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Review article

Induced sputum in the diagnostic of occupational asthma

Occupational asthma is, nowadays, the most frequent occupational lung disease in industrialised countries. Since its diagnosis implies aspects on worker's health and, also, medico-legal and economic aspects, a correct ethiopathogenic diagnosis is of great importance. Traditionally, the diagnostic procedure for occupational asthma is based on a compatible clinical and occupational history, positive immunological tests against the suspected agent (whenever indicated), and compatible functional respiratory tests. To date, the gold standard for the ethiological diagnosis is the specific bronchial challenge test with the suspected agents, considering it positive if a 20%, or greater, fall in FEV₁, from baseline values, is reached. In the last decade, the induced sputum technique has been started to be used as a complementary tool for the study of occupational asthma, with promising results. In this article, the literature and the current status of the topic are reviewed, and potential prospective uses of this technique are considered.

Key words: Occupational asthma. Induced sputum. Diagnosis. Review.

Utilidad del esputo inducido en el diagnóstico del asma profesional

El asma profesional es, hoy en día, la enfermedad respiratoria laboral más frecuente en los países industrializados. Su diagnóstico comporta aspectos de salud del trabajador y también aspectos médico-legales y económicos, por lo que un diagnóstico etiopatogénico correcto es de gran importancia. Tradicionalmente, el procedimiento diagnóstico del asma profesional está fundamentado en una historia clínico-laboral compatible, unas pruebas inmunológicas positivas frente al agente sospechado (cuando están indicadas) y unas pruebas funcionales respiratorias compatibles. Hasta la fecha, el patrón oro para el diagnóstico etiológico lo constituye la prueba de provocación bronquial específica con los agentes sospechados, que se considera positiva si se alcanza un descenso del FEV₁ superior al 20% del valor basal. En la última década, se ha comenzado a utilizar la técnica del esputo inducido como herramienta de apoyo al estudio del asma profesional, con resultados prometedores. En este artículo se realiza una revisión bibliográfica y del estado actual del tema y se consideran los usos potenciales futuros de esta técnica.

Palabras clave: Asma profesional. Esputo inducido. Diagnóstico. Revisión

Occupational asthma is at present the occupational respiratory disease with the highest prevalence in industrialised countries such as the USA and Canada¹, where it exceeds even the pneumoconioses. There are no reliable statistics for Spain but, in all probability, the situation is the same or a very similar one. There are a number of definitions of occupational asthma; in all of them the concept of reversible airway obstruction upon exposure to agents, which may be sensitising or not, that are present at the workplace. Most authors tend to exclude cases of pre-existing asthma or bronchial hyperreactivity that are exacerbated by chemical or physical irritants present at the workplace. The Editors of a very significant reference book, "Asthma in the Workplace", define occupational asthma as "a disease characterised by variable airflow restriction and/or bronchial hyperreactivity due to causes and conditions attributable to a concrete occupational environment and not to stimuli existing outside the workplace. Two types of occupational asthma may be differentiated based on the presence or absence of a latency period preceding its apparition: (1) immunologic (asthma with a latency period) and (2) non-immunologic (reactive airway dysfunction syndrome or irritant-induced asthma)"².

The diagnosis of occupational asthma must be established according to scientific criteria. However, in Spain, three minimum criteria must be met for the asthmatic disease evidenced by a worker to be legally considered to be occupational and thus for the worker to be eligible to disability compensation, to wit³:

(1) Asthmatic disease, immunological or not in mechanism, that has developed as a consequence of work performed under payment for others; (2) The occupational activity performed by the worker must be included as a possible cause in the listing of occupational diseases defined by Law, and (3) The disease must be brought about by the action of elements or conditions which, according to the said listing, are applicable in each particular case.

As regards the pathogenetic mechanism, there is still legal debate about whether the reactive airway dysfunction syndrome should be considered to represent an occupational disease or an occupational accident. This debate exceeds the boundaries of the present review.

Table I. Diagnostic procedure in occupational asthma

1. <i>Demonstration of the presence of bronchial asthma</i>
• Compatible clinico-occupational history
• Obstructive spirometric findings with positive bronchodilator test
• Positive bronchial hyperreactivity test
2. <i>Demonstration of the occupational pattern of bronchial asthma</i>
• Compatible clinico-occupational history
• Spirometric changes between the at-work and sick-leave situations
• Serial peak flow rate measurements in the at-work and sick-leave situations
• Changes in bronchial hyperreactivity between the at-work and sick-leave situations
3. <i>Demonstration of the aetiological agent</i>
• Clinico-occupational history concordant with exposure
• Concordant hygienic study
• Immunological study (high-molecular weight agents)
• Positive specific bronchial challenge test (low-molecular weight agents; eventually high-molecular weight agents)

Generally speaking, the diagnostic work-up and procedure for occupational asthma includes the following (Table I):

1) A compatible clinico-occupational history, with an adequate correlation between occupational exposure and the apparition of symptoms.

2) A compatible immunologic study in the case of high-molecular weight substances.

3) Compatible lung function tests: (a) spirometric exposure / no exposure variations; (b) exposure / no exposure variations in bronchial hyperreactivity, and (c) exposure / no exposure variations in the serial peak flow rate recordings. Regarding this particular point, Quirce et al.⁶ have already pointed out its scarce usefulness because of its easy manipulation by the worker.

4) The specific bronchial challenge test with the suspected aetiological agent(s). This test is still considered to represent the gold standard in the aetiological diagnosis of occupational asthma. It is arbitrarily accepted, as the criterion for a positive test, that a 20% fall in the FEV₁ must be induced after exposure to the causative agent as compared to the baseline value. However, a negative test does not conclusively exclude the diagnosis of occupational asthma, either because the adequate causative agent is not being used, because of errors in carrying out the test, or because the worker may ha-

Abbreviations:

TD: toluene diisocyanate; mRNA: messenger ribonucleic acid; IL-8: interleukin 8; IL-5: interleukin 5; FEV₁: Forced Expiratory Volume in the first second; PC₂₀: concentration of the agent used in the challenge test that causes a 20% fall in the FEV₁ as compared to the baseline value; PD₂₀: dose of the agent used in the challenge test that causes a 20% fall in the FEV₁ as compared to the baseline value; 95% CI: 95% confidence interval.

Table II. Summary of literature data on induced sputum in occupational asthma

Authors (year)	Subjects (Active/Control)	Occupational agent	Parámetros	When performed	Observed changes	Other tests
Maestrelli et al. ⁹ (1994)	A: 9 OA C: 4 (AA, NAA, NANA)	TDI or MDI	DCC	Pre-SBCT 8, 24 and 48 h post-SBCT	Significant Eos ↑ after 8 and 24 h Weak ↑ Eos / magnitude of the FEV1-SBCT response correlation No immediate / late BCT results differences	Metacholine
Park et al. ¹⁰ (1998)	A: 6 OA C: 6 (AA, mites)	Grain dust	IL-8 / Albumin	Pre-SBCT 7 h post-SBCT	Significant ↑ IL-8	ELISA IgG and IgE Bronchial biopsy Metacholine
DiFranco et al. ¹² (1998)	A: 24 OA (16 LMWA, 8 HMWA) C: 38 (24 NOA, 14 NANA)	TDI (14) MDI (2) Flour (5) Tobacco / Wood (3)	TCC DCC	Baseline No SBCT performed	Higher Neut % in OA-LMWA > OA-HMWA = NOA > NANA Higher Eos % in OA-HMWA > NOA > OA-LMWA	Serum ECP Eos in peripheral blood BHR with hypertonic saline
Lemière et al. ¹⁶ (1999)	A: 10 OA C: 6 OE without demonstrated OA	Not clearly specified: 6 LMWA 4 not determined	TCC DCC	After 4 weeks back at work (BAW) After 4 weeks' sick leave (SL)	↑ Eos BAW vs. SL ↑ ECP BAW vs. SL No ≠ Neut BAW vs. SL significant correlation Eos/BHR	Eos and ECP in peripheral blood Metacholine
Park et al. ¹¹ (1999)	A: 8 OA C: 5 OE without demonstrated OA	TDI	MPO / Albumin IL-8 / Albumin	Pre-SBCT 7 h post-SBCT	Significant correlation ECP/BHR Significant ↑ IL-8 and MPO Correlation ↑ IL-8 / ↑ MPO	Neut in peripheral blood Metacholine
Obata et al. ¹⁵ (1999)	A: 9 OA C: 8 OE without demonstrated OA	Plicatic acid	DCC	Pre-SBCT 6 and 24 h post-SBCT	Significant ↑ Eos after 6 and 24 h Significant neg. Correlation ↑ Eos at 6 h / FEV1 fall	Expired nitric oxide
Alvarez et al. ¹⁷ (1999)	A: 1 OA C: the same subject, with barley	L. destructor	DCC, ECP, Tryptase	Pre-SBCT 30 min and 18 h post-SBCT	↑ tryptase after 30 min ↑ Eos and epithelial cells after 18 h ↑ ECP after 18 h	Metacholine
Leigh and Hargreave ²¹ (1999)	1 case	Cutting fluids	DCC	Initial and serial / 6 months	Sterile neutrophylia Improvement SL vs. BAW Correlation with spirometric changes and PC20-Met	Metacholine
Lemière et al. ²⁰ (2000)	A: 15 OA (8 HMWA, 7 LMWA) C: 0	Flour (6) Red cedar (3) HDI (2) MDI (1) Guinea pig (1) Latex (1) Tea (1)	TCC, DCC IL-5-mRNA and Eotaxin-mRNA positive cells	7 h after: Control BCT SBCT at 1/2PD20 SBCT at PD20 SBCT at higher doses or longer times	↑ Eos, IL-5-mRNA and Eotaxin-mRNA positive cells as compared to control day inflammatory changes precede the 20% fall in FEV1 in SBCT no correlation ↑ Eos/magnitude of FEV1 fall	Metacholine

Table II. Summary of literature data on induced sputum in occupational asthma (continuacion)

Authors (year)	Subjects (Active/Control)	Occupational agent	Induced sputum		Other tests
			Parameters	When performed	
Alvarez et al. ¹⁸ (2001)	3 OA	Rapeseed flour	DCC ECP	Pre-SBCT 24 h post-SBCT	Metacholine ↑ Eos and ECP in all three cases ↑ PC20 in all three cases 1 case with negative SBCT
Quirce et al. ¹⁹ (2001)	2 OA	Cyanoacrylates	DCC	Pre-SBCT 3 and 24 h post-SBCT	Metacholine ↑ Eos 3 h post-SBCT ↑ BHR
Lemière et al. ¹³ (2001)	A: 17 OA C: 14 OE without demonstrated OA and 10 NOA	Flour (8) Isocyanates (9)	TCC, DCC	7 h after: control BCT SBCT with progressive ↑ doses in successive days	Metacholine ↑ Eos (total and %) and ↑ Neut (total) in OA ↑ Eos (total and %) in non-OA EL no immediate/late BCT differences establishment of test sensitivity for various cut-off points (see text) establishment of SBCT probability on the basis of IS and PC20-Met (see text)

ve been separated from the causative agent for a protracted period of time (although, in our own experience, the specific bronchial challenge test remains positive for many years after the cessation of exposure). Similarly, not all positive tests are indicative of the aetiology, particularly if the individual is challenged with irritant concentrations of the agent⁷.

Over the last few years there have been a number of attempts to validate the induced sputum technique for the diagnosis of asthma. This technique is relatively easy to carry out, and the procedure is described in detail elsewhere⁸. Basically, it consists in having the subject inhale hypertonic saline at increasing concentrations and during a definite period, a manoeuvre that stimulates expectoration. In this way, samples of sputum can be collected that are representative for the lower airways and which, when adequately processed, render possible the analysis of inflammatory cells and of various inflammatory markers, such as definite products of the eosinophil (i. e., eosinophil cationic protein, ECP) or different interleukins.

In 1994 the first publication by Maestrelli et al.⁹ appeared in which the use of this technique in the diagnosis of occupational asthma was described. Other groups of investigators have then studied various biologic parameters in induced sputum samples of subjects with occupational asthma. In all these studies the technique is first performed at baseline, before the worker is exposed to the suspected causative agent, and then again, after various time intervals, after the natural or voluntary (challenge) exposure of the worker. Overall, these studies describe an increase of the inflammatory infiltrate, usually with predominance of the eosinophil population, which very often correlates with an increase in the non-specific bronchial hyperreactivity. However, the use of this apparently promising technique in the diagnosis of occupational asthma must still be studied and examined in detail. The aim of the present paper is to review the various published works in which the induced sputum technique has been used as a diagnostic procedure in workers with occupational asthma (Table II), highlighting those aspects that should be examined in more detail and standardised.

INDUCED SPUTUM IN OCCUPATIONAL ASTHMA VS NON-EXPOSED SUBJECTS

It is convenient to review first of all the published data analysing the results achieved with the induced sputum technique in subjects with occupational asthma as

compared to control subjects not exposed to occupational agents. There are five studies available in the literature that include control groups formed by subjects with non-occupational atopic asthma (mites), non-asthmatic atopics and healthy subjects, who submitted to bronchial challenge testing with the same occupational agent as the subjects with occupational asthma (isocyanates, grain dust, flour, tobacco, woods) (Table II)⁹⁻¹³.

Maestrelli et al.⁹ performed differential cell counts on the induced sputum before and 8, 24 and 48 hours after the specific bronchial challenge test with isocyanates. They observed a significant increase in the eosinophil counts in the subjects with occupational asthma due to isocyanates as compared to the control subjects, between 8 and 24 hours after the challenge test. Even though an increase was seen in the neutrophil counts, these changes were non-significant as compared to those in the control subjects, who also evidenced slight increases in this cell population at the 24-hour timepoint. No significant changes were seen in the lymphocyte or macrophage counts in any group at any timepoint after the specific bronchial challenge tests.

Contrary to this, in the study of Park et al.¹⁰ a significant increase was seen in the neutrophil and mast cell counts and in the IL-8 (neutrophil chemotaxis) levels in induced sputum after the specific bronchial challenge test with grain dusts. Remarkably, these authors did not see changes in the levels of activated eosinophils or lymphocytes between the subjects with occupational asthma and the control subjects. This paper does not state whether there were any changes in the eosinophil counts.

This same group of investigators published a revision of a group of 15 subjects with occupational asthma [15 cases due to toluene diisocyanate (TDI) and 6 caused by grain dusts], in which a similar number of exposed subjects and also 6 patients with mite-induced asthma were used as controls¹¹. As compared to the latter six, the bronchial biopsies of the subjects with occupational asthma evidenced a significant increase of the neutrophil, mast cell and activated eosinophil populations. There was also increased neutrophil chemotactic activity in the sera of the subjects with occupational asthma (both TDI- and grain-dust-induced) 30 minutes after the specific bronchial challenge. These changes correlated with increases in the IL-8 and neutrophil myeloperoxidase levels in the induced sputum from subjects with occupational asthma collected after the challenge test.

Di Franco et al.¹² used a different study design. These authors used the induced sputum technique in baseline

conditions, without a specific bronchial challenge test, in four study groups: two groups of subjects with occupational asthma (caused by high-molecular weight agents in 16 cases and by low-molecular weight agents in 8; Table II) who were currently exposed, after a one-month period without corticosteroid therapy; one group of 24 subjects with non-occupational asthma (17 atopics and 7 non-atopics), and one group of 14 healthy subjects. The results achieved were quite remarkable. In the first place, there were no significant differences in the total inflammatory cell counts between the four groups of study subjects. However, the percentage neutrophil count was significantly higher in the patients with occupational asthma caused by low-molecular weight agents, without differences between those with occupational asthma due to high-molecular weight agents and those with non-occupational asthma. Quite to the contrary, the percentage eosinophil count was lower in low-molecular weight agent occupational asthma than in high-molecular weight agent occupational asthma or in non-occupational asthma. Similarly, the peripheral blood eosinophil counts were significantly increased in the latter two groups as compared to that of low-molecular weight agent occupational asthma, although there were no significant changes in the serum levels of eosinophil cationic protein in any of the four study groups. The percentage counts of macrophages and lymphocytes were lower in the healthy subjects as compared to the asthmatics of any aetiology, no significant differences being detectable between the latter.

Recently, Lemièrre et al.¹³ have demonstrated an overall significant increase of the eosinophil counts, both in absolute numbers and in percentages, and of the neutrophil counts in absolute numbers –but not in percentages– in a group of 17 subjects with occupational asthma and positive specific bronchial challenge test, while they observed no significant changes in any of the cell populations studied (eosinophils, neutrophils, lymphocytes and macrophages) in the induced sputum of subjects with non-occupational asthma exposed to frequently-used agents (cyanoacrylates and flour).

The data provided by these studies thus indicate that in subjects with occupational asthma, regardless of its being caused by high- or low-molecular weight agents, there is a significant increase in the eosinophil and/or neutrophil counts in the induced sputum after the specific bronchial challenge test as compared to subjects without occupational exposure. It would seem that both cell types can play a role in the pathogenesis of occupational asthma.

ma, although it is still unclear which type of cellularity is predominant in each type of exposure. Apparently, under conditions of natural exposure¹⁰ a neutrophilic infiltrate would predominate in subjects with low-molecular weight agent-induced occupational asthma, while eosinophils would predominate in asthma caused by high-molecular weight agents. Artificial exposure (the specific bronchial challenge test) seems to activate the inflammatory infiltrate, both neutrophilic and eosinophilic, in both types of occupational asthma. However, it should be stressed that in the study of Maestrelli et al.⁹ the neutrophil count also increased in control subjects after isocyanate challenge, although to a lesser degree and at a later timepoint (24 h) as in the group with occupational asthma. It is well known that the induced sputum technique *per se* may induce an increase in the neutrophil counts. For this reason, a slight increase in the neutrophil count in the induced sputum of an individual with a negative specific bronchial challenge test should be interpreted with caution.

In this context, the data should now be analysed that are derived from studies comparing subjects who, being exposed to the same causative agent in the occupational environment, differ in their response to the specific bronchial challenge test.

EXPOSED SUBJECTS: EXPOSURE / POSITIVE BRONCHIAL RESPONSE CORRELATION VS. EXPOSURE / NEGATIVE BRONCHIAL RESPONSE CORRELATION

Four published studies compare the results obtained in induced sputum in workers with occupational asthma caused by a known aetiological agent to those from workers in whom, while being exposed to the same agent(s) at their workplace and evidencing respiratory symptoms, either there is a negative specific bronchial challenge test or an occupational cause-effect relationship cannot be demonstrated^{11,13,15,16}.

The study by Park et al.¹¹ (Table II) includes eight subjects with occupational asthma caused by TDI, demonstrated by a positive specific bronchial challenge test, and five control subjects exposed to TDI who experienced respiratory symptoms while at their workplaces but whose specific bronchial challenge tests with that agent were negative. The subjects with positive specific challenge test evidenced a significant increase of the neutrophil chemotactic activity in serum and of the IL-8/albumin and mye-

loperoxidase/albumin ratios in induced sputum 7 hours after the specific bronchial challenge test as compared to their baseline values. The increase in the IL-8/albumin ratios ranged between 1.8 and 5.7-fold, and it was correlated to the increase in myeloperoxidase. It should be pointed out that the mean occupational exposure time of the subjects with positive specific bronchial challenge tests was considerably higher than that of those with negative tests, a factor that might have interfered in the results. It is possible that the latter group of workers might have in the end result developed occupational asthma due to TDI if the exposure had persisted. This study had been designed for the evaluation of the neutrophil response, which appears to be important in isocyanate occupational asthma¹⁴, and provides no data regarding the eosinophil infiltrate or regarding other cell types.

Obata et al.¹⁵ studied 17 sawmill workers with a minimum duration of exposure to red cedar (plicatic acid) of six months. Nine of them had a positive specific bronchial challenge test with plicatic acid, while eight had a negative test. Both groups had very similar mean values for age, duration of exposure and duration of symptoms. The authors carried out the induced sputum technique, with total and differential cell counts, at baseline and 6 and 24 hours after the specific bronchial challenge test. They also analysed the expired nitric oxide, but this will not be dealt with in detail in the present review. In the baseline assessments there were no significant differences either in the total or in the differential cell counts between specific challenge test responder and non-responders. After the specific bronchial challenge test with plicatic acid, there was in the responders a significant increase in the percentage count of eosinophils in induced sputum 6 and 24 hours after the test, without perceptible changes in the total cell count or in the counts of neutrophils or other cell types. In the non-responders (negative specific bronchial challenge test) there were no significant changes in the mean eosinophil count after the challenge, although three subjects in this group did evidence increased eosinophil counts in induced sputum 6 hours after the specific challenge test.

Lemière et al.¹⁶ used a different study design without specific bronchial challenge. They studied 16 workers, whom they classed into "occupational asthma" and "no occupational asthma" categories based on the worsening of symptoms while at work together with a greater than 20% fall in the FEV₁ and a greater than fourfold increase in the PC₂₀-metacholine during at-work as compared to sick lea-

ve periods. They carried out the induced sputum technique in all these workers within the last 48 hours of exposure of a period of four weeks at work and again after a 4-week sick leave period. They measured the total and differential cell counts and the eosinophil cationic protein levels in induced sputum and also the eosinophil counts and eosinophil cationic protein levels in peripheral blood. The causative agents were not clearly identified: in the "occupational asthma" group six subjects were exposed to low-molecular weight agents and four to unknown agents, while in the "no occupational asthma" one four subjects were exposed to low-molecular weight agents, one was exposed to cotton, and one to unknown agent(s). The authors observed a significant increase in the average eosinophil count and eosinophil cationic protein level in the induced sputum of the occupational asthma subjects during the at-work periods. These same subjects also evidenced a significant increase in the eosinophil count in peripheral blood (absolute numbers), even though with actual counts within the normality range, and a trend towards higher levels of serum eosinophil cationic protein. These changes were not seen in the "no occupational asthma" group. It bears pointing out that one subject in the "occupational asthma" group did not evidence changes either in the eosinophil counts or in the eosinophil cationic protein levels in the induced sputum collected 24 hours after the last exposure as compared to the sick leave values. On the other hand, the authors did not observe changes in the counts of neutrophils or of other cell types in the patients with "occupational asthma".

Recently, again the group of Lemièrè¹³ have observed, as already commented, an overall significant increase in both the absolute numbers and percentage counts of eosinophils, and of the absolute numbers –but not of the percentage counts– of neutrophils in a group of 17 subjects with occupational asthma and positive specific bronchial challenge test, while a group of 14 workers who were exposed to occupational agents and had respiratory symptoms, but with a negative specific bronchial challenge test, also showed a significant –though lesser in degree– increase of the eosinophil counts, both in absolute numbers and in percentage.

The analysed studies appear to show clearly that there is, in general terms, a significant increase of the eosinophyllic or neutrophyllic infiltrate according to the type of causative agent and of the activation markers (eosinophil cationic protein or myeloperoxidase, respectively) in subjects with occupational asthma as compared to workers

in whom, even though evidencing respiratory symptoms in relation to their workplace, the existence of occupational asthma cannot be demonstrated through specific bronchial challenge testing or other surrogate parameters (changes in FEV₁ or in the PC₂₀-metacholine). Thus, the changes in cell counts and in the activation markers that are detected in induced sputum appear to be specific for occupational asthma, and in any case more sensitive than the parameters determined in serum. However, and based on the study of Lemièrè et al.¹⁵, it should be borne in mind that these changes may be absent even when there is an evident diagnosis of occupational asthma through a positive specific bronchial challenge test, and, conversely, that they may also be present even in the absence of a positive specific test. Hence the need to have available sensitivity and specificity data, which will be discussed later on.

OTHER STUDIES WITHOUT CONTROL GROUP

In the reviewed literature there are five further studies in which the induced sputum technique has been used as diagnostic support in cases of occupational asthma¹⁷⁻²¹ (Table II).

In Spain Alvarez et al.¹⁷ published in 1999 a case report of occupational asthma caused by *Lepidoglyphus destructor* in a silo worker with ten years' exposure to various different grains. They observed increased eosinophils and epithelial cells but no increases of other cell types, and an increase in the eosinophil cationic protein in the induced sputum collected 18 hours after the specific bronchial challenge test with *L. destructor*. They also observed an increase of the mast cell tryptase in sputum 30 minutes after the challenge. This study is interesting, as it is the only one to show the specificity of the changes as related to the causative agent by having the same worker perform a challenge test with barley, in which no changes were seen in the sputum after the challenge with a product the worker usually manipulated.

Again Alvarez et al.¹⁸ published three cases of occupational asthma caused by rapeseed flour, in which an increase in the percentage eosinophil counts and in the eosinophil cationic protein levels were seen in the induced sputum collected 24 hours after the specific bronchial challenge test, together with a fall in the PC₂₀-metacholine. It is noteworthy that one of the cases, with sporadic exposure to rapeseed flour, evidenced an increase in the eosino-

phil cationic protein levels and in the eosinophil counts in induced sputum and a fall in the PC₂₀-metacholine after the challenge with rapeseed flour, even though the results of the challenge test with this agent were negative (as the fall in the FEV₁ was less than 20%).

Also in Spain, Quirce et al.¹⁹ published two cases of workers with occupational asthma in relation to exposure to cyanoacrylate adhesives. The specific bronchial challenge test with cyanoacrylate was positive in both cases, and an increase in the eosinophil counts in induced sputum as compared to the baseline one was evident already three hours after the bronchial challenge exposure. The specific bronchial challenge test in two asthmatics that were not exposed to the occupational agent did not elicit any form of reaction. In one of these two workers an increase in the non-specific bronchial hyperreactivity was demonstrated after the specific challenge test; in the other one no metacholine test was performed after the specific bronchial challenge one.

A most interesting study is the one published by Lemièrre et al.²⁰, with a quite different approach. These investigators selected 15 patients with already-diagnosed occupational asthma (caused by high-molecular weight agents in eight and by low-molecular weight agents in seven), in whom the individual PD₂₀ values for the respective aetiological agents were obviously already known. In each individual they carried out a control challenge test with inert substances and, in successive days, specific bronchial challenge tests at one-half of the known PD₂₀ ($\frac{1}{2}$ PD₂₀), or with higher doses or inhalation times if required. The induced sputum was collected and analysed 7 hours after each test, including the control ones, for assessment of the cell counts and of the counts of cells positive for IL-5 mRNA and for eotaxin mRNA. They also assessed the changes in bronchial hyperreactivity. Overall, and as compared to the control day, they observed an increase in the eosinophil counts and in the detection of IL-5 mRNA-positive and eotaxin mRNA-positive cells in the specific challenge days; there was also an increase in bronchial hyperreactivity. The most striking finding in this study is that the inflammatory changes preceded the 20% fall in FEV₁ in the specific bronchial challenge tests, without significant differences between the bronchial challenge at $\frac{1}{2}$ PD₂₀ and at full PD₂₀. These changes were more evident in the subjects with occupational asthma induced by low-molecular weight agents than in those with high-molecular weight inductors. As will be discussed later on, these inflammatory changes correlated with the increase in non-specific bronchial hyperreactivity.

Finally, Leigh et al.²¹ reported the case of a female worker with occupational asthma induced by exposure to metal-cutting fluids. In this particular patient, they demonstrated a neutrophilic infiltrate in induced sputum that reverted upon cessation of exposure and recurred upon repeated exposures, in association to the corresponding changes in the spirometric assessments and in the non-specific bronchial hyperreactivity (PC₂₀-metacholine).

CORRELATION BETWEEN INFLAMMATORY CHANGES AND NON-SPECIFIC BRONCHIAL HYPERREACTIVITY

In the various case reports published¹⁷⁻²⁰ an increase in non-specific bronchial hyperreactivity is described in association to the increase of the inflammatory infiltrate in the induced sputum samples.

In one of the papers by Lemièrre et al.¹⁶ there was a highly significant negative correlation between the PC₂₀-metacholine values and the eosinophil counts and eosinophil cationic protein levels in the induced sputum. These authors were later confirmed by the same authors²⁰, who observed, in 15 workers with occupational asthma, a significant negative correlation between the PC₂₀-metacholine fall after the positive specific bronchial challenge test and the increased counts and of IL-5 mRNA-positive and eotaxin mRNA-positive cells in the induced sputum.

However, in the last study published by this group the reported correlation data for the changes in eosinophils and neutrophils and the non-specific bronchial hyperreactivity are rather weaker ($r = -0.4$, $p = 0.03$ and $r = -0.4$, $p = 0.01$, respectively).

Finally, Di Franco et al.¹², in their study of 24 workers with occupational asthma, observed a significant negative correlation between the percentage count of eosinophils in induced sputum and the response to the inhalation of hypertonic saline expressed as the duration of that inhalation. However, and surprisingly, they found no correlation at all between the percentage eosinophil counts and the PD₂₀-metacholine values.

Even so, it must be pointed out that in our own experience²², as well as in that of others²⁰, the non-specific bronchial hyperreactivity test may be negative or show no changes despite a positive specific bronchial challenge test.

CORRELATION BETWEEN INFLAMMATORY CHANGES AND SPECIFIC BRONCHIAL RESPONSE

As regards the correlation between the inflammatory infiltrate and the specific bronchial response, the available data are also controversial.

Lemière et al.²⁰ found no correlation whatsoever between the eosinophil count and the FEV₁ fall expressed as the area under the curve or as the maximum FEV₁ fall on the day of the specific asthmatic reaction. Neither did they find any correlation between the increase in the IL-5 mRNA-positive and eotaxin mRNA-positive cells (comparing the control and reaction days) and the FEV₁ fall on the day of the specific bronchial challenge test.

Opposite to this, Maestrelli et al.⁹ did observe a correlation between the eosinophil count in induced sputum and the magnitude of the FEV₁ response in the specific bronchial challenge test expressed as the area under the reaction curve ($r = 0.71$, $p = 0.014$), although not between the eosinophil count and the maximum FEV₁ fall.

On the other hand, Obata et al.¹⁴, in their study of nine workers with occupational asthma induced by plicatic acid (Table II), did indeed find a significant negative correlation between the percentage fall in FEV₁ in the specific bronchial challenge test and the percentage change in the percentage eosinophil count in induced sputum 6 hours after the specific challenge test ($r = -0.62$, $p < 0.05$), but not 24 hours after the test ($r = -0.19$, $p = 0.51$).

Finally, and so as to further complicate the subject, in the last study published by Lemière et al.¹³ the authors did observe a significant correlation between the maximum FEV₁ fall and the changes in the eosinophil ($r = 0.5$, $p < 0.001$) and neutrophil ($r = 0.6$, $p < 0.001$) counts.

Thus, the data published are indicative of a significant correlation, variable in degree, between the increase in the inflammatory (eosinophyllic and/or neutrophyllic) infiltrate and the increase both in the non-specific and in the specific bronchial hyperreactivity. The disparity of the data begs reflection on which pathogenetic mechanisms contribute to the specific bronchial response. It is quite possible that inflammation itself and the consequent changes are not the only responsible mechanism, but that the release of neuropeptides and the stimulation of non-adrenergic non-cholinergic sensitive pathways, particularly by low-molecular weight causative agents, also contribute to the phenomenon of bronchoconstriction.

Finally, neither Maestrelli et al.⁹ nor Lemière et al.^{13,20} have found differences in the inflammatory changes between individuals with an immediate asthmatic reaction and those with a delayed/dual one. Thus, the induced sputum technique does not appear to be a useful predictive tool in this context, and it is quite possible that the clinical history might better predict the possibility of a delayed response in the specific bronchial challenge test.

SENSITIVITY AND SPECIFICITY OF INDUCED SPUTUM IN THE DIAGNOSIS OF OCCUPATIONAL ASTHMA

For an assessment of sensitivity, the design of the already-discussed study of Lemière et al.²⁰ (Table II) is useful. As of that study, it is known that the inflammatory infiltrate (at least the eosinophyllic one) precedes the 20% fall in FEV₁ in the specific bronchial challenge test. It thus seems that the induced sputum technique is highly sensitive for detecting early changes in the bronchial asthmatic response.

However, a number of authors have reported an increase in the inflammatory infiltrate in induced sputum after the specific bronchial challenge test without a 20% FEV₁ fall ensuing. Thus, Obata et al.¹⁵ reported increased eosinophils in the induced sputum of three workers whose specific bronchial challenge tests with plicatic acid were negative. The same way, Alvarez et al.¹⁸ published the case of a worker with a clear-cut clinical history of occupational asthma induced by rapeseed flour whose specific bronchial challenge test was negative, but in whom an increase in non-specific bronchial hyperreactivity and in the eosinophil counts and eosinophil cationic protein levels in induced sputum could be demonstrated. Lemière et al.²⁰, in six subjects with known and diagnosed occupational asthma observed an increase in airway inflammation without changes in non-specific bronchial hyperreactivity, and in two subjects with negative specific bronchial challenge tests they observed a clinically significant increase in the eosinophil counts¹³. the explanation of this phenomenon might be that (a) these workers could have spent a long time without exposure to the causative agent –although this seems to be rather improbable, as in occupational asthma patients the specific bronchial challenge test may remain still positive for many years of avoidance of the agent and, furthermore, in the study of Obata et al.¹⁵ the longest period without contact with the causative agent had been

three months; (b) the subject might have been exposed to doses that were sufficient for inducing inflammatory cell chemotaxis but insufficient for inducing bronchospasm, or (c) because of the more extensive use of lower airway cellularity analysis due to the greater ease and absent invasiveness of the induced sputum technique, a greater number of cases of eosinophilic bronchitis may be being detected. The latter clinical entity, described by Gibson et al.²⁴, courses with eosinophilic bronchial inflammation but without the functional changes that are characteristic of asthma.

In the attempts to clarify the problem of the sensitivity of this technique, the last study by Lemière et al.¹³ has capital importance. That study compared the inflammatory changes in induced sputum and in the non-specific bronchial hyperreactivity (metacholine test) after the specific bronchial challenge test vs. the control day in three groups of subjects: (1) patients with occupational asthma and with a positive specific bronchial challenge test (FEV_1 fall >20%) (n = 17), (2) subjects with a history suggestive of occupational asthma and with a negative specific bronchial challenge test (n = 14), and (3) subjects with asthma of non-occupational aetiology and with a negative specific bronchial challenge test with cyanoacrylates or flours. Through the analysis of the diagnostic efficacy (ROC) curves the authors evaluated the optimal clinically significant cut-off points, that is those able to predict a 20% FEV_1 fall in the specific bronchial challenge test. These cut-off points are (a) an increase in the eosinophil counts in induced sputum by at least $0.26 \times 10^9/l$ (sensitivity 82%, 1 - specificity 8.3%) and (b) an at least 1.8-fold fall in the PC_{20} -metacholine as compared to the baseline value (sensitivity 73%, 1 - specificity 14%). Furthermore, by combining these two parameters, they determined the following probabilities of having a positive specific bronchial challenge test:

- Change in both parameters: 96% probability of a positive specific bronchial challenge test (95% CI: 70-99%).
- No change in either parameter: 4% probability of a positive specific bronchial challenge test (95% CI: 0.5-26%).
- Clinically significant change in the eosinophil count without clinically significant change in PC_{20} -metacholine: 66% probability of a positive specific bronchial challenge test (95% CI: 26-91%).
- Clinically significant change in the PC_{20} -metacholine without clinically significant change in the eosinophil

counts: 38% probability of a positive specific bronchial challenge test (95% CI: 12-74%).

These data derived from selected subjects may become very useful in day-to-day clinical practise. As Lemière et al.²⁰ demonstrated according to the reported data, an eosinophilic infiltrate already at $^{12}PD_{20}$ in the specific bronchial challenge test, it is conceivable that there may be no need to go on to higher doses in order to consider a specific bronchial challenge test to be positive, with a consequent reduction of the risk to the subject. On the other hand, the possibility should be studied to transfer these data to the normal daily situation in which the subject is exposed to the causative agent at his/her workplace in a natural manner. Stated otherwise it should be investigated whether a similar magnitude of changes in the induced sputum and in the PC_{20} -metacholine recorded during at-work periods as compared to sick leave periods provides a similar probability of a positive specific bronchial challenge test. Should this be the case, many specific bronchial challenge tests performed in the laboratory might turn out to be unnecessary and not required.

As for the specificity of the induced sputum technique, a highly illustrative study is that of Alvarez et al.¹⁷ on a worker with *L. destructor*-induced occupational asthma in whom inflammatory changes were seen after the positive specific bronchial challenge test with this mite, but without any change at all during the negative bronchial challenge test with barley, a product to which that worker was also occupationally exposed. Neither Maestrelli et al.⁹ nor Lemière et al.¹³ demonstrated any variation in the induced sputum composition as compared to the baseline one in subjects without occupational asthma who were exposed to isocyanates or flours or cyanoacrylates respectively.

Another situation described in the literature and that may occur in clinical practise is that of a worker with a positive specific bronchial challenge test and without inflammatory changes in the induced sputum after the said test²⁰, or perhaps that of a worker with a clinical history compatible with occupational asthma and with a drop in the PC_{20} -metacholine during at-work as compared to sick leave periods, but without inflammatory changes between those periods in the induced sputum¹⁶. This might perhaps have its explanation in a primary irritant effect of the involved agent that would cause a direct increase in bronchial hyperreactivity, or because there exist mechanisms different from the purely inflammatory ones, which we are as yet unable to identify in the pathogenesis of occupational asthma.

TIMING OF THE INDUCED SPUTUM TECHNIQUE

Table III shows when the induced sputum technique might be indicated nowadays in the diagnostic procedure of occupational asthma. Obviously, if one wishes to measure any change in a given parameter, an initial value for comparison is required. Thus, a baseline induced sputum test must be performed prior to the specific bronchial challenge test. Ideally, the induced sputum test should be carried out at baseline, after the day of the control test with an inert agent, and in the successive days after the specific challenge tests. Considering that, for this technique, salbutamol may be optionally administered prior to the inhalation of hypertonic saline so as to prevent bronchospasm, the technique cannot be applied at baseline on the same day the specific challenge test will be performed, as there is a risk of false-negative results.

The timing of the induced sputum test after the specific bronchial challenge test varies on the basis of the type of analysis to be performed. Thus, for instance, Alvarez et al.¹⁷ collected induced sputum samples as early as 30 minutes after the specific bronchial challenge test in order to measure the tryptase levels. If the aim is to measure

Table III. Application of the induced sputum technique to the diagnostic procedure in occupational asthma

1. *Demonstration of the presence of bronchial asthma*

- Compatible clinico-occupational history
- Obstructive spirometric findings with positive bronchodilator test
- Positive bronchial hyperreactivity test

2. *Demonstration of the occupational pattern of bronchial asthma*

- Compatible clinico-occupational history
- Spirometric changes between the at-work and sick-leave situations
- Serial peak flow rate measurements in the at-work and sick-leave situations
- Changes in bronchial hyperreactivity between the at-work and sick-leave situations
- Changes in the inflammatory infiltrate (and in the inflammation markers) in induced sputum between the at-work and sick-leave situations

3. *Demonstration of the aetiologic agent*

- Clinico-occupational history concordant with exposure
- Concordant hygienic study
- Immunological study (high-molecular weight agents)
- Positive specific bronchial challenge test (low-molecular weight agents; eventually high-molecular weight agents)
- Inflammatory changes in induced sputum after vs. Before specific bronchial challenge test, even with a FEV1 fall <20%

cell population changes most authors perform the technique between 6-8 hours and 24 hours after the bronchial challenge. Nevertheless, Quirce et al.¹⁹ observed an increase in the eosinophil counts in induced sputum already 3 hours after the specific bronchial challenge test. Maestrelli et al.⁹ performed serial analyses of induced sputum samples after the bronchial challenge, and observed a return of the eosinophil counts to practically the baseline values by 48 hours. Thus, it appears to be logical not to delay the induced sputum technique more than 24 hours after the specific bronchial challenge test, as longer delays would increase the probability that eventual cell population changes go undetected.

Another possibility is to apply the induced sputum technique after a 4-week at-work period and again after a 4-week sick leave period and compare the results of both

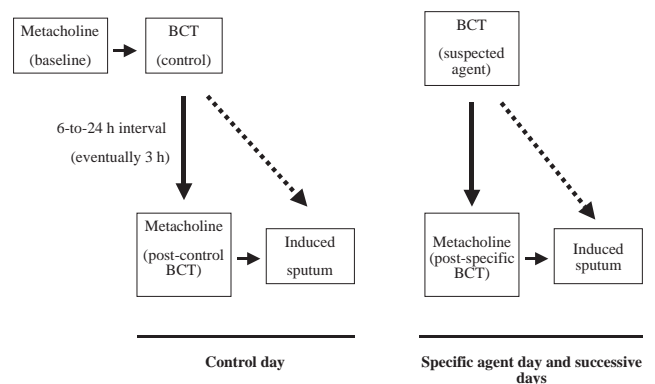


Fig. 1. Planning schedule for diagnostic bronchial tests with in-the-laboratory exposure. The option exists of carrying out the induced sputum technique without the foregoing metacholine test. BCT = bronchial challenge test.

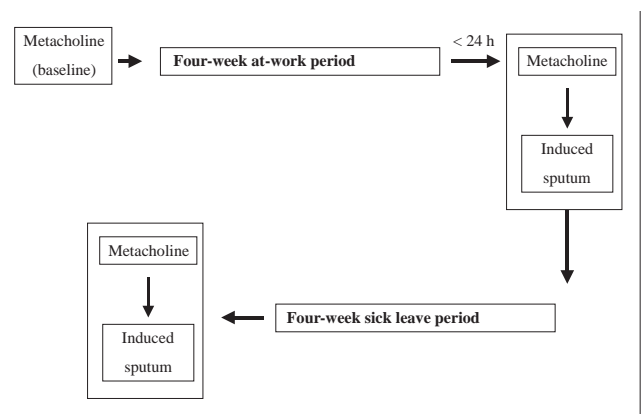


Fig. 2. Planning schedule for diagnostic bronchial tests with natural exposure.

analyses¹⁶. this modality, together with a non-specific bronchial hyperreactivity test, might represent a great help in the diagnosis of occupational asthma, particularly when it is not clearly known which is the aetiological agent involved, and might render unnecessary performing a variable number of specific bronchial challenge tests, these being more dangerous, more expensive and requiring more time.

Figures 1 and 2 illustrate possible options for the sequence of the various bronchial tests (specific and non-specific bronchial challenge tests and induced sputum), with "artificial" exposure in the laboratory or with "natural" exposure at the worker's usual workplace.

FURTHER USES OF THE INDUCED SPUTUM TECHNIQUE IN OCCUPATIONAL ASTHMA

In the same manner that a regression of the degree of bronchial hyperreactivity after cessation of occupational exposure to the causative agent has been observed²⁵⁻²⁹, the induced sputum technique might be used for indirectly assessing the degree of control of the bronchial inflammation in subjects who have withdrawn from exposure. Perhaps, however, this technique might be more useful for the follow-up of subjects who remain exposed and who receive therapy with inhaled corticosteroids³⁰, or for evaluating whether the institution of corrective measures aimed at reducing occupational exposure to the causative agent (such as a change in the workplace, an improvement of the industrial hygiene conditions, the use of individual protection equipment, etc.) is being really effective in preventing the persistence of occupational asthma.

Table IV. Application of the induced sputum technique to the diagnostic procedure in occupational asthma

- Support in the diagnosis of occupational asthma
- Evolutive follow-up of the workers with occupational asthma and of their response to therapy
- Assessment of the effects of:
 - Cessation of occupational exposure
 - Change of workplace
 - Adequacy of industrial hygiene measures
 - Application of individual protection equipment
- Aetiopathogenic investigation of existing and new occupational agents
- Replacement of the specific bronchial challenge test in the laboratory for the diagnosis of occupational asthma?

In addition, the induced sputum technique affords the possibility to measure inflammatory markers such as the eosinophil cationic protein, myeloperoxidase, interleukins, etc., which, in combination with other analytical methods (such as the quantitation of the expired nitric oxide¹⁵) may allow us to investigate the pathogenetic mechanisms of occupational asthma induced by existing or new occupational agents (Table IV).

SUMMARY

In summary, occupational asthma is the most frequent occupational respiratory disease, and its correct diagnosis, both syndromic and aetiologic, is highly important from the aspect of the worker's health and also from the medico-legal and economic ones. The induced sputum technique is an easy to perform diagnostic tool that is relatively inexpensive, minimally invasive and associated to little risk, and that provides considerable information about the inflammatory changes in the lower airways when it is adequately carried out and the subject is able to expectorate. These characteristics have led to its being increasingly used in the study of occupational asthma. The various studies reviewed document the possibility of detecting an increase in the inflammatory infiltrate after specific exposure to the causative agent. Although the eosinophyllic infiltrate seems to predominate, some authors have pointed out the importance of the neutrophyllic one; probably, however, both cell types participate to a greater or lesser extent according to the type (high or low molecular weight) of the causative agent. These changes may be detected as early as three hours after the specific bronchial challenge test, but not later than 24 hours after it. The induced sputum technique appears to have high sensitivity and high specificity for an increment in excess of $0.26 \times 10^9/l$ eosinophils/l. However, it should be borne in mind that unusual situations may occur, such as that of an eosinophyllic infiltrate with a negative specific bronchial challenge test or that of a positive specific bronchial challenge test without cell population changes in the induced sputum. The association of an increment in excess of $0.26 \times 10^9/l$ eosinophils/l and a greater than 1.8-fold drop in the PC_{20} -metacholine imply a 96% probability of having a positive specific bronchial challenge test. Even though these recently published data have been obtained in selected subjects, and they must still be confirmed in clinical practise, they will quite probably become very useful for the diagnosis of occupational asthma without the need to perform a specific bronchial challenge test that is associated to a greater risk and requires more time and more money. At present, the

certainty aetiological diagnosis of occupational asthma is still achieved through the specific bronchial challenge test and the –arbitrary– measurement of a greater than 20% fall in the FEV₁. The induced sputum technique has confirmed the agent-specific inflammatory mechanism but, as more experience is accumulated with it, it is quite possible that the procedure or the criteria for the diagnosis of occupational asthma may change within a few years. The usefulness of this technique in the diagnosis of occupational asthma under conditions of natural occupational exposure should be examined in more detail.

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