwiche JP, Jamart J, Van de Weyer R. Increase in non-specific bronchial hyperresponsiveness as an early marker of bronchial response to occupational agents during specific inhalation challenges. *Thorax* 1996; 51: 472–478.

51. Malo JL. The case for confirming occupational asthma: why, how much, how far? (revisited). *Eur Respir J* 1999; 13: 477–478.

52. Kharitonov SA, Yates D, Robbins RA, Logan-Siclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996; 153: 454–457.

53. Dupont LJ, Rochette F, Demedts MG, Verleden GM. Exhaled nitric oxide correlates with airway hyperresponsiveness in steroid-naive patients with mild asthma. *Am J Respir Crit Care Med* 1998; 157: 894–898.

54. Allmers H, Chen Z, Barbinova L, Marcynski B, Kirschman V, Baur X. Challenge from methacholine, natural rubber latex, or 4,4′-diphenylmethane diisocyanate in workers with suspected sensitization affects exhaled nitric oxide (change in exhaled NO levels after allergen challenges). *Int Arch Occup Environ Health* 2000; 73: 181–186.

55. Kopferschmitt-Kubler MC, Ameille J, Popin E, *et al.* Occupational asthma in France: a 1-yr report of the Observatoire National de Asthmes Professionnels project. *Eur Respir J* 2002; 19: 84–89.

56. Moscato G, Dellabianca A, Maestrelli P, *et al.* Features and severity of occupational asthma upon diagnosis: an Italian multicentric case review. *Allergy* 2002; 57: 236–242.