

Bronchial responsiveness to ultrasonic “fog” in occupational asthma due to low molecular weight chemicals

Antonio Dellabianca, MD; Pinuccia Omodeo, MD; Maria Cristina Colli, MD; Patrizia Bianchi, MD; Joseph Scibilia, MD; and Gianna Moscato, MD

Background: In a previous work we have demonstrated that the use of an indirect stimulus such as ultrasonically nebulized distilled water (UNDW, “fog”) challenge, in addition to methacholine, in the assessment of occupational asthma due to TDI improves our ability in detecting sensitized subjects.

Objective: In the present study we aimed to determine the validity of “fog” in the assessment of occupational asthma induced by various low molecular weight substances.

Methods: Forty subjects exposed to low molecular weight substances with work-related respiratory symptoms underwent methacholine, UNDW and specific bronchial challenge tests. Diagnosis of occupational asthma was made in 20 of 40 patients who developed bronchoconstrictive responses to the specific challenges (reactors). Sensitivity and specificity of UNDW alone, of methacholine alone, and of the combination of the two tests were determined at two different cutpoints of $PD_{20}FEV_1$ of methacholine, with the results of the specific challenge as the “gold standard.”

Results: Frequency and severity of bronchial hyperresponsiveness both to UNDW and to methacholine were significantly higher in reactors than in nonreactors. Ultrasonically nebulized distilled water had higher specificity (80% versus 60%) but lower sensitivity (65% versus 75% to 90%) than methacholine. As compared with methacholine alone, the combination in series of the two challenges (both challenges positive) substantially improved specificity (85% versus 60%).

Conclusions: In conclusion, in the assessment of low molecular weight chemical-induced asthma diagnosed with the specific challenge as the “gold standard,” UNDW challenge proves more specific than methacholine for occupational asthma, but it is considerably less sensitive. The combination of the two challenges results in very high sensitivity and high specificity for occupational asthma, therefore, although further investigations are required for the definitive diagnosis, it should be regarded as a useful means to confirm objectively a suggestive history.

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INTRODUCTION

Diagnosis of occupational asthma requires a stepwise approach that includes objective testing to confirm the history and permit diagnosis.^{1,2} The

crucial points are to demonstrate that the patient does indeed have asthma and that the asthma has a causal relationship with work.

Measurement of nonspecific bronchial hyperresponsiveness to pharmacologic agents is an important step, and in recent years it has assumed a central role in the diagnostic workup.^{3,4} The absence of bronchial hyperresponsiveness in a patient who has worked for 2 weeks under his usual conditions virtually rules out the diagnosis of occupational asthma. If bronchial hyper-

responsiveness is found, further testing is required to confirm that asthma is related to work. In fact, methacholine or histamine challenge is highly sensitive, but poorly specific for occupational asthma.^{4–6}

Bronchial responsiveness can be evaluated by various stimuli that act through different bronchoconstrictor mechanisms. Methacholine and histamine are so-called “direct” stimuli since they act directly on bronchial smooth muscle.⁷ “Indirect” stimuli, such as exercise, cold air and ultrasonically nebulized distilled water (“fog,” UNDW), act through intermediate mechanisms, such as release of mediators by cells in the bronchial lumen and submucosa.⁷ In nonoccupational asthma UNDW inhalation has been proven to be more specific than methacholine challenge in identifying asthma.^{8,9}

Recently, we evaluated whether the use of UNDW challenge could be of advantage in the assessment of isocyanate-induced asthma diagnosed with specific challenge as the gold standard.¹⁰ We found that UNDW challenge is more specific, but less sensitive than methacholine, and that the combination of both tests positive improves our ability in identifying subjects with TDI asthma.

In the present study we extended the above observations to occupational asthma induced by low molecular weight chemicals other than isocyanates. In particular, we aimed to evaluate whether “fog” could be useful in the clinical assessment of low molecular weight chemical-induced asthma diagnosed by specific challenge tests.

Specialization Schools of Allergology and Clinical Immunology and of Occupational Medicine, University of Pavia; Salvatore Maugeri Foundation, Occupational and Rehabilitation Clinic, IRCCS, Medical Center of Pavia, Department of Allergy and Clinical Immunology, Italy.

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MATERIALS AND METHODS

Subjects

We examined 40 consecutive subjects (19 men and 21 women) referred to our center because of probable occupational asthma due to low molecular weight chemicals (Table 1). Recruitment of subjects was discontinued when we had included 20 subjects with positive responses to the specific challenge (reactors) and 20 with negative responses (nonreactors).

Each subject had a history of occupational exposure to low molecular weight chemicals and reported respiratory symptoms (shortness of breath, wheezing, chest tightness or dry cough) related in some way to exposure. Most subjects were employed in the plastic resin industry, others were employed in chemical industries or were tanners, welders, or grinders. Other subjects worked as nurses, hairdressers or self-employed carpenters. Data on the type of exposure were derived by safety data sheets and/or results of previous environmental surveys provided by employers. Each subject was examined at the end of a working period of at least 2 weeks.

Each subject underwent clinical interview in order to establish the type of job and exposure, the symptoms, the relationship between occupational exposure and symptoms, the duration of occupational exposure before the onset of symptoms, the duration of symptoms before diagnosis, and smoking habits. As for the latter, subjects were divided into current smokers, ex-smokers (with at least 6 months' abstinence and a previous consumption of more than 3 pack-years) and nonsmokers.¹⁰

At the time of the diagnosis of occupational asthma, each subject underwent pulmonary function tests, non-specific bronchial challenges with methacholine (Mch) and ultrasonic "fog," and specific bronchial challenge.

All subjects had been free of clinical symptomatic respiratory infection for at least 2 weeks. No subject took any anticholinergic drug, adrenergic bron-

chodilator, or sodium cromoglycate within the 24 hours preceding each study day, nor did they take slow release theophylline or nonsteroidal antiinflammatory drugs during the 48 hours that preceded each study day. No subject was taking corticosteroids at the time of testing.

Thirty-four subjects underwent intradermal skin tests with a panel of 11 common allergenic extracts. A subject was considered atopic if there was a personal history of eczema or allergic rhinitis, or a positive skin reaction (wheal > 5 mm) to one or more allergens.¹⁰

Pulmonary Function Tests, Methacholine and UNDW Challenge
Spirometry was performed by means of a computerized (M 24 Olivetti) water-sealed spirometer (Biomedin,

Padova, Italy). Forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were taken as the best of three satisfactory respiratory tracings.

Methacholine challenge was carried out on a day before the "fog" challenge, during the same week. Methacholine (Lofarma Allergeni, Milano, Italy) was delivered by a nebulizer connected to a dosimeter (Mefar, Brescia, Italy). A cumulative, doubling dose schedule was used. Three nebulizers (n.1, 2, and 3), each with a different output (n.1 and n.2: 11 μ L; n.3: 17 μ L), were used. Each nebulizer was filled, respectively, with 2 mL of diluent control (n.1), 2 mL of 0.2% methacholine solution (n.2), and 2 mL of 1% methacholine solution (n. 3), after its passage through a 0.2 milli-

Table 1. Clinical Features of the Subjects

	Reactors	Nonreactors
N	20	20
Age, yr*	33 $\pm$ 2.37	38.2 $\pm$ 2.57
FEV ₁ % of predicted*	95.8 $\pm$ 3.6	103.55 $\pm$ 4.15
Atopy, %†	25	16.7
Smoking habits		
Current smokers (%)	15	24
Nonsmokers (%)	40	43
Ex-smokers (%)	45	33
Duration of symptoms before diagnosis, yr*	2.26 $\pm$ 2.86	2.37 $\pm$ 1.73
Duration of exposure before onset of symptoms, yr*	13.31 $\pm$ 1.89	13.35 $\pm$ 1.5
Type of asthmatic response		
Immediate (n)	12 (60%)	
Late (n)	6 (30%)	
Dual (n)	2 (10%)	
Positive responses to methacholine, %‡	75	40
Positive responses to methacholine, %§	90	40
PD ₂₀ FEV ₁ methacholine (μ g)*	951.2 $\pm$ 258.3¶	1993.9 $\pm$ 322.3¶
Positive responses to UNDW, %	65**	20**
FEV ₁ maximal fall after UNDW, %*	17.7 $\pm$ 2.3††	7.55 $\pm$ 1.38†

* Mean $\pm$ SEM.

† Data for 34 subjects.

cutpoint: 1560 μ g (PD₂₀ FEV₁).

§ cutpoint: 2625 μ g (PD₂₀ FEV₁).

** $P < .01$, chi square test.

†† $P < .01$, Mann-Whitney U test.

¶ $P < .02$, Mann-Whitney U test.

|| $P < .05$, chi square test.

pore filter. The patient carried out five inhalations with phosphate buffered-saline from nebulizer n.1, and one to four inhalations from nebulizer n.2. With each series of inhalations the dose of methacholine was doubled until a cumulative dose of 176 μg was reached. Thereafter, one inhalation from nebulizer n.3 was administered, thus reaching a cumulative dose of 3460 μg . Subsequently, the dose of methacholine was doubled at each step until a maximal cumulative dose of 3460 μg was reached. Each dose was administered at 2-minute intervals until reaching the maximal cumulative dose or until a fall in $\text{FEV}_1 \geq 20\%$ from the highest postsaline control level was observed. FEV_1 was measured two minutes after each series of inhalations. The final datum obtained was the methacholine dose causing a 20% fall in FEV_1 . A subject was considered to be hyperresponsive if the $\text{PD}_{20} \text{FEV}_1$ was $\leq 1560 \mu\text{g}$.¹¹ Hyperresponsiveness was excluded completely when $\text{PD}_{20} \text{FEV}_1$ was $\geq 2625 \mu\text{g}$.³ $\text{PD}_{20} \text{FEV}_1$ between 1561 and 2625 μg was considered intermediate bronchial hyperresponsiveness.

Ultrasonically nebulized distilled water was generated by an ultrasonic nebulizer (Devilbiss, Somerset, Pennsylvania, USA) at a flow of 4 L/min.^{12,13} FEV_1 was measured before and at 5, 15, and 30 minutes after inhalation. Patients inhaled through a mouthpiece connected to a three-way valve breathing at tidal volume for five minutes in a single dose fashion. The output of the nebulizer was measured by simulating ventilation by means of a pump. The pump was connected to the nebulizer through a 3-way valve. The weight of the canister containing distilled water, tubing and valve was measured before and after simulated ventilation. Ten determinations at different levels of simulated ventilation were carried out. The mean difference in weight was 1.35 g/L of airflow, SEM 0.14, thus the output of the nebulizer was 5.4 g of water per minute. FEV_1 values after challenge were expressed as the percent FEV_1 variations as compared to baseline [$100 \times (\text{post-}$

$\text{pre})/\text{pre}]$. The test was considered positive if FEV_1 dropped at least 15% after the challenge.

Specific Bronchial Challenges

Specific bronchial challenges were carried out in a 7.2 m³ exposure chamber.^{10,14} In this chamber, a controlled atmosphere was generated by evaporation or aerosolization of appropriate quantities of each chemical. Challenges were performed with the following chemicals: epoxy resin (n.7), colophony (n.4), phthalic anhydride (n.4), styrene (n.3), formaldehyde (n.8), glutaraldehyde (n.3), nickel sulphate (n.3), chrome salts (n.2), ammonium persulphate (n.4), and chloramine-T (n.2). Each challenge was carried out using an incremental dosing pattern over several days. The increase in dose was preferably obtained by increasing the exposure time rather than the concentration of the substance. For epoxy resin, colophony and phthalic anhydride the atmosphere in the chamber was generated by allowing evaporation by heat of appropriate quantities of each substance. Styrene was allowed to evaporate at room temperature.¹⁵ For nickel sulphate, chrome salts, formaldehyde, glutaraldehyde, ammonium persulphate and chloramine-T the atmosphere in the chamber was created by intermittent aerosolization of the due quantities by means of a pneumatic nebulizer. In the absence of specific recommendations in the literature the quantity of each substance was adjusted to reach a starting mean concentration corresponding to a quarter of the threshold limit value established by the American Conference of Governmental Industrial Hygienists,¹⁶ when available, or a concentration proved to have no irritant effect on a control group of nonexposed asthmatic subjects. The weighted mean environmental concentration of each substance was monitored by means of sampling pumps (flow rate between 0.5 and 1 L/min). Aldehydes were collected on an activated (dinitrophenylhydrazine) XAD substrate. Ammonium persulphate, nickel and chrome salts were collected on nitrocellulose microfibers.

Four styrene charcoal tubes (jumbo) were used. The other substances were collected on glass wool filters and charcoal tubes or silica-gel tubes. Analyses were performed by means of atomic absorption spectroscopy equipped with graphite furnace for nickel sulphate and chrome salts, high resolution GC-FID for styrene, HPLC-Diode Array for aldehydes and chloramine-T, and HR GC-MS for all other substances. For each analytical method the detection limit, accuracy, and precision were determined at different concentration levels. The final concentration never exceeded half the threshold limit value. Exposure was terminated after 20 minutes on day 1. In the absence of response, the challenge was repeated after a 2-day interval, and the exposure time was increased up to a maximum of three hours. For western red cedar the challenge was performed using an occupational method¹⁷ in the same chamber.

During each challenge the exposure was immediately stopped if symptoms of asthma occurred.

Respiratory function was monitored measuring FEV_1 before (0) and at 15 and 30 minutes, then hourly after exposure for the next eight hours. The test was considered positive when FEV_1 decreased at least 15% from the baseline within 30 minutes (immediate response or immediate component of a dual response), and/or one or more hours after exposure (late response or late component of a dual response).

To exclude a spontaneous variation of pulmonary function, FEV_1 was recorded at the same time points of the specific challenges on a nonchallenge control day in each patient. To exclude a nonspecific response each challenge was repeated on a different day after inhalation of a placebo. The choice of placebo varied according to the characteristics of the active substance tested.

Expression and Analysis of Data

The methacholine dose capable of causing a 20% fall in FEV_1 ($\text{PD}_{20}\text{FEV}_1$) was calculated by interpolating, on a semilogarithmic dose-re-

Table 2. Clinical Features of the Reactors According to the Type of Asthmatic Response

	Immediate Response	Late Response	Dual Response
N subjects	12	6	2
Age, yr*	29.7 ± 3.2	36.8 ± 2.1	41 ± 12
FEV ₁ % of predicted	94.4 ± 4.1	99.5 ± 9.3	93 ± 6.0
Atopy, %	30	25	0
Smoking habits			
Current smokers, %	8.3	33.3	0
Nonsmokers, %	41.7	16.7	100
Ex-smokers, %	50.0	50.0	0
Duration of symptoms before diagnosis, yr*	1.8 ± 0.7	3.5 ± 1.8	1.7 ± 0.25
Duration of exposure, yr*	10.8 ± 2.7	15.8 ± 1.1	18.5 ± 8.5
Positive responses to methacholine, % [#]	66.6	83.3	100
Positive responses to methacholine, % [§]	83.3	100	100
PD ₂₀ FEV ₁ methacholine, μg*	1163.8 ± 381.1	806.2 ± 369.7	110.5 ± 23.5
Positive responses to UNDW, %	75	50	100
FEV ₁ maximal fall after UNDW, %*	20.2 ± 3.2	11 ± 3.3	23 ± 0

* Mean ± SEM.

[#] Cutpoint: 1560 μg (PD₂₀ FEV₁).[§] Cutpoint: 2625 μg (PD₂₀ FEV₁).

sponse curve plot, the point corresponding to the dose causing the FEV₁ fall just below 20% with the point corresponding to the dose causing the FEV₁ fall just above it. Data were analyzed at the two different cut-points for PD₂₀FEV₁ of methacholine described above (1560 and 2625 μg).

Sensitivity (percent of subjects with TDI asthma with a positive test) and specificity (percent of subjects without TDI asthma with a negative test) of methacholine and UNDW challenge were determined with the results of the specific challenge as previously described.^{7,10} The two tests were also combined to enhance sensitivity or specificity.^{15,18} Combination in series considered a subject positive only if he tested positive to both methacholine and UNDW, negative if he tested negative to either of the two challenges; combination in parallel labelled a subject positive if he tested positive to either of the two challenges. Combination in series enhances the specificity of the testing, combination in parallel enhances sensitivity.

Statistical analysis was carried out by Mann-Whitney U test, Kruskal-Wallis nonparametric analysis of variance, Spearman's rank correlation test and chi square test where it was appropriate. A level greater than 95% probability was considered to be significant.

RESULTS

No significant difference was found in age, prevalence of atopy, smoking habits, average duration of symptoms before diagnosis and duration of occupational exposure between the group of reactors (subjects who developed a bronchoconstrictive response to the specific challenge) and the group of nonreactors. The mean basal FEV₁ was in the normal range both in reactors and in nonreactors. Among the nonreactors only four subjects (20%) had FEV₁ lower than 80% of that predicted. Asthmatic responses to the specific challenge were immediate in 12 (60%), late in 6 (30%), and dual in 2 (10%) cases (Tables 1 and 2). Among the three subgroups with different

types of asthmatic responses there was no significant difference in age, sex, atopy, average duration of symptoms before diagnosis and duration of occupational exposure.

The frequency of positive responses to methacholine was significantly higher ($P < .05$) in reactors than in nonreactors at both cutpoints (Table 1). The mean PD₂₀FEV₁ was significantly ($P < .02$) lower in reactors (μg 951.2, SEM 258.3) than in nonreactors (μg 1993.9, SEM 322.3) (Table 1). At the lower cutpoint (PD₂₀FEV₁ 1560 μg) 15 reactors (75%) and eight nonreactors (40%) were hyperresponsive to methacholine. The degree of bronchial hyperresponsiveness was similar in the two subgroups (mean PD₂₀FEV₁ μg 344.2, SEM 49.7 in the reactors, μg 370.6, SEM 53.8 in nonreactors). The positive responses were 18 (90%) in reactors, and 8 (40%) in nonreactors when the intermediate responses (higher cutpoint, PD₂₀FEV₁ 2625 μg) were included. No difference was found in the frequency or in the distribution of positive responses to methacholine among the immediate, dual and late responders to the specific challenge (Table 2).

The frequency and severity (maximal mean percent decrease in FEV₁ after UNDW challenge) of the responses to UNDW were significantly ($P < .01$) higher in reactors than in nonreactors (Table 1, Fig 1), whereas no difference was found among the three subgroups of reactors with different pattern of response to the specific challenge (immediate, late or dual) when comparing the subgroups with each other (Table 2). A significant correlation was found between the PD₂₀FEV₁ of Mch, and the maximal decrease in FEV₁ after UNDW inhalation (Spearman correlation coefficient = 0.473, $P < .01$). At the lower cutpoint (PD₂₀FEV₁ 1560 μg) the concordance between the two nonspecific bronchial challenges was 70%, at the higher cutpoint (PD₂₀FEV₁ 2625 μg) it was 67.5%.

Combination of positive and negative responses to methacholine and UNDW had a different distribution in

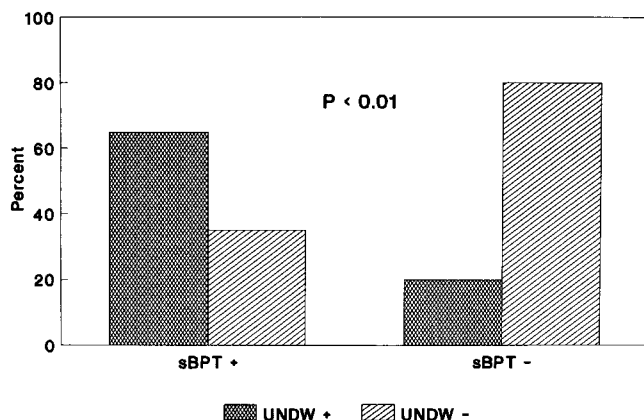


Figure 1. Distribution of the responses to UNDW challenge in reactors and nonreactors to the specific challenge.

reactors and nonreactors (Tables 3 and 4), but no difference was found among the three subgroups of subjects with different patterns of response to the specific challenge.

The sensitivity and specificity of UNDW and methacholine in the diagnosis of low molecular weight chemical induced-asthma with the specific challenge as "the gold standard" are shown in Figures 2 and 3. Ultrasonically nebulized distilled water challenge offered the best specificity (80%) but lacked sensitivity (65%). Methacholine challenge proved to be more sensitive (75% and 90% at the lower and higher cutpoint, respectively), but less specific (60% at both cutpoints).

Combination in parallel (positivity to any of the two tests) of methacholine

Table 3. Distribution of the Combination of Positive and Negative Responses to Methacholine and UNDW in Reactors and Nonreactors at the 1560- μ g Cutpoint for PD₂₀ FEV₁ of Methacholine

	Reactors*	Non-reactors*
Mch +, UNDW + (%)	11 (55)	3 (15)
Mch +, UNDW - (%)	4 (20)	5 (25)
Mch -, UNDW + (%)	2 (10)	1 (5)
Mch -, UNDW - (%)	3 (15)	11 (55)

* $P < .05$, chi square test.

Table 4. Distribution of the Combination of Positive and Negative Responses to Methacholine and UNDW in Reactors and Nonreactors at the 2625- μ g Cutpoint for PD₂₀ FEV₁ of Methacholine

	Reactors*	Non-reactors*
Mch +, UNDW + (%)	12 (60)	3 (15)
Mch +, UNDW - (%)	6 (30)	5 (25)
Mch -, UNDW + (%)	1 (5)	1 (5)
Mch -, UNDW - (%)	1 (5)	11 (55)

* $P < .01$, chi square test.

line and UNDW had considerably greater sensitivity (85% and 95% at the lower and higher cutpoint, respectively) than UNDW alone (65%), but slightly changed the sensitivity of methacholine alone (85% versus 75% at the lower cutpoint, 95% versus 90% at the higher). Combination in series (both tests positive) had considerably greater specificity (85%) than methacholine alone (60%), but only slightly changed UNDW specificity (85% versus 80%) (Figs 2 and 3).

DISCUSSION AND CONCLUSIONS

The results of the present study show that, in a group of occupationally exposed subjects with a suggestive history, the UNDW challenge is more

specific than methacholine in identifying the subjects with occupational asthma due to low molecular weight chemicals, diagnosed with the specific challenge as the "gold standard." The UNDW test, however, is considerably less sensitive. The combination of methacholine and "fog" results in very high sensitivity and high specificity for occupational asthma.

The role of nonspecific bronchial responsiveness in occupational asthma has been widely evaluated by means of histamine or methacholine challenge test. In a clinical setting, these challenges have proved to be useful for the diagnosis of asthma, for the assessment of work-relatedness of asthma in association with other function tests, for the determination of the initial and subsequent concentrations of occupational sensitizing agents for specific challenge test, and for the assessment of permanent impairment/disability.^{19,20} Recently, the measurement of airway responsiveness to methacholine has assumed a central role in the diagnostic procedure of occupational asthma. In fact, the absence of a positive response to methacholine, after the worker has been at work for at least 2 weeks under the usual work conditions, is considered by most authors to be sufficient for virtually ruling out occupational asthma, and halt the diagnostic work-up.^{3,4} If airway hyperresponsiveness is found, there is indication for further workup in order to confirm the relationship between asthma and work. In occupational asthma the measurement of nonspecific airway responsiveness to methacholine has strong practical implications. For this reason, the cutpoint for hyperresponsiveness is chosen at a very sensitive endpoint (PC₂₀ 10 mg/mL, ie, PD₂₀ 2625 μ g),³ which is higher than that indicated for the definition of hyperresponsiveness in nonoccupational asthma (PC₂₀ 8 mg/mL, ie, PD₂₀ 1560 μ g).¹¹

Like histamine, methacholine is a stimulus which acts directly on bronchial smooth muscle cells to cause bronchial contraction.⁷ Ultrasonically nebulized distilled water challenge is an indirect stimulus whose mechanism

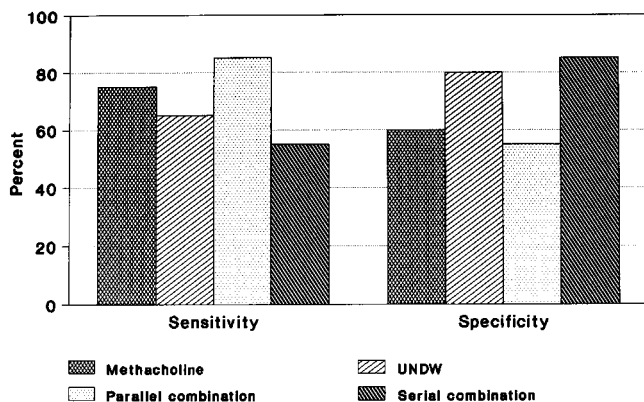


Figure 2. Sensitivity and specificity of Mch and UNDW, alone and in combination, at the 1560- μ g cutpoint for PD₂₀ FEV₁.

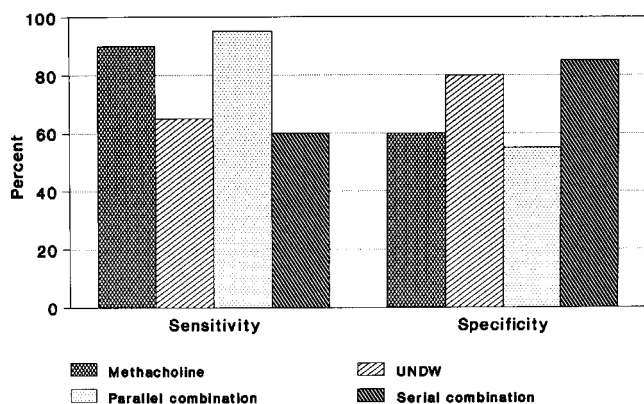


Figure 3. Sensitivity and specificity of Mch and UNDW, alone and in combination, at the 2625- μ g cutpoint for PD₂₀ FEV₁.

of action seems to invoke intermediate events such as the release of inflammatory mediators in the airway lumen and in the submucosa,^{8,12,13,21} thus, the two stimuli provoke a response by two different pathways. We wondered whether the use of "fog," which has been proved highly specific in nonoccupational asthma^{8,9} and may more clearly reflect clinical disease,⁷ could improve the diagnostic accuracy in the investigation of occupational asthma. In a previous study on a group of subjects occupationally exposed to TDI with work-related symptoms,¹⁰ we found that UNDW challenge was more specific than methacholine in assessing TDI asthma. In the present study we extended those observations to a group of subjects occupationally exposed to a heterogeneous group of chemicals all

known as etiologic factors of occupational asthma.²² Data were analyzed at two cutpoints for methacholine: the cutpoint more commonly used clinically for the diagnosis of asthma (PD₂₀ 1560 μ g),¹¹ and the more sensitive cutpoint for the diagnosis of occupational asthma (PD₂₀ 2625 μ g).³

In this study, the clinical features of the subjects studied were similar to those of the TDI study.¹⁰ The group of subjects who developed a bronchoconstrictive response to the specific challenge (reactors) did not differ from the group of nonreactors in age, or characteristics of occupational history. Atopy did not seem to influence the development of low molecular weight induced-asthma. In most reactors respiratory function was not impaired. Only three subjects had baseline FEV₁ lower

than 80% of predicted. The asthmatic responses to the specific challenge were immediate, late and dual. In contrast to the TDI study that revealed a higher frequency of late reactions (73.3% of the subjects), this study showed a higher frequency of the immediate component (60% of subjects). According to the type of response, the three subgroups of subjects did not differ significantly with respect to age, sex, occupational history, and basal FEV₁. In contrast to the TDI study, current smokers were all distributed in the groups of either immediate or late reactors.

At both cutpoints, the distribution of bronchial hyperresponsiveness to methacholine was significantly different in the reactors and nonreactors, with no difference regarding the type of asthmatic response. At the lower cutpoint (PD₂₀ 1560 μ g), five reactors (25%) had a negative response to methacholine. At the higher cutpoint, only two reactors (10%) had a negative methacholine challenge. At both cutpoints, 40% of the nonreactors showed hyperresponsiveness to this pharmacologic stimulus, confirming the poor specificity of the test in occupational asthma.⁴⁻⁶

The frequency and severity of positive responses to UNDW inhalation were significantly higher in the reactors to the specific challenge, as compared with nonreactors, and in the proportion of reactors who showed an immediate component of the asthmatic response (immediate plus immediate component of the dual considered together), as compared with nonreactors. In the present study, as well as in a former study,¹⁰ a positive response to UNDW seemed to be correlated with a positive specific challenge with an immediate component (immediate alone or dual). According to nonoccupational asthma^{8,9} and TDI asthma,¹⁰ UNDW was found highly specific (80%) for occupational asthma, but poorly sensitive (65%).

We evaluated whether the use of both nonspecific challenges could give any advantage. We found that at the higher cutpoint for PD₂₀FEV₁ the com-

bination of the two challenges resulted in very high sensitivity and high specificity for occupational asthma.

In conclusion, in the assessment of low molecular weight chemical induced-asthma diagnosed with the specific challenge as the "gold standard" UNDW challenge proves more specific than methacholine for occupational asthma, but it is considerably less sensitive. In a clinical setting, in subjects with a suggestive history and exposed to possible causal agents such as low molecular weight chemicals the combination of the two challenges results in very high sensitivity and high specificity for occupational asthma. Further investigations, however, are required for the definitive diagnosis of occupational asthma, but UNDW challenge should be regarded as a useful method to confirm objectively a suggestive history.

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Request for reprints should be addressed to:
A Dellabianca, MD
Clinica del Lavoro Foundation
Via Boezio 26
27100 Pavia
Italy